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Bacteria-mediated reduction of As(V)-doped lepidocrocite in a flooded soil sample

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ABSTRACT

Understanding the processes involved in the control of arsenic (As) dynamics within soils has become a challenging issue for soil and water quality preservation. Interactions between mineralogical phases, organic ligands and bacterial communities — closely linked to the chemical conditions of the medium — were thus investigated through a geochemical and microbiological experimental study involving the reduction of As(V)-doped lepidocrocite within the soil. Reducing conditions were established as soon as the experiment started, followed by a release of dissolved organic carbon corresponding to a release of acetate. Scanning electron microscopy observations pointed out a large bacterial colonization occurring on the lepidocrocite leading to a 3-dimensionally shaped biodissolution of lepidocrocite. The taxonomic diversity evolved throughout the experiment, and thus it demonstrated the evolution of the metabolic activities of the bacteria. At the beginning, lepidocrocite was mainly colonized by bacteria belonging to the *Geobacter* genus (Deltaproteobacteria) (26%) and *Bacillus* and *Oxalophagus* fermentative related genera (Firmicutes) (72%). After two weeks, *Geobacter* spp. and Firmicutes represented 54% and 30% of the bacterial community, respectively. Although still dominated by *Geobacter* spp. (34%) at the end of the experiment, the bacterial diversity had increased. After 3 and 8 weeks of incubation, the presence of the *arsB* and *ACR3(1)* genes, encoding transporters involved in As detoxification processes, indicated that this community harbored As-resistant or As-transforming genera able to contribute to the transformation of As(V) into As(III).

1. Introduction

Iron(III) oxides are ubiquitous mineral constituents of soils, sediments, aquifers and geological materials. Their respective stability, specific surface area, porosity, dissolution rate as well as transformation kinetics are controlled by their mineral structure (Cornell and Schwertmann, 2003). The precipitation and dissolution of Fe oxides are dependent on the pH, redox state and occurrence of organic ligands (McBride, 1994), which are themselves controlled by microbial activities. The key role of microorganisms in the biogeochemical cycling of Fe has been well assessed (Lovley and Phillips, 1986; Lovley, 1991; Lovley et al., 1991; Wahid and Kamalam, 1993; Roden and Zachara, 1996). Fe-reducing bacteria, which are ubiquitous in waterlogged soils, couple the oxidation of organic matter with the reduction of various Fe(III) oxyhydroxides for their metabolism (Lovley and Phillips, 1986; Francis and Dodge, 1990; Chuan et al., 1996; Charlatchka and Cambier, 2000; Quantin et al., 2001, 2002; Green et al., 2003; Grybos et al., 2007). This functional group is phylogenetically diverse. It comprises bacteria that conserve energy to support growth from Fe(III) reduction and those that do not possess this potential (Lovley, 2006).

A direct consequence of Fe(III) reduction is the release of the associated trace metals into the soil solution (e.g. Schwertmann and Taylor, 1989; Lovley and Coates, 1997; Davranche and Bollinger, 2000, 2001; Quantin et al., 2001; Zachara et al., 2001; Van Geen et al., 2004; Burnol et al., 2007; Jönsson and Sherman, 2008). Therefore, the biogeochemical cycles and fate of Fe and the associated trace metals are closely linked (Francis and Dodge, 1990; Lovley, 1991). As a consequence, the sorption and redox chemistry of Fe(III) oxides have been widely studied in near-surface geochemical systems (Charlatchka and Cambier, 2000; Davranche and Bollinger, 2000; Davranche et al., 2003; Bonneville et al., 2004; Grybos et al., 2007). Under oxic conditions, crystallized or amorphous Fe(III) mineral phases are able to incorporate or scavenge toxic trace elements such as As, Cr, Cd or Pb (Bousserrhine et al., 1999; Morin et al., 1999, 2002; Brown and Sturchio, 2002; Bonneville et al., 2004; Morin and Calas, 2006). Secondary Fe(II)–Fe(III)-containing minerals, such as green rusts and magnetite (Lovley et al., 1987; Ona-Nguema et al., 2002, 2004; Zachara et al., 2002; Glasauer et al.,

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2003; Zegeye et al., 2005), have been shown to scavenge trace elements (e.g., Cooper et al., 2000; Coker et al., 2006; Root et al., 2007; Wang et al., 2008; Ona-Nguema et al., 2009). Among the trace metals, arsenic (As) is strongly adsorbed onto Fe-oxides (Manning et al., 1998; Raven et al., 1998; Dixit and Hering, 2003), which are probably the largest carriers of As in aquifers and soils (Morin et al., 2002; Cancès et al., 2005, 2008; Morin and Calas, 2006). Arsenic occurs at concentrations exceeding potable levels in major aquifers in several parts of the world, especially in South Asia (Smedley and Kinniburgh, 2002; Islam et al., 2004; Van Geen et al., 2008). High As concentrations in subsurface waters have been shown to often result from the reductive dissolution of hydrous Fe-oxides and the subsequent release of associated As (Nickson et al., 2000; Bose and Sharma, 2002; Van Geen et al., 2004). When As(V) is released, a subsequent bioreduction to As(III) can be performed by microorganisms via two processes: the first involves dissimilatory arsenate-respiring prokaryotes (DARPs) that will reduce As(V) using organic matter as an electron donor to obtain energy and to support growth, whereas the second is the consequence of detoxification strategies that expel As(III) from the cell after As(V) has entered the microbial cell through phosphate transporters (Lloyd and Oremland, 2006).

In the natural environment, the mineral soil matrix could also strongly influence the mechanism of the reductive dissolution and therefore the nature of the secondary minerals (for example, due to Fe(II) adsorption or As solubilization). Until recently, it was still a challenge to study the processes involved in bioreduction directly in soils despite a large set of experimental studies (Jorgensen and Willems, 1987; Voegelin et al., 2002; Jenkinson and Franzmeier, 2006). However, Fakih et al. (2008) developed a method inspired from Birkefeld et al. (2005) to monitor the transformation of Fe-oxides directly within soils and to quantify their reductive dissolution. Iron oxides are precipitated onto acrylic slides, which can be directly inserted into the soil.

Considering the crucial need for addressing interdisciplinary approaches combining geochemistry and microbiology to understand Fe-oxide bioreduction mechanisms, we used the monitoring tool recently developed by Fakih et al. (2008, 2009) to investigate the reductive dissolution of As(V)-doped lepidocrocite directly in a soil sample. The aims of this study were as follows: (i) to quantify Fe and As release over time, (ii) to unravel the role of bacteria in Fe-oxide bioreduction, and (iii) to describe over time the bacterial consortia involved and their respective functional role with regards to Fe and As dynamics at the soil/water interface. One of the key questions confronting geochemical and microbiological data is as follows: who is doing what, as well as how and when, with regards to Fe and associated As?

2. Materials and methods

All of the chemicals used were of analytical grade. The solutions were prepared with double de-ionized water (Milli-Q system, Millipore). The containers used were (i) soaked in 10% ultrapure HNO_3 for 48 h at 60 °C to remove all possible contaminant sources, (ii) soaked in Milli-Q water for 24 h at 60 °C, and finally (iii) dried at 30 °C.

2.1. Iron oxide-covered slides

A technique based on slides coated by Fe-oxyhydroxides (Birkefeld et al., 2005; Fakih et al., 2008) was used. The tool consists of small (2 × 2 × 0.2 cm) striated polymer plates covered by synthetic As(V)-lepidocrocite (As-Lp). The detailed methodology for the production of the slides, their characterization and the method validation are further detailed in Fakih et al. (2008). Lepidocrocite was synthesized in the presence of As(V) according to the protocol given by Schwertmann and Cornell (2000). The average amount of Fe coating the slides was 0.7 ± 0.2 mg per slide (Fakih et al., 2009). The mass ratio of As and Fe was 0.005 (i.e. 3.5 μg of As per slide). Although the question of the form into which As was incorporated into the lepidocrocite was not addressed through this study, As was probably mostly sorbed onto lepidocrocite via an inner-sphere complex (Randall et al., 2001; Pedersen et al., 2006; Ona-Nguema et al., 2009). The surface area of the lepidocrocite, measured by a multi-point BET method (using a Micromeritics FlowSorb II 2300 apparatus equipped with a LECO TC-436 analyzer), was determined as 65.1 $m^2\ g^{-1}$. The As(V)-lepidocrocite mineralogy was assessed by XRD analyses performed on a Siemens D500 diffractometer (Fig. A.1, supporting information). The BET and XRD analyses were performed at the Chemical Sciences Department at the University of Rennes 1.

2.2. Soil sampling

Soil was sampled from the Mercy wetland located in the Naizin-Kervidy/Coët-Dan subcatchment in Brittany, Western France (Curmi et al., 1997). This catchment is particularly well adapted to study the reductive dissolution of Fe because redox cycles involving Fe have been identified in these soils (Dia et al., 2000; Olivié-Lauquet et al., 2001). Wetland soil was sampled in the uppermost organo-mineral horizon (Ah) of a planosol (according to the WRB international classification). The soil was collected in January 2010 at the beginning of the water table rise and the wetland reduction period. The collected soil sample was dried at 30 °C for 72 h, and then sieved to 2 mm (AFNOR, 2004). After fusion of the sample with $LiBO_2$ flux and acidic dissolution with HNO_3, the major and trace element composition was determined at the CNRS Analytical Research Facility — SARM (France), by inductively-coupled plasma optical emission spectrometry and mass spectrometry (ICP-OES, Thermo Elemental IRIS radial and ICP-MS, Agilent 7700X) for the major and trace elements, respectively (Table 1). The soil organic carbon content was determined using a carbon–sulfur analyzer (Leco SC144 DRPC) (Table 1). The major element concentrations in the soil sample are given in Table 1. The soil sample contained 1.40 wt.% of Fe, 6.64 wt.% of organic carbon and 7.64 μg g^{-1} of As. Water extraction performed for a soil/water ratio of 20 (in weight) showed that 26 mg L^{-1} versus 105 mg L^{-1} of DOC could be solubilized under oxidative conditions at pH 3 and 7, respectively. In a previous study conducted by Davranche et al. (2011), a comparable soil sample recovered within the same uppermost organo-mineral horizon (Ah) was sequentially leached following the modified BCR (European Communities Bureau of Reference) extraction scheme (Mossop and Davidson, 2003). The distribution of Fe showed that 23% of Fe lies in the reducible fraction (as amorphous Fe(III)-oxyhydroxides), 24% in the oxidizable fraction (as organic complexes or sulfides) and 52% in the residual fraction (Fe as well crystallized Fe(III)-oxyhydroxides and/or Fe in clays and/or Fe in relict primary minerals).

2.3. Experimental set-up

Columns suited for anaerobic conditions are described in detail in Fakih et al. (2009). Briefly, two reservoirs are connected by a flexible tygon tube with an internal diameter of 0.2 mm. The columns were operated in up-flow mode. The solution percolated gently through the soil sample from the bottom of the column and was returned back to the solution reservoir. The solution (800 mL) was continuously flushed through the soil column at a flow rate of 20 mL h^{-1} using a peristaltic pump (Ismatec Ecoline). The solution circulated in a closed system. Forty grams of a dried and sieved soil sample were placed into a 250 mL polypropylene reservoir. Two horizontal perforated Teflon disks (with a pore size of 69 μm) retained the soil sample particles in the reservoir. The total porosity in the soil columns (i.e., pore water volume) was 56%. Four coated As(V)-Lp slides were inserted in the soil sample. The chemical composition and ionic strength of the percolating solution were chosen to be comparable to that of the pore water in the Mercy wetland just at the beginning of the flooded period (30.71 mg L^{-1} of NaCl, 30.91 mg L^{-1} of $NaNO_3$ and 10.62 mg L^{-1} of Na_2SO_4 and pH = 5.9). The incubation experiments were performed