

Influence of clay minerals on sorption and bioreduction of arsenic under anoxic conditions

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Abstract Adsorption of As(V) on various clay minerals including kaolinite (KGa-1), montmorillonite (SWy-1) and nontronites (NAU-1 and NAU-2), and subsequent bioreduction of sorbed As(V) to As(III) by bacterium *Shewanella putrefaciens* strain CN-32 were investigated. Nontronites showed relatively higher sorption capacity for As(V) primarily due to higher iron oxide content. Freundlich equation well described the sorption of As(V) on NAU-1, NAU-2 and SWy-1, while As(V) sorption isotherm with KGa-1 fitted well in the Langmuir model. The bacterium rapidly reduced 50 % of dissolved As(V) to As(III) in 2 h, followed by its complete reduction (>ca. 98 %) within 12 h. In contrast, sorption of As(V) to the mineral surfaces interferes with the activity of bacterium, resulting in low bioreduction of As(V) by 27 % for 5 days of incubation. *S.*

putrefaciens also promoted the reduction of Fe(III) present in the clay mineral to Fe(II). This study indicates that the sorption and subsequent bioreduction of As(V) on clay minerals can significantly influence the mobility of As(V) in subsurface environment.

Keywords Adsorption · Arsenic · Bioreduction · Clay minerals · *Shewanella putrefaciens*

Introduction

Arsenic (As) is a common toxic element found in the environment, which is well known for its high carcinogenicity. It exists as oxyanions of trivalent arsenite [As(III)] or pentavalent arsenate [As(V)] in natural waters. Redox potential and pH are the most important factors influencing arsenic speciation. As(V) predominates under oxidizing conditions as deprotonated oxyanions of arsenic acid (H_xAsO_{4-x-3}), while As(III) is thermodynamically stable and exists as arsenious acids ($H_3AsO_3^0$, $H_2AsO_3^-$ and $HAsO_3^{2-}$) under mildly reducing conditions (Manning and Goldberg 1997). Both As(V) and As(III) can adsorb onto sediments and minerals in the natural environment, but As(V) generally binds more strongly than As(III) (Smedley and Kinniburgh 2002). Adsorption is one of the most crucial processes that influence the bioavailability and mobilization of As in aqueous environments (Polizzotto et al. 2008). The factors

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influencing the adsorption process may inevitably affect the fate of As in aqueous system (Zhang et al. 2012).

The mobility and speciation of As in the environment is also significantly influenced by various metabolic processes of microorganisms (Stolz and Oremland 1999; Ona-Nguema et al. 2009), including oxidation (Joanne et al. 2002), reduction (Ahmann et al. 1997) and methylation (Bentley and Chasteen 2002). Dissimilatory arsenate-reducing bacteria (DARBs) can utilize As(V) as a terminal electron acceptor and reduce it to As(III) under anoxygenic conditions, increasing As mobility in groundwater environments (Kocar et al. 2008, 2010). Iron-reducing bacteria (IRB) that catalyze the reduction of Fe(III) in As-bearing Fe minerals also affect As mobility in anoxic condition (Herbel and Fendorf 2006). Cummings et al. (1999) reported that a dissimilatory iron reducer, *Shewanella alga* strain BrY, promoted As(V) mobilization from both synthetic scorodite ($\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$) and As-contaminated natural sediments. Both As(V) and As(III) can bind strongly to hydrous ferric oxides under oxic conditions (Fendorf et al. 1997), and the adsorbed As can be mobilized by the activity of dissimilatory iron-reducing bacteria (DIRB) upon establishment of anoxic condition (Nicholas et al. 2003).

Clays and clay minerals are ubiquitous in soils, sediments and sedimentary rocks, which are key constituents in environmental processes that affect the fate and transport of contaminants (Dong 2012). Both iron oxyhydroxides and clay minerals are common arsenic scavengers in soil due to their large active surface area, large abundance and strong affinity for arsenic (Zhang et al. 2012). Although a few studies have reported the bioreduction of As(V) adsorbed on iron oxyhydroxides under anoxic conditions (Ona-Nguema et al. 2009; Huang et al. 2011; Zhang et al. 2012; Jiang et al. 2013), bioreduction and mobilization of As(V) adsorbed on clay minerals, especially iron clay minerals, need to be further investigated for better understanding the effect of bioreduction on mobility and bioavailability of As(V) adsorbed on Fe-rich clay minerals.

In this study, *Shewanella putrefaciens* strain CN-32 was incubated under anoxic conditions with aqueous As(V) and sorbed As(V) on different clay minerals including nontronite (NAU-1 and NAU-2), montmorillonite and kaolinite, to evaluate and compare the

ability of CN-32, a dissimilatory iron-reducing bacterium (DIRB), to reduce As(V) when it is the sole electron acceptor in an aqueous solution and when it is adsorbed on the surface of clay minerals.

Materials and methods

Mineral preparation

The clay minerals used in this study were obtained from the Clay Minerals Society Source Clay Repository, Indiana, USA; Kaolinite (KGa-1) from Washington County, USA; sodium montmorillonite (SWy-1) from Crook County, Wyoming; and two nontronites (NAU-1 and NAU-2) from Uley Graphite Mine near Port Lincoln, Australia. Clay minerals were ground to pass through 50- μm sieve. The two nontronites differed in Fe(III) site occupancy (Gates et al. 2002) and in the crystal chemical environment of octahedral Fe(III) and the extent of bioreduction on two kinds of nontronites.

The BET surface area of the clay minerals was determined by N_2 adsorption isotherm using an automatic surface analyzer (Quantachrome, Autosorb-1 instrument, USA). Characterization of clay minerals was conducted with an XRF (X-ray fluorescence spectrometer) using a Philips PW1480 wavelength-dispersive XRF spectrometer.

As(V) adsorption experiments

As(V) adsorption on clay minerals was measured in 10 mM PIPES (1,4-piperazine-*N,N'*-bis-2-ethanesulfonic acid) buffer solution at pH 7.0. For all the four sorbents, adsorption isotherms of As(V) were determined as $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (Sigma) with the initial concentrations of 0.05–0.2 mM in PIPES buffer, and solid-to-solution ratio was 27 g L^{-1} . The adsorption equilibrium from adsorption kinetics experiment was considered for 48 h. The results of adsorption isotherm were used to choose appropriate As(V) concentration for subsequent bioreduction experiments.

Microbial As(V) reduction experiments

Shewanella putrefaciens strain CN-32 obtained from ATCC BAA-1097 (Manassas, USA) was grown for 24 h to a late exponential phase under aerobic

conditions in tryptic soy broth at 30 °C. Cells were harvested by centrifugation (2100g, 15 min at 4 °C), washed three times with 10 mM PIPES buffer (piperazine-*N,N'*-bis(ethanesulfonic acid); 1,4-piperazinediethanesulfonic acid) (pH 7.0) and re-suspended in the same buffer (Royer et al. 2004; Huang et al. 2011). Bioreduction experiments were performed at 25 °C in a 97 % N₂/3 % H₂ atmosphere within an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lakes, Mich.) equipped with a palladium catalyst to remove trace O₂.

PIPES basal medium (10 mM, pH 7.0) was prepared for microbial reduction of aqueous As(V) in the absence of sorbents. One hundred milliliters of the medium was transferred to each serum bottle (capacity 150 mL), which were capped with thick butyl rubber stoppers and aluminum seals, followed by autoclaving at 121 °C for 15 min. Serum bottles inside the anaerobic chamber were degassed by connecting to an oxygen trap (Jeon et al. 2004a, 2005) to ensure complete removal of trace O₂ (i.e., $<7.5 \times 10^{-9}$ atm O₂). As(V) at concentration of 0.1 mM as sodium arsenate (Na₂HAsO₄), 0.2 mM Na lactate from filter-sterilized solutions (0.2 μm) and approximately 1×10^8 *S. putrefaciens* cells mL⁻¹ were added to all solutions [1 % of re-suspended cells (OD₆₀₀ = 2.0)] (Jiang et al. 2013). The bottles were incubated on a rotary incubator shaker (150 rpm) for 12 h. Aliquots (1 mL) of samples were withdrawn from the suspensions with a sterile syringe at predefined time, and the samples were analyzed for As(V) and As(III).

For bioreduction experiments in the presence of sorbents, suspensions of clay minerals (27 g L⁻¹) were sterilized by autoclaving and batch adsorption of As was examined by shaking minerals suspensions containing 0.1 mM As(V) up to 48 h. The suspensions were centrifuged at 2100g for 30 min and then filtered through membrane filter (0.2 μm). The filtrate was then used for determination of sorbed As(V) as the difference between the initial and final equilibrium As(V) concentrations. After complete adsorption of As(V) onto minerals, bioreduction experiments were conducted in which lactate (25 mM) served as the electron donor, and *S. putrefaciens* CN-32 cells ($\sim 1 \times 10^8$ cells mL⁻¹) were added to the bottles at the end. The bottles were incubated in the dark at 25 °C under anaerobic condition. All experiments were run in triplicate. For each experiment, an

uninoculated bottle was considered as the control. Aliquots (1 mL) of samples were taken from individual bottles every 5 days over a total of 30 days and were analyzed for concentrations of aqueous and total As(V), aqueous As(III), and soluble and total Fe(II).

Analytical assay

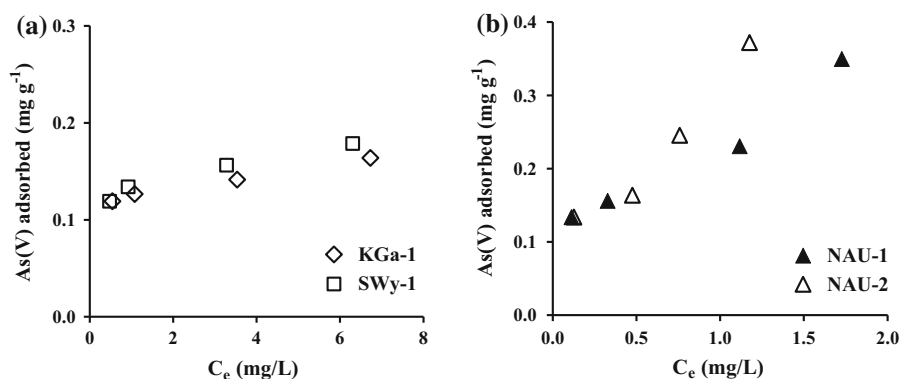
The extent of microbial reduction of Fe(III) in the minerals was determined by measuring the concentration of aqueous Fe(II) and acid-extractable Fe(II) by colorimetric analysis using UV–Vis spectrophotometer. To examine the presence of aqueous Fe(II), an aliquot (0.1 mL) of the filtrate (0.2-μm syringe filter) was added to 5 mL of ferrozine reagent (1 g L⁻¹ ferrozine in 50 mM HEPES buffer). After 10 min of incubation, the absorbance at 562 nm was determined by UV–Vis spectrophotometer (WPA S2000) (Stookey 1970). The acid-extractable Fe(II) was determined by adding a 0.5 mL aliquot of suspension to 5 mL of 1 N HCl. It was allowed to stand for 24 h (Zachara et al. 1998; Dong et al. 2003). The samples were filtered through 0.2-μm syringe filter, and Fe(II) concentration was measured using the ferrozine method (Stookey 1970). Samples of dissolved As(V) and As(III) were filtered, and 1 mL of the filtrate was added to 9 mL of distilled and deionized water (DDW) in the anaerobic chamber. Arsenic speciation was performed by passing the filtered samples through arsenic speciation cartridges (Meng and Wang 1998). The cartridges selectively remove As(V) from water in the pH range of 4.0–9.0. Total concentrations of As(V) and As(III) were analyzed by ICP-OES (inductively coupled plasma-optical emission spectrometer, Varian 730-ES, USA). For total As(V) measurement, 1 mL of 1 N HCl was added to 1 mL of sample, and the sample was filtered through 0.2-μm syringe filter after 20 h of incubation followed by ICP analysis (Lee et al. 2011).

Results and discussion

Adsorption of As(V) onto different clay minerals

Figure 1 represents the adsorption isotherms of As(V) with respect to different mineral clays. The amounts of As(V) adsorbed on the sorbents decreased in the following order as NAU-1 \geq NAU-2 > SWy-

Fig. 1 Equilibrium isotherms of As(V) adsorption on KGa-1 and SWy-1 (a), and NAU-1 and NAU-2 (b) (shaking time = 48 h, pH = 7.0, solid-to-solution ratio = 27 g L⁻¹ at 25 °C)



1 ≥ KGa-1. Mineral clays containing Fe(III) oxides on their surfaces promote the sorption of As(V) (Mohanpatra et al. 2007), which explains the higher sorption of As(V) onto NAU-1 and NAU-2 with 35.16 and 37.63 % of Fe₂O₃ compared to KGa-1 and SWy-1 with 0.13 and 3.35 % of Fe₂O₃, respectively (Table 1). Lin and Puls (2000) reported that chlorite, an iron-rich mineral (with 4.1 % Fe₂O₃ and 21.3 % FeO), exhibited a high sorption affinity for As(V). In addition to the iron content, NAU-2 had a higher specific surface area (32.1 m² g⁻¹) than the other two minerals (Table 1) providing more sorption sites (Huang et al. 2011).

Sorption of anions on clay minerals can also occur due to protonation of broken Al–OH bonds exposed at the particle edges that generate positive surface charges (Manning and Goldberg 1996). Nontronites (NAU-1 and NAU-2) are finely grained particles and possess more broken Al–OH bonds at the particle edges compared to KGa-1 and SWy-1 minerals (Manning and Goldberg 1996), which explains relatively higher As(V) sorption to nontronites.

The sorption of As(V) on NAU-1, NAU-2 and SWy-1 was well described by the Freundlich equation [$\log q_e = \log (K_F) + 1/n \log (C_e)$], while the data for KGa-1 were well fitted into the Langmuir equation [$C_e/q_e = C_e/b + 1/(K_L b)$], where q_e and C_e are the amounts of As(V) adsorbed per unit mass of clay minerals (mg g⁻¹) and the equilibrium concentration of As(V) (mg L⁻¹), respectively (Zeng 2004). Freundlich and Langmuir isotherm parameters for As(V) adsorption onto different mineral clays used in this study are shown in Table 2. Based on the results of adsorption isotherm, initial concentration of As(V) for bioreduction experiments was selected as 0.1 mM.

Microbial reduction of dissolved As(V)

The reduction kinetics of dissolved As(V) by *S. putrefaciens* CN-32 in the absence of mineral sorbents has been presented in Fig. 2. The bacterium reduced approximately 50 % of dissolved As(V) to As(III) for initial 2 h, followed by its reduction to near or less than the detection limit within 12 h. The microbial reduction of As(V) increased the concentration of As(III). At all sampling times, the summation of As(V) and As(III) concentrations (As_{total}) was in good accordance with the initial As(V) concentration (0.1 mM) (Fig. 2). As(III) was not measurable in the uninoculated bottles throughout the experiment, indicating that the reduction of As(V) was only due to microbial activity. Zobrist (2000) reported that up to 95 % of dissolved As(V) was rapidly and thoroughly reduced to As(III) by bacterium *Sulfurospirillum barnesii* within initial 3 h, with simultaneous oxidation of lactate to acetate. *S. putrefaciens* strain ATCC 12099 (1.0 × 10¹⁰ CFU mL⁻¹) has been also reported to reduce approximately 80 % of dissolved As(V) to As(III) using methanoate as the electron donor within initial 10 h at a bacterial reduction rate of 6 × 10⁻¹¹ μmol h⁻¹ CFU⁻¹ (Ona-Nguema et al. 2009). Thus, DIRB consistently showed the capacity of rapid and thorough reduction of dissolved As(V) in the presence of appropriate electron donor.

Microbial reduction of As(V) in the presence of sorbents

Prior to the bioreduction experiments, 0.1 mM As(V) was preloaded to clay minerals (27 g L⁻¹, 48 h), resulting in complete sorption of added As(V) to the mineral phases (Fig. 3). Significantly

Table 1 Chemical properties of clay minerals

| Minerals | SiO ₂ | Al ₂ O ₃ | TiO ₂ | Fe ₂ O ₃ | MgO | CaO | Na ₂ O | K ₂ O | P ₂ O ₅ | LOI ¹ (1050 °C) | SA ² (m ² g ⁻¹) | APD ³ (Å) | LC ⁴ |
|--------------------|------------------|--------------------------------|------------------|--------------------------------|------|------|-------------------|------------------|-------------------------------|-------------------------------|--|-------------------------|-------------------|
| KGa-1 ^a | 44.4 | 39.4 | 1.51 | 0.13 | 0.04 | 0.02 | 0.021 | 0.035 | 0.034 | 12.5 | 10.1 | 32.51 | 0.06 ⁵ |
| SWy-1 ^b | 62.7 | 19.8 | 0.08 | 3.35 | 3.03 | 1.69 | 1.54 | 0.52 | 0.05 | 1.59 | 18.5 | 48.64 | 0.55 ⁵ |
| NAU-1 ^c | 51.34 | 8.18 | 0.02 | 35.16 | 0.23 | 3.52 | 0.05 | 0.01 | ND ⁶ | 0.83 | 12.5 | 50.6 | 1.05 ⁷ |
| NAU-2 ^d | 56.28 | 3.11 | 0.02 | 37.63 | 0.19 | 2.31 | 0.12 | 0.01 | ND ⁶ | 0.22 | 32.1 | 67.15 | 0.72 ⁷ |

^a (Mg_{0.02}Ca_{0.01}Na_{0.01})(Al_{3.86} Fe(III)_{0.02} Mntr Ti_{0.11})(Si_{3.83}Al_{0.17})O₁₀(OH)₈ (provided by Clay Minerals Society)

^b (Ca_{0.12}Na_{0.32}K_{0.05})(Al_{3.01} Fe(III)_{0.41} Mn_{0.01} Mg_{0.54}Ti_{0.02})(Si_{7.98} Al_{0.02})O₂₀(OH)₄ (provided by Clay Minerals Society)

^c M_{1.05}⁺ (Si_{6.98}Al_{1.02})(Al_{0.29}Fe_{3.68}Mg_{0.04})O₂₀(OH)₄ where, *M: the interlayer cation (Na/Ca/K) (Keeling et al. 2000)

^d M_{0.72}⁺ (Si_{7.55}Al_{0.16}Fe_{0.29})(Al_{0.34}Fe_{3.54}Mg_{0.05})O₂₀(OH)₄ where, *M: the interlayer cation (Na/Ca/K) (Keeling et al. 2000)

¹ Loss on ignition

² Surface area

³ Average pore diameter

⁴ Layer charge

⁵ Given by the Clay Minerals Society

⁶ Not determined

⁷ Gates et al. (2002)

Table 2 Adsorption isotherm parameters for As(V) adsorption by studied clays

| Minerals | Freundlich | | | | Langmuir | | | |
|----------|---|----------------|----------------|-------|--------------------------------------|--|----------------|-------------------|
| | K _F ^a (mg g ⁻¹) | n ^b | R ² | RMSE | b ^c (mg g ⁻¹) | K _L ^d (L g ⁻¹) | R ² | RMSE ^e |
| KGa-1 | 0.125 | 6.169 | 0.874 | 0.011 | 0.203 | 1.14 | 0.968 | 0.023 |
| SWy-1 | 0.134 | 5.52 | 0.976 | 0.009 | 0.22 | 1.26 | 0.944 | 0.022 |
| NAU-1 | 0.28 | 2.5 | 0.869 | 0.057 | 0.535 | 1.322 | 0.756 | 0.079 |
| NAU-2 | 0.326 | 1.937 | 0.831 | 0.057 | 0.799 | 0.78 | 0.501 | 0.054 |

^a Freundlich constant related to the sorption capacity

^b Measure of the sorption intensity

^c The Langmuir constant related to the adsorption energy

^d A constant related to binding energy

^e Root-mean-square error (RMSE) = [Σ(q_t-q_t^{*})²/(n - m)]^{0.5} where q_t and q_t^{*} are the amount of As(V) adsorbed (mg g⁻¹) at time t (min), obtained from laboratory experiments and predicted by the models, respectively. The n value is the number of measurements, and m is the number of fitted parameters (Zhang and Selim 2005; Halajnia et al. 2012)

slower and limited bioreduction of sorbed As(V) (Fig. 4a) was observed compared to rapid and complete bioreduction of dissolved As(V) (Fig. 2). Bioreduction of sorbed As(V) to As(III) was continuously monitored, and comparable amount of dissolved As(III) was produced for all tested clay minerals (Fig. 4b). Concentration of As(III) in the solution phase was rapidly increased during the initial 5 days followed by slow increase up to 20 days and thereafter remained almost constant up to 30 days. The final concentration of dissolved As(III) after

30 days was 0.064, 0.052, 0.053 and 0.045 mmol L⁻¹ in the presence of KGa-1, SWy-1, NAU-1 and NAU-2, respectively (Fig. 4b). The total As(V) concentration measured in the uninoculated bottles (control) was 0.1 mmol L⁻¹ which remained constant over time, indicating that no As(V) reduction was observed in the abiotic experiments. It has been reported that microbial reduction of As(V) to As(III) may occur through dissimilatory reduction (respiration) or via detoxification (Zobrist et al. 2000; Jones et al. 2000). The detoxification pathway is controlled by the *ars* gene

that encodes for the enzyme As(V) reductase. The enzyme catalyzes As(V) reduction to As(III), which is further pumped out from the cell via an efflux pump (Campbell et al. 2006).

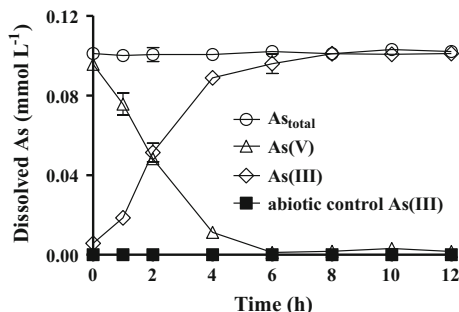


Fig. 2 Concentrations of dissolved As(V), As(III) and total As species (As_{total}) during reduction of 0.1 mM As(V) by *S. putrefaciens* strain CN-32 in the absence of mineral sorbents (pH 7.0)

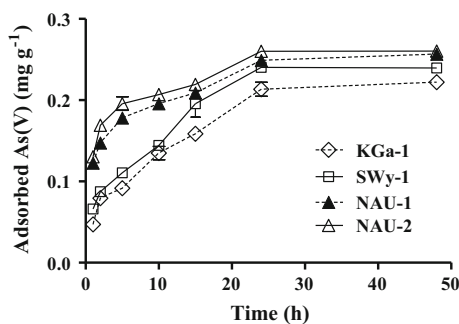


Fig. 3 As(V) adsorption on kaolinite (KGa-1), montmorillonite (SWy-1) and two nontronites (NAU-1 and NAU-2) as a function of time [initial As(V) concentration = 0.1 mM, suspension density = 27 g L⁻¹ at 25 °C and pH 7.0]

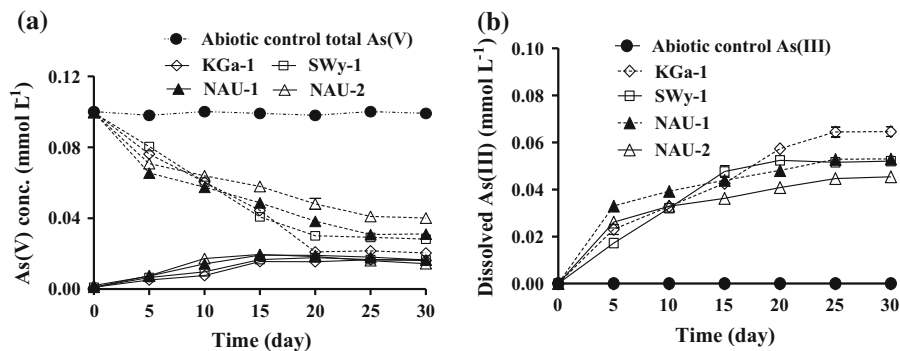


Fig. 4 Dissolved As(V) and total As(V) concentrations (a) and dissolved As(III) concentrations (b), with time during As(V) reduction by *S. putrefaciens* strain CN-32 in the presence of

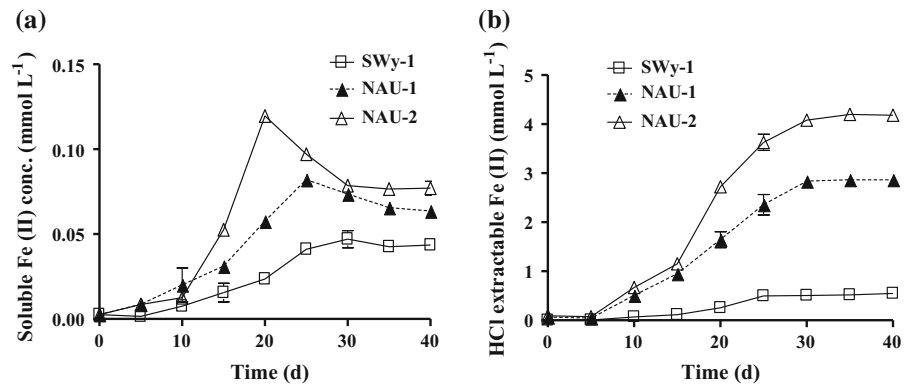
The results clearly demonstrate that the dissolved As(V) was rapidly reduced to As(III), while the presence of sorbents strongly retarded the microbial reduction of As(V). Microbial accessibility to As(V) adsorbed on the clay minerals is accountable for the impeded reduction of As(V). The reason(s) for the incomplete reduction of As(V) adsorbed on clay minerals cannot be fully explained with the available data. Jeon et al. (2004b) reported that the incomplete bioreduction of U(VI) adsorbed to natural Fe(III) oxide-bearing soils was due to association of U(VI) with inaccessible micropores within the solids, interfering the enzymatic reduction.

Bioreduction of Fe(III) in As-iron-bearing clay minerals

Fe(II) was not measurable in the control (iron minerals without cells and 25 mM lactate) including KGa-1 (which does not contain structural iron), indicating the absence of abiotic electron transfer (data not shown). The extent of microbial Fe(III) reduction showed significant variation among the three iron oxide containing clay minerals (SWy-1, NAU-1 and NAU-2). Microbial reduction of structural Fe(III) in nontronites (NAU-1 and NAU-2) and SWy-1 was remarkably increased after 10 days when the most reduction of accessible sorbed As(V) to As(III) was observed (Figs. 4, 5). This indicates that bioreduction of structural Fe(III) in iron-bearing clay minerals might occur after the reduction of sorbed As(V) and that propensity of *S. putrefaciens* for adsorbed As(V) is higher than that for Fe(III) present in the

27 g L⁻¹ of clay minerals (pH 7.0). [Total As(V) and dissolved As(V) are shown in *dash* and *continuous lines*, respectively, with the same symbols for clay minerals]

Fig. 5 Soluble Fe(II) (a) and HCl extractable Fe(II) (b) concentrations with time during bioreduction by *S. putrefaciens* strain CN-32 in the presence of 27 g L⁻¹ of clay minerals (pH 7.0)



structure of minerals under anoxic conditions. Ona-Nguema et al. (2009) demonstrated that the bacterium *S. putrefaciens* strain ATCC 12099 was able to reduce both As(V) adsorbed onto lepidocrocite and Fe(III) present within lepidocrocite structure. Zhang et al. (2012) implied that the bioreduction of Fe(III) in Fe(OH)₃ started slightly before the cessation of As reduction. Campbell et al. (2006) reported that the reduction of As(V) commenced before Fe(III) reduction by *Shewanella* sp. ANA-3 in As-equilibrated hydrous ferric oxide (HFO) and described that the utilization of As(V) as a terminal electron acceptor prior to or concurrently with Fe(III) is consistent with calculations of the thermodynamic driving force for As(V) and Fe(III) reduction couple with lactate oxidation. Different species of *Shewanella* can substitute As(V) and Fe(III) for O₂ as the terminal electron acceptor to gain energy for growth and metabolism (Jiang et al. 2013; Lovley 2013).

The concentration of Fe(II) in the aqueous phase with SWy-1, NAU-1 and NAU-2 showed an overall increasing tendency, eventually attaining peak concentration after 20–25 days and decreasing slightly at the endpoint to reach a steady state (Fig. 5a). Lee et al. (2007) and Jiang et al. (2013) reported that the decrease in Fe(II) at the endpoint was possibly due to re-adsorption of Fe(II) and formation of Fe₃^{II} (As^VO₄)₂. The concentration of acid-extractable Fe(II) for the studied minerals gradually increased from 5 to 10 days up to 30 days (Fig. 5). Fe(II) concentration was increased up to 0.26, 1.64 and 2.72 mmol L⁻¹ for SWy-1, NAU-1 and NAU-2 in 20 days, respectively (Fig. 5b). The aqueous and acid-extractable Fe(II) in the cultures of SWy-1 was significantly lower than NAU-1 and NAU-2 (Fig. 5a, b) because of lower iron

content of SWy-1 throughout the experiments (Table 1). In addition to different Fe(III) site occupancy in the two nontronites, the high layer charge of NAU-1 (1.05) compared to NAU-2 (0.72) (Table 1) might also contribute for the lower Fe(III) reduction in NAU-1 than NAU-2 (Jaisi et al. 2005). The sorption and bioreduction processes on clay minerals significantly influence the mobility of As(V) in subsurface environment.

Conclusion

Bioreduction of As(V) sorbed on different clay minerals (NAU-2, NAU-1, SWy-1 and KGa-1) by *S. putrefaciens* was investigated in this study. The higher iron oxide content in nontronites (NAU-1 and NAU-2) primarily resulted in a higher sorption of As(V) compared to SWy-1 and KGa-1. *S. putrefaciens* strain CN-32 effectively reduced aqueous As(V) to near or below detection level within 12 h. The sorption of As(V) on clay minerals impeded the activity of bacterium, limiting the rate and extent of bioreduction such that only 27 % of As(V) was reduced for 5 days. Reduction of structural Fe(III) from iron clay minerals (NAU-2, NAU-1 and SWy-1) was also catalyzed by the bacterium. The present study emphasizes that clay minerals in the natural environment can significantly influence on speciation and mobility of arsenic through sorption and subsequent bioreduction processes.

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