Reduction of adsorbed As(V) on nano-TiO₂ by sulfate-reducing bacteria

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HIGHLIGHTS

- As(V), either adsorbed or dissolved, was reduced in the presence of SRB.
- Reduction was faster for adsorbed As(V) than for dissolved As(V).
- SRB promoted As(V) desorption from TiO₂ compared with abiotic sulfide.
- As(V) desorption due to competition with phosphate surface complexation

GRAPHICAL ABSTRACT

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ABSTRACT

Reduction of surface-bound arsenate [As(V)] and subsequent release into the aqueous phase contribute to elevated As in groundwater. However, this natural process is not fully understood, especially in the presence of sulfate-reducing bacteria (SRB). Gaining mechanistic insights into solid-As(V)-SRB interactions motivated our molecular level study on the fate of nano-TiO₂ bound As(V) in the presence of *Desulfovibrio vulgaris* DP4, a strain of SRB, using incubation and in situ ATR-FTIR experiments. The incubation results clearly revealed the reduction of As(V), either adsorbed on nano-TiO₂ or dissolved, in the presence of SRB. In contrast, this As(V) reduction was not observed in abiotic control experiments where sulfide was used as the reductant. Moreover, the reduction was faster for surface-bound As(V) than for dissolved As(V), as evidenced by the appearance of As(III) at 45 h and 75 h, respectively. ATR-FTIR results provided direct evidence that the surface-bound As(V) was reduced to As(III) on TiO₂ surfaces in the presence of SRB. In addition, the As(V) desorption from nano-TiO₂ was promoted by SRB relative to abiotic sulfide, due to the competition between As(V) and bacterial phosphate groups for TiO₂ surface sites. This competition was corroborated by the ATR-FTIR analysis, which showed inner-sphere surface complex formation by bacterial phosphate groups on TiO₂ surfaces. The results from this study highlight the importance of indirect bacteria-mediated As(V) reduction and release in geochemical systems.

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1. Introduction

Arsenic (As) is a toxic metalloid naturally occurring as As(V) and As(III). Mounting evidence over the past decade suggests that coupled redox and adsorption/desorption processes regulate the concentration and speciation of As in groundwater (Smedley et al., 2002; Islam et al.,

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2004; Kumar et al., 2016a, 2016b). Nevertheless, a great challenge in the biogeochemical study of As is to decipher the reduction and release process at the molecular level in the complex real environment.

In natural environments, As is primarily bound to soil minerals, especially to iron (hydr)oxides, which are responsible for As release or stability in soils and sediments (Daus et al., 1998). When iron (hydr)oxides dissolve, As is released, which leads to elevated As in groundwater (Islam et al., 2004). Meanwhile, the reduced iron (hydr)oxides may form various secondary iron minerals, which can re-uptake the released As (Tufano and Fendorf, 2008; Muehe et al., 2016; Wang et al., 2014).

Under anoxic conditions, microorganisms such as sulfate-reducing bacteria (SRB) play an important role in the biogeochemical cycling of As (Burton and Kocar, 2014; Kumar et al., 2016a, 2016b; ThomasArrigo et al., 2016). SRB reduce sulfate to sulfide (Oremland et al., 2000; Burton et al., 2011), and the biogenic sulfide is capable of reducing As(V) to As(III) at appreciable concentrations, particularly in sulfidic environments (Xu et al., 2016; Wilkin et al., 2003; Oremland et al., 2005). The detailed knowledge of As reduction and desorption processes on metal oxides in the presence of microbes is the key to understanding As contamination.

Great efforts have made to explore the impact of microbes on As reduction and release from metal oxides (Stuckey et al., 2016; Zobrist et al., 2000; Zhang et al., 2016; Sun et al., 2016). Some studies concluded that As(V) bio-reduction occurred predominantly in solution, rather than on mineral surfaces (Jones et al., 2000; Langer and Inskeep, 2000). For instance, Huang et al. (2011a, 2011b) suggested that dissolved As(V) reduction by *Shewanella putrefaciens* strain CN-32 followed first-order kinetics with a 3 h half-life, and the addition of goethite resulted in a significant decrease in the reduction rate (32 h As(V) half-life). In contrast, some studies hypothesized that adsorbed As(V) was directly reduced on solids by bacteria. For example, Ohtsuka et al. (2013) reported that a dissimilatory arsenate-reducing bacterium (*Geobacter pelophilus*, OR-18) directly reduced As(V) on a paddy soil. Rochette et al. (1998) also found that As(V) was rapidly reduced to As(III) and remained mostly associated in the solid phase.

The complexity of the As redox and release processes has motivated the application of in situ spectroscopic techniques such as in situ ATR-FTIR (Yan et al., 2016a, 2016b). Previous ATR-FTIR studies implied that the functional groups of microbes such as phosphate and phosphodiester may play an important role in the attachment of bacterial cells to iron oxide minerals through the formation of covalent bonds (Omoike et al., 2004; Parikh and Chorover, 2008; Burnett et al., 2006). Then, bacteria may facilitate As desorption by competing with As for coordination at surface sites.

The objective of the present study was to investigate the reduction and desorption of adsorbed As(V) on nano-TiO₂ in the presence of SRB using batch incubation experiments and in situ ATR-FTIR spectroscopy. Nano-TiO₂ is an effective As adsorbent (Luo et al., 2010; Yan et al., 2015; Yan et al., 2016a, 2016b), and its physicochemical stability enables it to be a good template to de-couple the As reduction and release processes. The insights gained from this study shed new light on the risk assessment of As-laden solids in the environment.

2. Materials and methods

2.1. Nano-TiO₂, artificial groundwater and SRB preparation

Nano-TiO₂ used in this study was prepared with titanium sulfate. The basic properties of synthetic nano-TiO₂ can be found in our previous work (Luo et al., 2010). Artificial groundwater was composed of 0.5 mM NaHCO₃, 0.02 mM NH₄Cl, 0.07 mM KCl, 9 mM Na-lactate, 21 mM MgSO₄, and 1.5 mM CaCO₃ (Luo et al., 2013). The electron acceptor was sulfate (MgSO₄), and the electron donor was lactate (Na-lactate). The SRB, named as *Desulfovibrio vulgaris* DP4, was isolated from a soil sample collected at a naturally-occurring As-contaminated site

in China, which was described in detail in our previous study (Luo et al., 2013).

2.2. Batch incubation experiments of adsorbed/dissolved As(V) in the presence of SRB and sulfide

2.2.1. Adsorption experiments

Suspensions containing 50 mL As(V) (3 mg L⁻¹, Na₂HAsO₄·7H₂O) and 1 g L⁻¹ nano-TiO₂ were prepared in 50 mL centrifuge tubes. The background solution was artificial groundwater. The pH of batch experiments was adjusted to 8.0 ± 0.1 using NaOH and H₂SO₄ solutions. Suspensions were mixed on a rotator for 24 h, and then centrifuged. As-laden nano-TiO₂ solids were thus obtained. The supernatant was filtered by a 0.22-μm membrane filter for soluble As(V) analysis. The final adsorption capacity of As(V) on nano-TiO₂ was approximately 3 mg g⁻¹.

Reduction experiments of adsorbed As(V) on nano-TiO₂ were conducted with SRB and an abiotic reducer sulfide (21 mM, Na₂S) in the glovebox. The reduction experiments were conducted in 500 mL serum bottles. After the As-laden nano-TiO₂ solid was obtained, the solid (As adsorption capacity 3 mg g⁻¹, TiO₂ 1 g L⁻¹) was suspended in artificial groundwater, and SRB were added at 1 mL per 100 mL solution (Luo et al., 2013). For comparison with the reduction induced by SRB, the sulfide as an abiotic reducer was added to the suspension. Reduction experiments of dissolved As(V) in the presence of SRB and sulfide were conducted as well in order to compare with adsorbed As(V). As(V) (3 mg L⁻¹) solution was prepared with Na₂HAsO₄·7H₂O and artificial groundwater. As(V) solution was transferred to 500 mL serum bottles, and SRB were added at 1 mL per 100 mL solution. Moreover, the sulfide was added to the As(V) solution to examine As(V) reduction. All of the experimental solutions were adjusted to pH 8.0 ± 0.1 with NaOH and H₂SO₄. Finally, the bottles were sealed and transferred out of the glovebox and incubated at 25 °C for 4 d. During this period, homogenized samples were extracted using a syringe and passed through a 0.22 μm filter at predesigned intervals. Eh and pH were also monitored during the incubation experiments. All batch incubation experiments were performed in triplicate.

2.3. Analysis

The concentrations of soluble As were determined using a furnace atomic absorption spectrometer (Perkin-Elmer AAS-800) with a detection limit of 0.7 μg/L. The As speciation was analyzed with high performance liquid chromatography atomic fluorescence spectrometry (HPLC-AFS, Jitian, China) with a detection limit of 0.6 μg/L. Separation of As was carried out in a Hamilton PRP-X100 anion exchange column, using 10 mM phosphate buffer at pH 5.8 as mobile phase. Sulfate concentrations were determined using a DX-1100 ion chromatograph (Dionex, Sunnyvale, CA) with an AS11-HC Ion Pac column. Sulfide concentrations were measured using the methylene blue method (Luo et al., 2013) immediately following the sample collection.

2.4. In situ ATR-FTIR spectroscopy flow cell experiments

In situ ATR-FTIR experiments were performed for direct monitoring of the reduction of adsorbed As(V) on nano-TiO₂ in the presence of SRB. The ATR-FTIR spectra were recorded on a Perkin-Elmer Spectrum 100 IR spectrometer equipped with a liquid N₂-cooled MCT/A detector and an optics compartment that was continuously purged with high purity nitrogen.

ATR-FTIR experiments were conducted by a flow cell technique. Firstly, a nano-TiO₂ thin layer was prepared on the ZnSe ATR crystal. After 5 g L⁻¹ nano-TiO₂ was added to ultrapure water, the suspension was sonicated for two or three hours. Then about 300 μL of the nano-TiO₂ suspension was dropped onto the ZnSe ATR crystal with a pipette, and the colloidal TiO₂ thin layer was sealed in a flow cell. The flow cell

was placed on the ATR stage of the spectrometer and connected through plastic tubes to a reaction vessel containing 250 mL of artificial groundwater solution. A peristaltic pump was used to pump solution from this vessel through the flow cell at a rate of 15 mL h^{-1} for 3 h in order to equilibrate the TiO_2 thin layer with the background solution.

Following pre-equilibration, a background scan consisting of the combined absorbances of the ZnSe crystal, TiO_2 film, and artificial groundwater background solution was collected as the average of 256 scans at 4 cm^{-1} resolution. Next, the reaction solution was replaced with an As(V) solution ($\text{pH } 8.0 \pm 0.1$), which was prepared by spiking the appropriate amount of $\text{NaH}_2\text{AsO}_4 \cdot 7\text{H}_2\text{O}$ stock solution into artificial groundwater. The initial As(V) concentrations were chosen as 10 mg L^{-1} , 100 mg L^{-1} , and 1000 mg L^{-1} . The adsorption of As(V) on the TiO_2 film was monitored by observing the absorbance of As(V) in the spectral range $700\text{--}1300 \text{ cm}^{-1}$. After about 3 h, no further increases in As(V) absorbance were seen, and the final spectrum of adsorbed As(V) was collected. Next, 250 mL of a SRB suspension with a cell density of $10^8 \text{ cells mL}^{-1}$ was pumped through the flow cell for equilibration with As(V)-laden TiO_2 film for 19 h. The characteristic absorbance of adsorbed As(V) species and functional groups associated with SRB cells was recorded in the $700\text{--}1300 \text{ cm}^{-1}$ range throughout this time period by collecting spectra as the average of 250 scans at 4 cm^{-1} resolution every 5 min. The FTIR frequencies of adsorbed As(V) after SRB introduction were recorded simultaneously to study the effect of SRB cells on As(V) reduction at the TiO_2 surface in this system.

Finally, ATR spectra were obtained as follows: (1) the background spectrum of the TiO_2 film and the synthetic groundwater; (2) a series of spectra of As(V) adsorption on the TiO_2 film with three initial concentration levels at 10, 100, and 1000 mg L^{-1} ; (3) a time series of spectra showing the reduction kinetics of As(V) adsorbed on the TiO_2 film in the presence of SRB.

3. Results and discussion

3.1. Reduction of dissolved and adsorbed As(V)

Fig. 1 presents the reduction of dissolved and adsorbed As(V) in the presence of SRB and sulfide. SRB clearly facilitated the reduction of As(V) to As(III), both for As(V) initially adsorbed on nano- TiO_2 and dissolved As(V) (Fig. 1A). For the adsorbed As(V) system, reduced As(III) was detected at 45 h and its concentration increased to $68 \text{ }\mu\text{g/L}$ at 92 h, accounting for 2.3% of total As (Table 1). For the dissolved As(V) system, As(III) was observed at 75 h and increased to $12 \text{ }\mu\text{g/L}$ at 92 h, accounting for 0.4% of total As (Table 1). In contrast, As(III) was not detected for adsorbed or dissolved As(V) systems in the presence of sulfide as an abiotic control (Fig. 1B, Table 1).

The appearance of As(III) was earlier for adsorbed As(V) (45 h) than for dissolved As(V) (75 h) in the presence of SRB (Fig. 1A), indicating that the reduction of As(V) was faster in adsorbed form than in dissolved form. Some strains of SRB such as *Desulfovibrio vulgaris* (ATCC strain 7757) can reduce As(V) to As(III) by biogenic sulfide at high pH (Burton et al., 2011; Saalfield and Bostick, 2009; Hoefft et al., 2004). Some SRB strains can directly reduce As(V) and sulfate, for example, *Desulfosporosinus auripigmenti* (Newman et al., 1997). The SRB strain (*Desulfovibrio vulgaris*) used in this study cannot directly reduce As(V) (Luo et al., 2013; Saalfield and Bostick, 2009), whereas the biogenic sulfide generated by SRB reduction can reduce As(V) to As(III). In this study, the level of biogenic sulfide was higher for adsorbed As(V) than for dissolved As(V) (Fig. 3B), and the sulfide concentration was 5.9 mg L^{-1} for adsorbed As(V), but not detected for dissolved As(V) at 45 h (Fig. 3B). At 75 h, the sulfide concentration increased to 5.3 mg L^{-1} for dissolved As(V) (Fig. 3B), and then As(V) reduction occurred (Fig. 1A). The molar ratio of sulfide to As(V) was about 5:1 for

Fig. 1. The As(III) concentration in solution with initial adsorbed As(V) on TiO_2 and dissolved As(V) in the presence of SRB (A) and sulfide (abiotic reducer) (B). The As(V) concentration in solution with initial adsorbed As(V) on TiO_2 and dissolved As(V) in the presence of SRB (C) and sulfide (abiotic reducer) (D). Dashed lines indicate reaction time at 45 h and 75 h in Fig. 1A. Vertical lines on symbols indicate error bars in Fig. 1A and B.

Table 1

Distribution of As species in batch experiments of adsorbed As(V) and dissolved As(V) in the presence of SRB and sulfide.

	As(V) desorption (%) ^a		Detected As(III) (%) ^a		Total As desorption (%) ^a	
	End ^b	Mean	End ^b	Mean	End ^b	Mean
Adsorbed As-SRB	32.0	28.5	2.3	1.0	34.3	28.9
Dissolved As-SRB	/	/	0.4	0.2	/	/
Adsorbed As-sulfide	11.2	11.4	N.D. ^c	N.D. ^c	11.2	11.4
Dissolved As-sulfide	/	/	N.D. ^c	N.D. ^c	/	/

^a Percentage of total arsenic.

^b Batch incubation finish.

^c Not detected.

adsorbed As(V) at 45 h and 4:1 for dissolved As(V) at 75 h. Moreover, a significant correlation was observed between As(III) and biogenic sulfide for adsorbed ($R^2 = 0.997$) and dissolved As(V) ($R^2 = 0.776$) in the presence of SRB (Fig. S1). Therefore, the rate of As(V) reduction was dependent on biogenic sulfide processes. The reason for the low level of sulfide in the dissolved As(V) system might be ascribed to the As toxicity to SRB. In fact, the activity of SRB was found to be adversely affected by dissolved heavy metals (Hao et al., 1994). Thus, the dissolved As(V) (3 mg L^{-1}) might restrain the activity of SRB, whereas the toxicity of As(V) was significantly diminished once it was adsorbed.

Previous studies suggested that the reduction rate of adsorbed As(V) was much slower than that of dissolved As(V) (Zobrist et al., 2000; Jones et al., 2000; Langer and Inskeep, 2000). Jones et al. (2000) reported that the rate of microbial As(V) reduction in goethite suspensions was about 1200 times slower than for dissolved As(V) in the presence of As(V)-reducing bacteria *Clostridium* sp. Strain CN-0. The authors

proposed that the rate of As(V) reduction was controlled by its desorption rate, implying that As(V) reduction occurred predominantly in the aqueous phase after As(V) desorption. This As(V) desorption was promoted by microbes due to their attachment to solid surfaces. In this study, however, we speculated that adsorbed As(V) reduction occurred directly on solid surfaces, resulting in a faster As(V) reduction rate for adsorbed As(V) than for dissolved As(V). The surface interactions between As, oxide surface, and bacteria might accelerate the As(V) reduction.

Fig. 2 presents Eh and pH values during the incubation. The pH in biotic systems slightly decreased from the initial value of about 8.1 to 7.6 and 7.7, respectively, for adsorbed and dissolved As(V) systems at 92 h. This pH decrease can be attributed to the accumulation of biogenic H_2S due to SRB activity (Cruz Viggi et al., 2010; Luo et al., 2013). In the abiotic control, the pH slightly increased from 8.1 to 8.4 for both adsorbed and dissolved As(V) systems at 92 h. Concurrently, Eh dropped from -224 mV to around -345 and -238 mV , respectively, for adsorbed and dissolved As(V) systems in the presence of SRB. In the abiotic control, the change in Eh was negligible, and the Eh on average was $-405 \pm 12 \text{ mV}$ for the adsorbed As(V) system and $-404 \pm 14 \text{ mV}$ for dissolved As(V).

In the abiotic control, no As(V) reduction was observed for adsorbed and dissolved As(V) systems in the presence of sulfide (Fig. 1B). The reduction of As(V) induced by dissolved sulfide is highly pH dependent (Rochette et al., 2000). Sulfide can act as an electron donor for abiotic As(V) reduction at low pH. Rochette et al. (2000) reported that As(V) was reduced to As(III) at pH 4–5 with a sulfide to As(V) molar ratio of 2:1. During the As(V) reduction, sulfide first replaced an oxygen atom on As(V) to form thioarsenate species, and then thioarsenate was reduced to As(III), which is facilitated by H^+ (Rochette et al., 2000). However, no As(V) reduction was observed at pH 7 after 7 days, even

Fig. 2. pH and Eh as a function of incubation time for initial adsorbed As(V) and dissolved As(V) in the presence of SRB (A, C) and sulfide (abiotic reducer) (B, D).

with an initial sulfide to As(V) molar ratio of 100:1. Therefore, pH plays an important role in the As(V) reduction. The pH decreased to 7.6 in the presence of SRB compared to the increase to 8.4 in the abiotic control (Fig. 2), which favors the As(V) reduction in the biotic system. Coincident with the previous study, our study shows that As(V) is not reduced by sulfide, the abiotic reducer, at the more alkaline condition (pH 8.1), even with a high molar ratio of sulfide to As(V) up to 524:1.

In the presence of SRB, sulfate concentrations decreased over time, and sulfide accumulated accordingly (Fig. 3). The concentration of sulfate decreased more sharply for adsorbed As(V) than for dissolved As(V) (Fig. 3A). The sulfate concentration fell to 1.74 g L^{-1} from the initial 2 g L^{-1} (21 mM) for adsorbed As(V) compared with 1.97 g L^{-1} for dissolved As(V) during 92 h incubation. The biogenic sulfide increased to about 98.8 mg L^{-1} for adsorbed As(V) compared with 10.2 mg L^{-1} for dissolved As(V) near the end of the incubation experiments. In the abiotic control, sulfide concentrations slightly decreased from around 600 mg L^{-1} to 491 mg L^{-1} and 440 mg L^{-1} , respectively, for adsorbed and dissolved As(V) systems (Fig. S2). As shown in the sulfur mass balance in Table 2, 27.1% and 3.9% of sulfate was reduced, and 4.9% and 0.5% sulfide was detected, respectively, for adsorbed and dissolved As(V) systems in the presence of SRB. On the other hand, in the abiotic control, 81.6% and 72.7% sulfide were detected for adsorbed As(V) and dissolved As(V). Then, about 22.2% and 3.4% of total sulfur existed as neither sulfide nor sulfate for adsorbed and dissolved As(V), respectively, in the presence of SRB. Similarly, about 18.4% and 27.3% of total sulfur was not detected for adsorbed and dissolved As(V), respectively, in the

Table 2

Sulfur mass balance in batch experiments of adsorbed As(V) and dissolved As(V).

	Loss of sulfate (%) ^a		Detected sulfide (%) ^a	
	End ^b	Maximum	End ^b	Maximum
Adsorbed As-SRB	27.1	27.1	4.9	4.9
Dissolved As-SRB	3.9	4.4	0.5	0.5
Adsorbed As-sulfide	/	/	81.6	96.5
Dissolved As-sulfide	/	/	72.7	98.5

^a Percentage of total sulfur.^b Batch incubation finish.

abiotic control, which could exist in other forms of sulfur such as an As-S complex (Wilkin et al., 2003; Rochette et al., 2000).

3.2. In situ reduction of adsorbed As(V) on nano-TiO₂

To justify our speculation that the reduction of adsorbed As(V) occurred on nano-TiO₂ surfaces, in situ ATR-FTIR flow cell experiments were performed with three As(V) levels (10, 100, 1000 mg L⁻¹), and the spectra at adsorption equilibrium are presented in Fig. 4. The adsorbed As(V) exhibited a peak at 856 cm^{-1} corresponding to $\nu(\text{As-O})$, in line with previous reports that the peak centered at $854\text{--}861 \text{ cm}^{-1}$ could be assigned to the stretching mode of As—O bonds (Voegelin and Hug, 2003; Pena et al., 2006; Goldberg and Johnston,

Fig. 3. Dissolved sulfate and sulfide concentrations as a function of incubation time for initial adsorbed As(V) and dissolved As(V) in the presence of SRB. Vertical lines on symbols indicate error bars in Fig. 2A and B.

Fig. 4. FTIR spectra of As(V) adsorbed on nano-TiO₂ film after equilibrium. As(V) absorbance increased linearly with the initial As(V) concentration from 10 to 1000 mg L⁻¹. TiO₂-ZnSe is TiO₂ film on ZnSe crystal without As(V).

2001). Increasing the As(V) concentration from 10 to 1000 mg L⁻¹ resulted in a linear increase in the absorbance for adsorbed As(V) on the TiO₂ film.

The flow cell ATR-FTIR spectra of adsorbed As(V) reduction in the presence of SRB are presented in Figs. 5 and 6. The first spectrum (time = 0) showed the equilibrium of As(V) (1000 mg L⁻¹) adsorption on TiO₂ with an As—O stretching band at 856 cm⁻¹ (Fig. 5). Immediately after this spectrum was collected, SRB were introduced, and additional spectra were collected during the following 19 h. During this period, a new peak appeared at 794 cm⁻¹ which was assigned to As(III) (Fig. 5A) (Voegelin and Hug, 2003; Pena et al., 2006). Similarly, Voegelin and Hug (2003) reported that the peak for surface-bound As(III) on ferrihydrite was centered at 774 cm⁻¹. Compared to the decrease in As(V) peak intensity, As(III) absorbance increased with the reaction time. Fig. 5B

shows that As(V) was reduced gradually to As(III) as evidenced by the peak shift from 856 cm⁻¹ to 794 cm⁻¹ after 19 h. Meanwhile, the peak attributed to adsorbed As(III) after SRB introduction was also observed with initial As(V) 100 mg L⁻¹ (Fig. 6) and 10 mg L⁻¹ (Fig. S3). These results indicated that the presence of SRB facilitated the reduction of adsorbed As(V) to As(III) on TiO₂ surfaces.

Interestingly, pronounced increases in FTIR absorbance were observed for functional groups associated with SRB coordination to the TiO₂ surface (1049 cm⁻¹) as time progressed (Figs. 5, 6, and S3). The peak at 1049 cm⁻¹ was also observed when SRB were added without As(V) on the TiO₂ film (Fig. S4). According to previous studies, the P—O stretching bands range from 1000 to 1200 cm⁻¹ (Parikh and Chorover, 2006; Tejedor-Tejedor and Anderson, 1999; Persson et al., 1996). Parikh and Chorover (2006) reported that the interaction of

Fig. 5. ATR-FTIR spectra collected during the reduction of As(V) (1000 mg L⁻¹) on the surface of TiO₂ film after the addition of SRB cells (A). Spectra were collected every 5 min. After SRB cell addition, the growth of the As(III) peak can be observed in situ. The right small fig. (B) showed the ATR-FTIR spectra at time 0 h and 19 h.

Fig. 6. ATR-FTIR spectra collected during the reduction of As(V) (100 mg L^{-1}) on the surface of TiO_2 film after the addition of SRB cell. Spectra were collected every 5 min. After SRB cell addition, the growth of the As(III) peak can be observed in situ.

bacteria with $\alpha\text{-Fe}_2\text{O}_3$ films resulted in a strong inner-sphere P-O-Fe complexation as evidenced by the 1041 cm^{-1} peak. Tejedor-Tejedor and Anderson (1999) assigned the bands located at 1096 and 1044 cm^{-1} to the deprotonated bidentate-binuclear surface complex, $\equiv\text{Fe}_2\text{PO}_4^-$. Persson et al. (1996) suggested that peaks at 1122 and 1049 cm^{-1} could be ascribed to orthophosphate adsorption to goethite as monodentate complexes. Thus, the peak at 1049 cm^{-1} in this study was assigned to the P—O—Ti vibrational band (Omoike et al., 2004; Parikh and Chorover, 2006; Huang et al., 2011a, 2011b; Barja et al., 1999). The FTIR results suggested that P-O-Ti inner-sphere coordination of phosphate groups, either present in surface biomolecules or in materials exuded from the bacteria such as extracellular polymeric substances, might be formed at TiO_2 surfaces.

3.3. Influence of SRB on As(V) desorption

The results of As(V) desorption from nano- TiO_2 in the presence of SRB and abiotic control (sulfide) are presented in Fig. 1C and D. The amount of desorbed As(V) was greater in the presence of SRB than that in the abiotic control. For instance, the concentration of desorbed As(V) was 0.86 mg L^{-1} on average with SRB, compared with

0.35 mg L^{-1} with sulfide. In total, 34.3% As was released in the presence of SRB, including 32% As(V) and 2.3% As(III) (Table 1). Nevertheless, only 11.2% of total As desorbed from TiO_2 in the presence of sulfide. The results clearly showed that SRB enhanced As(V) desorption from nano- TiO_2 , which was consistent with our previous study (Luo et al., 2013). The reason might be the displacement of adsorbed As(V) through competitive complexation of bacteria cells at nano- TiO_2 surface sites, which was corroborated by the new FTIR peak at 1049 cm^{-1} (Figs. 5 and 6). Previous studies using ATR-FTIR spectroscopy indicated that As(V) desorption from metal oxide surfaces was promoted due to the attachment of phosphate and phosphodiester groups of bacterial cells (Omoike et al., 2004; Parikh and Chorover, 2008; Huang et al., 2011a, 2011b). Huang et al. (2011a, 2011b) found that As(V) desorption from hematite was induced by *Shewanella putrefaciens*, due to the formation of an inner-sphere P-O-Fe complex. The inner-sphere coordination of bacteria phosphate groups and the concurrent desorption of As(V) in our incubation experiments were consistent with the displacement of As(V) from surface sites by SRB functional groups.

To clarify the mobility of adsorbed As(V/III) on TiO_2 surface in the presence of SRB, the variation of the peak intensity for adsorbed As(V) and As(III) as a function of time is presented in Fig. 7 with an initial As(V) concentration of 1000 mg L^{-1} . Compared to the increase in As(III) peak intensity, the absorbance of As(V) on TiO_2 decreased gradually. The intensity of As(III) was equal to that of As(V) at about 5 h. Then, the As(III) intensity became greater, implying that the amount of As(III) on TiO_2 surfaces was more than the amount of As(V).

4. Conclusion

The present study revealed that SRB play an important role in the reduction of both adsorbed and dissolved As(V) compared with the abiotic reducer (sulfide) in an anaerobic environment at alkaline pH. The reduction rate of adsorbed As(V) was faster than that of dissolved As(V), and was controlled by the biogenic sulfide process. FTIR results provided direct proof that the reduction of adsorbed As(V) occurred on nano- TiO_2 surfaces. In addition, the desorption of As(V) from TiO_2 was promoted by SRB due to the competition from bacterial phosphate groups. In natural environments, the reduction, desorption, and adsorption reactions between As(V/III), solids, and bacteria are complex and coupled. Therefore, decoupling these reactions and understanding the molecular-level mechanism are of paramount importance exploring the As biogeochemical cycle.

Fig. 7. IR peak intensity as function of reaction time for initial As 1000 mg L^{-1} . Dashed lines indicate reaction time at 5 h.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.04.157>.

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