Coupling of arsenic mobility to sulfur transformations during microbial sulfate reduction in the presence and absence of humic acid

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A B S T R A C T

Microbial sulfate reduction is an important terminal electron accepting process in arsenic-contaminated sub-surface environments. Humic acids are ubiquitous in such environments, yet their impact on arsenic mobility under sulfate-reducing conditions is poorly understood. In this study, we examined the effects of microbial sulfate reduction and humic acid on arsenic mobilization via a series of advective-flow column experiments. The initial solid-phase in these experiments comprised quartz sand that was coated with As(III)-sorbed goethite (α-FeOOH). The effect of humic acid was assessed by comparing columns that received artificial groundwater in which humic acid was either absent or present at 100 mg L^{-1}, whilst the effect of microbial sulfate reduction was investigated by comparing columns that were inoculated with the sulfate-reducer *Desulfovibrio vulgaris* (ATCC strain 7757) versus abiotic control columns. The presence of high concentrations of humic acid alone did not enhance the overall extent of arsenic release from either the abiotic or the inoculated (sulfate reducing) columns. This is consistent with similar arsenic concentrations in porewaters filtered to both <0.45 μm and <3 kDa, demonstrating that aqueous arsenic did not form mobile colloidal humic acid complexes. In contrast, microbial sulfate reduction was found to mobilize substantial levels of arsenic relative to those observed in the corresponding abiotic control columns. Iron and sulfur K-edge X-ray absorption spectroscopy (XAS) showed that reaction between goethite and microbially-produced sulfide lead to accumulation of mackinawite (FeS) and elemental S. Microbial sulfate reduction also caused important changes in arsenic speciation, especially the formation of aqueous dithioarsenate and monothioarsenate. However, arsenic K-edge XAS showed that arsenic sulfide mineral phases (orpiment and realgar) did not form during the 60 day advective-flow experiment. The formation of poorly-sorbing thioarsenate species appeared to contribute to the observed enhancement of arsenic mobilization from the inoculated columns. Dithioarsenate and monothioarsenate were relatively stable, and were found to make up >40% of aqueous arsenic even at very low porewater sulfide concentrations (i.e. <10 μmol L^{-1}). Accordingly, the formation, stability and sorption–desorption of thioarsenate species need to be considered when evaluating and predicting subsurface arsenic mobility.

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1. Introduction

Arsenic (As) is a toxic and carcinogenic metalloid, and a common contaminant of soils, sediments and ground waters (Smedley and Kinniburgh, 2002). The widespread enrichment of As in shallow groundwater systems has made understanding subsurface As biogeo-chemistry a topic of international urgency (Harvey and Swartz, 2002; Nordstrom, 2002). The occurrence of As-rich groundwater is often a consequence of processes which mobilize As from the solid-phase, leading to accumulation of pore-water As (Fendorf et al., 2010; Charlet et al., 2011). This issue is particularly apparent in floodplain sediments, where As mobilization is associated with the onset of

reducing conditions (McArthur et al., 2004; Burton et al., 2008; Kocar et al., 2008; Pollizotto et al., 2008).

Arsenic mobilization has been widely attributed to the dissolution of binding phases, particularly reductive dissolution of ferric (hydr)oxides (Nath et al., 2009; Fendorf et al., 2010; Johnston et al., 2010). As a consequence, the effect of dissimilatory Fe(III) reduction on sub-surface As behavior has received a great deal of attention (Cummings et al., 1999; Islam et al., 2005; de Lemos et al., 2006; Quicksall et al., 2008). In contrast, the impact of dissimilatory sulfate reduction has received much less attention, despite sulfate-reducing microorganisms being ubiquitous in anoxic subsurface environments (Canfield et al., 2005; Burton et al., 2011a).

Microbial sulfate reduction produces sulfide which can react to precipitate As as As sulfide minerals, coprecipitate it with iron, or form iron sulfide phases which sequester As (O'Day et al., 2004; Wolthers et al., 2005a; Wilkin and Ford, 2006; Root et al., 2009).

Several earlier studies found that As solubility and extractability decrease under anoxic, sulfate-reducing conditions in marine and estuarine sediments (Huerta-Diaz and Morse, 1992; Sullivan and Aller, 1996). More recently, Kirk et al. (2004) demonstrated that sulfate reduction in deeper aquifer systems was also associated with low As concentrations. Accordingly, microbial sulfate reduction is generally thought to cause decreased As mobility in subsurface environments (e.g. Fendorf et al., 2010).

In contrast to earlier studies, recent work shows that under some conditions sulfate reduction can trigger increased As mobility (Keimowitz et al., 2007; Kocar et al., 2010; Burton et al., 2011b). In experimental studies examining As behavior in advective flow systems, both Kocar et al. (2010) and Burton et al. (2011b) found that microbial sulfate reduction caused substantial As release during replacement of ferrihydrite (Fe(OH)$_3$) by mackinawite (FeS). Likewise, Kirk et al. (2010) found that mackinawite sequestered very little As, in contrast to the formation of pyrite (FeS$_2$), which appears to have a comparatively strong affinity for As uptake (Bostick and Fendorf, 2003; Wolthers et al., 2005b).

Arsenic mobility is controlled largely through sorption processes, which are known to be strongly influenced by aqueous As speciation (Charlet et al., 2011). Under oxic and suboxic conditions, aqueous As exists primarily as oxyanions of As(III) (H$_{3-x}$As^{III}O$_3^{-x}$; arsenite) and As(V) (H$_{3-x}$As^{V}O$_4^{-x}$; arsenate). On the other hand, aqueous thioarsenic species become important in sulfide-bearing solutions (Wilkin et al., 2003; Stauder et al., 2005; Planer-Friedrich et al., 2007; Wallschlaeger and Stadey, 2007). Sorption of the arsenite and arsenate oxyanions has been extensively studied, particularly with regard to ferric (hydr)oxides (e.g. Raven et al., 1998; Dixit and Hering, 2003). In comparison, the sorption behavior of thioarsenic species and the associated implications for subsurface As mobility has received limited attention (Wilkin, 2001; Bostick and Fendorf, 2003; Suess and Planer-Friedrich, in press).

Humic acids are ubiquitous in the shallow subsurface and can affect As mobility in a number of ways. In particular, humic acids can form stable complexes with ferric (hydr)oxide surfaces and can, as a result, compete with As for sorption sites (Grafe et al., 2001, 2002; Redman et al., 2002; Bauer and Blodau, 2006). Humic acids can also interact with aqueous As to form stable colloidal complexes (Liu et al., 2011). The formation of ternary As-Fe-humic acid colloids may play a particularly prominent role in enhancing As mobility (Bauer and Blodau, 2009; Sharma et al., 2010, 2011). Despite being relatively well studied in oxidizing Fe(III)-rich systems, the role of colloid-facilitated transport in sulfate-reducing environments has received little attention (Weber et al., 2009).

The objective of this study was to examine the influence of microbial sulfate reduction and humic acid on subsurface As mobility. To achieve this objective, we carried out a series of advective-flow column experiments with an initial solid-phase comprising As(III)-bearing goethite-coated quartz sand. The effect of humic acid was evaluated by comparing columns that received artificial groundwater in which humic acid was either absent or present at 100 mg L^{-1}. The effect of microbial sulfate reduction was investigated by comparing As, S and Fe behavior in columns that were inoculated with the dissimilatory sulfate reducer *Desulfovibrio vulgaris* (American Type Culture Collection strain 7757) in comparison to abiotic control columns.

Goethite was used as the initial As host-phase because, unlike less crystalline ferric (hydr)oxides (e.g. ferrihydrite), goethite is known to persist in sulfate-reducing systems (Burton et al., 2007, 2008). Furthermore, the energy yield from microbial sulfate reduction can exceed microbial reduction of goethite-derived Fe(III) at near-neutral pH, thereby allowing sulfate reduction to competitively precede goethite-reduction (Postma and Jakobsen, 1996; Burton et al., 2007; Kocar and Fendorf, 2009). We used arsenite as the initial sorbed species because arsenate reduction can also precede dissimilatory reduction of both sulfate and goethite-bound Fe(III), making arsenite the principal

As species at the onset of sulfate reduction in the presence of crystalline ferric (hydr)oxides, such as goethite (Kocar and Fendorf, 2009).

2. Methods

2.1. General methods

Glass- and plastic-ware was cleaned by soaking in 5% (v/v) HNO$_3$ for at least 24 h, followed by repeated rinsing with deionized water. All chemicals were analytical reagent grade and all reagent solutions were prepared with deionized water having a resistivity of 18.2 MΩ. Deoxygenated solutions were prepared by purging with high-purity N$_2$ for at least 1 h. Oxygen-free experimental set-up conditions were achieved through the use of a Coy anaerobic chamber (1–5% H$_2$ in N$_2$), maintained at <1 ppm O$_{2(g)}$ with a Pd catalyst. Solid-phase results are presented on a dry weight basis.

2.2. Preparation of goethite-coated sand

Goethite was synthesized using the standard method of Cornell and Schwertmann (2003). Briefly, this involved adding 1.8 L of 5 M KOH to 1 L of 1 M Fe(NO$_3$)$_3$, then diluting the resulting suspension to 20 L and holding at 70 °C for 60 ho. The goethite suspension was rinsed several times with deionized water, mixed with clean quartz sand (size range of approx. 100 to 250 μm) and dried at 80 °C with regular manual mixing to homogenize the material and aid drying. The dry goethite-sand mixture was then repeated rinsed with deionized water until the rinsewater was free of goethite particles. The goethite-coated sand was then dried at 80 °C. The total Fe concentration (determined as described below) in the dry goethitecoated sand was 38 ± 1 μmol g^{-1}.

The goethite-coated sand was resuspended (with a solid: solution ratio of approx. 1) in artificial groundwater that was buffered at pH 6.5 by 0.01 M MES/MOPS. These organic buffers are non-complexing (Kandegedara and Rorobacher, 1999) and are therefore not expected to interfere with arsenic mobility or iron/sulfur transformations. The artificial groundwater was composed of 1 mM KCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 1 mM NH$_4$Cl, 0.08 mM KH$_2$PO$_4$, 6 mM Na$_2$SO$_4$, 6 mM lactate, 0.1 mL L^{-1} Wolfe's mineral supplement (Balch et al., 1979) and 0.025 g L^{-1} yeast extract. This composition was selected in order to be representative of natural groundwater in sulfate-reducing environments (e.g. Wilkin and Ford, 2006; Root et al., 2009; Johnston et al., 2010, 2011).

The suspension was then transferred to an anaerobic chamber and allowed to equilibrate for 24 h. The solution was decanted and replaced with fresh deoxygenated, artificial groundwater that had been amended with soluble As(III), prepared from NaAsO$_2$, at a loading of approx. 0.66 mmol As(III) per kg of sand. This mixture was allowed to equilibrate, with regular manual homogenization, under O$_2$-free conditions for 4 days before removal of the supernatant solution (Kocar et al., 2010; Burton et al., 2011b). The final goethite-coated sand used in the experiment described here had a total As concentration of 460 ± 5 nmol g^{-1} (determined as described below). This relatively low total As concentration (i.e. 34.5 ± 0.4 mg kg^{-1}) is comparable to natural background concentrations in soils and sediments.

A culture of *D. vulgaris*, strain 7757 obtained from the American Type Culture Collection (ATCC) was grown to late exponential phase under anaerobic conditions in Barr's medium at room temperature (ATCC strain 7757 is a well-characterized sulfate-reducing microorganism, which does not reduce ferric iron or arsenate). The culture was centrifuged, decanted and resuspended several times in deoxygenated, artificial groundwater and then used to inoculate 4 x 1 kg portions of the As(III)-bearing goethite-coated sand (herein termed "inoculated" or "sulfidic" columns). An additional 4 x 1 kg portions of the sand were left un-inoculated, in order to serve as abiotic