



Biological attenuation of arsenic and iron in a continuous flow bioreactor treating acid mine drainage (AMD)

L. Fernandez-Rojo, M. Héry, P. Le Pape, C. Braungardt, A. Desoeuvre, E. Torres, V. Tardy, E. Resongles, E. Laroche, S. Delpoux, et al.

► To cite this version:

L. Fernandez-Rojo, M. Héry, P. Le Pape, C. Braungardt, A. Desoeuvre, et al.. Biological attenuation of arsenic and iron in a continuous flow bioreactor treating acid mine drainage (AMD). Water Research, 2017, 123, pp.594 - 606. 10.1016/j.watres.2017.06.059 . hal-01818488

HAL Id: hal-01818488

<https://brgm.hal.science/hal-01818488>

Submitted on 16 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biological attenuation of arsenic and iron in a continuous flow bioreactor treating Acid Mine Drainage (AMD)

Fernandez-Rojo L.¹, Héry M.¹, Le Pape P.², Braungardt C.^{3,1}, Desoeuvre A.¹, Torres E.¹, Tardy V.¹, Resongles E.¹, Laroche E.¹, Delpoux S.¹, Jouliau C.⁴, Battaglia-Brunet F.⁴, Boisson J.⁵, Grapin G.⁶, Morin G.², Casiot C.¹

¹ *Hydrosciences Montpellier, UMR 5569 CNRS-IRD-UM, CC57, 163 rue Auguste Broussonet, 34090, Montpellier, France*

² *Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), UMR 7590 CNRS-UPMC-IRD-MNHN, 4 place Jussieu, 75252 Paris cedex 05, France*

³ *School of Geography, Earth and Environmental Sciences (Faculty of Science & Engineering), Plymouth University, United Kingdom*

⁴ *French Geological survey (BRGM), 3 avenue Claude Guillemin, 45060, BP 36009, Orléans Cedex, France*

⁵ *IRH Ingénieur Conseil, 197 avenue de Fronton, 31200, Toulouse, France*

⁶ *IRH Ingénieur Conseil, 427 rue Lavoisier - CS 50155, 54714, Ludres Cedex, France*

Abstract

Passive water treatments based on biological attenuation can be effective for arsenic-rich Acid Mine Drainage (AMD). However, the key factors driving the biological processes involved in this attenuation are not well-known. Here, the efficiency of arsenic (As) removal was investigated in a bench-scale continuous flow channel bioreactor treating As-rich AMD (~30-40 mg L⁻¹). In this bioreactor, As removal proceeds *via* the formation of biogenic precipitates consisting of iron- and arsenic- rich mineral phases encrusting a microbial biofilm. Fe(II) oxidation and Fe and As removal rates were monitored at two different water heights (4 and 25 mm) and with/without forced aeration. An eighty percent As removal was achieved within 500 min at the lowest water height. This operating condition promoted intense Fe(II) microbial oxidation and subsequent precipitation of As-bearing schwertmannite and amorphous ferric arsenate. Higher water height slowed down Fe(II) oxidation, Fe precipitation and As removal, in relation with limited oxygen transfer through the water column. The lower oxygen transfer at higher water height could be partly counteracted by aeration. The presence of an iridescent floating film that developed at the water surface was found to limit oxygen transfer to the water column and delayed Fe(II) oxidation, but did not affect As removal. The bacterial community structure in the biogenic precipitates in the bottom of the bioreactor differed from that of the inlet water and was influenced to some extent by water height and aeration. Although potential for microbial mediated As oxidation was revealed by the detection of *aioA* genes, removal of Fe and As was mainly attributable to microbial Fe oxidation activity. Increasing the proportion of dissolved As(V) in the inlet water improved As removal and favoured the formation of amorphous ferric arsenate over As-sorbed schwertmannite. This study proved the ability of this bioreactor-system to treat extreme As concentrations and may serve in the design of future *in-situ* bioremediation system able to treat As-rich AMD.

Keywords: arsenic precipitation, bioremediation, iron oxidation, water treatment, biogenic precipitate, ferric arsenate, *aioA* genes, schwertmannite

1. Introduction

Arsenic is ubiquitous in acid mine drainage (AMD) (Williams, 2001; Paikaray, 2015) and represents a severe threat for freshwater resources downstream from mining sites. It is therefore essential to develop treatment processes able to remove arsenic from mine waters. In this respect, co-precipitation with iron is a well-established method (Carlson et al., 2002; Kim et al., 2003; Asta et al., 2010a). When enough iron is originally present in the AMD, addition of a neutralizing agent, such as lime, allows rapid Fe(II) oxidation and subsequent precipitation of simple ferric arsenate (FeAsO_4) and ferric hydroxide ($\text{Fe}(\text{OH})_3$), or basic ferric arsenate ($\text{FeAsO}_4 \cdot x\text{Fe}(\text{OH})_3$), depending on As/Fe molar ratios (Lawrence and Higgs, 1999). At acid pH, As(III) is poorly retained on Fe-solids. Chemical or photochemical oxidation of As(III) into As(V) might increase As removal efficiency (Emett and Khoe, 2001; Hug and Leupin, 2003).

Alternatively, biological oxidation of iron and arsenic occurs naturally in AMD and has been observed at various mining sites worldwide (Casiot et al., 2003; Asta et al., 2010b; Egal et al., 2010; Chen and Jiang, 2012; Paikaray, 2015). Bacteria involved in iron and arsenic oxidation have been isolated and their metabolic capacities investigated (Battaglia-Brunet et al. 2002, Bruneel et al., 2003). Treatments based on these natural biological oxidation processes are promising because they offer an alternative to active chemical oxidation that is costly and requires periodic maintenance. However, there have been only few attempts to exploit these biological processes for the treatment of As-rich AMD (Battaglia-Brunet et al., 2006; Elbaz-Poulichet et al., 2006; Macías et al., 2012; Ahoranta et al., 2016) and the factors controlling the efficiency of this natural process, ranging from 7% to nearly 100% As removal, are still poorly understood. Lessons learnt from AMD observations drew attention to the remarkable efficiency of terraces and waterfalls, as compared to pools, for arsenic retention (Asta et al., 2010b; Chen and Jiang, 2012; Macías et al., 2012). Chen and Jiang (2012) hypothesized that

turbulent flow conditions and shallow water in waterfall sections maximized the iron oxidation rate by improving oxygen diffusion. In this respect, Brown et al. (2011) also found in laboratory experiments that greater Fe(II) oxidation occurred at lower water height. Conversely, quiescent pools, by favouring the development of an iridescent oil-like floating film at the water surface (Kleja et al., 2012), were suspected to limit the efficiency of the treatment (Elbaz-Poulichet et al., 2006).

In the present study, a bench-scale continuous flow bioreactor was used to identify the major factors controlling the efficiency of microbially-mediated arsenic removal from AMD. The system was supplied with AMD from the Carnoulès mine (Southern France), where extreme arsenic concentrations and natural arsenic attenuation involving biological Fe and As oxidation had been evidenced (Leblanc et al., 1996; Bruneel et al., 2003; Casiot et al., 2003; Morin et al., 2003; Egal et al., 2010). The microbial, chemical and mineralogical composition of the biogenic precipitate naturally developing on the bottom of the channels was also investigated. The As molecular level speciation and redox state in the solid and aqueous phases was investigated using X-ray absorption spectroscopy and high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS), respectively. The results yield evidence for the effect of water height and floating film on the rate of microbial Fe(II) oxidation and Fe and As precipitation at acid pH.

2. Materials and methods

2.1 Continuous flow bioreactor design

Experiments were conducted in a bench-scale bioreactor (Fig. 1A), reproducing the shallow sheet flow commonly seen across natural terraced iron formations (TIFs) described in AMD (Sánchez-España et al., 2007; DeSa et al., 2010; Brown et al., 2011; Larson et al., 2014). It

was composed of four rectangular polyvinyl chloride channels (1 m length \times 0.06 m width \times 0.06 m depth), each equipped with a double envelope for temperature control. A biodegradable canvas of polylactic acid (BIO DURACOVER) was placed on the bottom of each channel to provide a rough surface for the adhesion of the biogenic precipitate. Preliminary tests showed that the mineralogy, concentrations of As and Fe, and microbiology (number of bacterial cells and bacterial community structure) in the precipitate formed on this material were similar to the one formed on an inert plastic net (Direct-Filet.com, mesh size of 1.4 mm \times 0.83 mm). Thus, the cost-effective biodegradable canvas was chosen.

A four-channel peristaltic pump (Gilson, Minipuls 3) simultaneously supplied the four channel inlets with AMD at specific flow rates that determined the water residence time (\sim 20-1800 min). A second peristaltic pump was used to remove the fluid from the experimental set-up through holes drilled in the terminal sections of the channels, and to maintain the water level at specific heights. C-Flex® tubing (i.d. 3.2 mm), was joined by polypropylene connectors (i.d. 3.1 mm) to peristaltic pump tubing (Tygon® i.d. 3.17 mm) and to the channel inlets and outlets. A 3-way valve was positioned at the end of each outlet tube for sample collection. Treated effluent from the experiments was collected and periodically returned to the mining site. The temperature in the double envelope of the channels was maintained at 20 ± 0.8 °C by continuous circulation of a cooler-heating fluid (Julabo Thermal G) in a closed circuit, connected to a temperature control unit (Julabo F34-EH). Air conditioning was settled to 20 °C in the laboratory. The channels were illuminated with a neon lamp (T5 Superplant 216W CROISSANCE 6500 K) in the 400-500 nm spectra, with a photon flux density of $0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, and a day/night cycle of 12 h.

2.2 AMD feed water sampling and preservation

AMD was collected on the 20th January, 12th February, and 23rd March 2015, from the spring of Carnoulès Creek. Containers (20 L, high density polyethylene) were decontaminated with

concentrated HNO_3 , rinsed three times with double deionized water (DDW, Milli-Q®) and finally with *in-situ* AMD before sample collection. The filled containers were immediately transported to the laboratory and then purged with N_2 until DO decreased below 1 mg L^{-1} , in order to avoid iron oxidation. Containers were stored at 20°C in the laboratory and used in turn under N_2 purge during the course of the experiment to supply water to the bioreactor. The main physico-chemical parameters, Fe and As dissolved concentrations (total and individual species) were checked at the opening of each new container.

2.3 Flow bioreactor experiments

Two experiments referred to as Exp 1 and Exp 2 were conducted in the bioreactor. During the setting up stage of the experiments, lasting 10-15 days, the water height was maintained at 4 mm and the flow rate was fixed at 0.5 mL min^{-1} , equivalent to a residence time (RT) of $324 \pm 30 \text{ min}$. Inlet and outlet water was collected at regular time intervals for dissolved Fe(II) and total dissolved Fe determination until steady-state was reached with respect to Fe(II) oxidation (Fig. 2). After this timeframe, kinetic studies were carried out for Fe(II) oxidation, total Fe precipitation, and As(III), As(V), and total As removal (Fig. 3). For that purpose, the flow rate was set to successively achieve specific residence times: $\sim 20 \text{ min}$, $\sim 50 \text{ min}$,... $\sim 1800 \text{ min}$. The exact residence times were calculated as described in paragraph 2.4.2. After each change in flow rate, the system was allowed to adjust for a minimum of three residence times before inlet and outlet water chemistry (Fe(II), total Fe, As(III), As(V) and total As in the dissolved phase) were determined, using routine sampling and preservation procedures described in section 2.4.1 and in the Supporting Information file (SI-Experimental part).

In both Exp 1 and Exp 2, a thin iridescent, oil-like film, referred as “pellicle biofilm” or “floating film” in the literature, spontaneously formed on the water surface.

Exp 1 was conducted at 4 mm water height and investigated the effect of the floating film. Accordingly, the kinetic study Exp 1_(FF) was first conducted in presence of a floating film

(FF), on four replicate channels. Six days after the beginning of the kinetic study, the experiment was stopped in two of the channels and the biogenic precipitate was recovered for analysis. In the two other channels the floating film was removed with a spatula and a kinetic study was carried out in open air (OA) for 17 days more (Exp 1_(OA)).

Exp 2 was conducted at two water heights in parallel, 4 mm (Exp 2_(4 mm)) and 25 mm (Exp 2_(25 mm)), with two replicate channels each. To prevent the formation of the floating film, the water surface was agitated daily with a spatula. A thin plastic film (HP transparency film, 60 mm x 60 mm) was positioned vertically in the channels parallel to the flow direction, in order to sample the biogenic precipitate accumulated along a vertical profile during the experiment. After 8 days of kinetic study, one of the 25 mm-water-height channels was aerated using an aquarium pump and ceramic air diffusers (Hagen, Marina A983), positioned at 3 cm and 50 cm from the inlet, and another kinetic study (Exp 2_(25 mm, Air)) was performed.

2.4 *Experiment monitoring*

2.4.1 *Water chemistry analysis*

Water chemistry analyses included measurement of the DO, temperature, pH, conductivity and redox potential as well as the determination of concentrations of dissolved Fe(II) and sulphate using spectrophotometry, and total dissolved Fe and As using ICP-MS, after 0.2 µm filtration. Arsenic redox speciation was determined using HPLC-ICP-MS. Dissolved oxygen depth profiles in the water column of bioreactor channels were acquired using microelectrodes. Details of these analytical procedures are reported in SI-Experimental part.

2.4.2 *Calculation of residence times and reaction rates*

The RT, in seconds, was determined for each flow rate value dividing the empirical volume of water (in mL) recovered from the channel, by the flow rate (in mL s⁻¹) measured at the

channel inlet. Fe(II) oxidation, Fe- and As-removal rates (in mol L⁻¹ s⁻¹) were calculated using Eq. (1);

$$\text{Rate} = \frac{([X]_{\text{inlet}} - [X]_{\text{outlet}})}{RT} \quad (1)$$

where [X] was the dissolved concentration of Fe(II), total Fe, total As, As(III) or As(V), respectively, in mol L⁻¹. It was not possible to calculate with the Eq. (1) the As(III) oxidation rate for a specific residence time, because dissolved As(III) may be removed from solution by precipitation of As(III)-Fe(III) minerals, oxidation to As(V) and/or precipitation of As(V)-Fe(III) minerals. Thus, only As(III) and As(V) removal rates were determined.

2.4.3 *Water microbial analysis*

At the beginning of the experiments, subsamples of bioreactor feed water were collected for microbiological characterization. Triplicates of 1 mL subsamples were analysed by flow cytometry for bacterial quantification. In addition, triplicates of 300 mL-subsamples were filtered (pore size 0.2 µm, cellulose acetate), and the filter membranes stored at -80 °C for further DNA extraction and bacteria community analysis by automated ribosomal intergenic spacer analysis (ARISA) fingerprints (see SI-Experimental part for details).

2.4.4 *Biogenic precipitate characterization*

The biogenic precipitates from Exp 1_(FF), Exp 1_(OA), Exp 2_(4 mm), Exp 2_(25 mm) and Exp 2_(25 mm, Air) were recovered from the bottom of the channels at the end of each experiment and characterized for their mineralogy, As and Fe content, As speciation, bacterial cell number, bacterial genetic fingerprint, and *aioA* genes quantification. For this purpose, water was removed from each experimental channel and collected in a graduated cylinder to determine water volume. The biogenic precipitate that formed at the bottom of the channel was recovered by scraping the biodegradable canvas with a sterilized spatula and transferred to Eppendorf tubes (2 mL). Three aliquots were used as replicates for the bacterial

quantification, one aliquot was used for DNA extraction, ARISA, rRNA 16S and *aioA* genes quantification (SI-Experimental part), one for total Fe and As determination after acid digestion, and the last aliquot for mineralogy, including X-ray diffraction (XRD), X-ray absorption near edge structure (XANES) at the As K-edge, and extended X-ray absorption fine structure (EXAFS) at both the As and Fe K-edges (see SI-Experimental part for details).

Typical floating films were sampled at the water surface in the bioreactor, dried in a desiccator under vacuum atmosphere, coated with Pt (SC7620 Quorum technologies) and examined with a scanning electron microscope (SEM; Hitachi S4800). The floating film from the Exp 1_(FF) was analysed by EXAFS at the As and Fe K-edges.

The vertical plastic films inserted in Exp 2 were transferred into a plastic box and dried in N₂ atmosphere with minimum disturbance for direct spatially resolved XANES and micro-X-ray fluorescence measurements and for electron microscopy analyses using a SEM-FEG Zeiss® Ultra55 microscope (see SI-Experimental part for details).

3. Results

3.1 Chemistry of inlet AMD water

Chemical composition of the AMD feed waters (Table 1) exhibited natural variability in the Carnoulès mine drainage (Casiot et al., 2003; Egal et al; 2010). The mean values for pH (3.0-3.4), conductivity (2.87-3.21 mS cm⁻¹), total dissolved Fe concentration (441-484 mg L⁻¹), dissolved Fe(II) (432-480 mg L⁻¹) and total dissolved As (mean 30-39 mg L⁻¹) varied only slightly among the AMD sampling campaigns. In contrast, oxidation state of dissolved arsenic varied among the sampling campaigns and evolved over time (mean 16-57 % As(V)/total As).

3.2 Setting up the steady-state in the continuous flow bioreactor

During the setting up stage of the experiments, the outlet Fe(II) concentration decreased as a function of time, while the Fe(II) concentration in the feed water remained nearly constant (Fig. 2). Concomitantly, the amount of Fe precipitated increased (Fig.2) and a thin orange deposit gradually covered the bottom of the channels (Fig. 1B). After ~ 10 days (Exp 1) and ~ 15 days (Exp 2), the Fe(II) concentration at the outlet remained stable, showing that steady-state conditions were reached regarding Fe(II) oxidation. Under steady-state, the dissolved Fe(II) concentration in the outlet was higher in Exp 2 (~250 mg L⁻¹) than in Exp 1 (~ 50 mg L⁻¹).

3.3 Variation of the amount of oxidized Fe(II) and precipitated Fe and As in kinetic studies

Increase in residence time was found to systematically improve Fe oxidation, Fe precipitation and As removal, however, there were substantial differences between experiments. Exp 1_(OA) showed the highest rate of Fe oxidation and precipitation (Fig. 3AB, Table 2). In Exp 1_(FF), delayed Fe(II) exhaustion and Fe removal were observed for residence times below 150 minutes (Fig. 3A). Exp 2_(4 mm) exhibited lower rates of Fe(II) oxidation and Fe and As removal than Exp 1_(OA) (Fig. 3ABC, Table 2). Finally, in Exp 2_(25 mm), Fe(II) oxidation, Fe precipitation and As removal were drastically slower than in the other experiments (Fig. 3ABC, Table 2); only the highest residence times (> 1000 min) showed significant Fe(II)-, Fe-, and As-exhaustion and pH decrease. After forced aeration in Exp 2_(25 mm, Air), the average rates of Fe(II) oxidation and Fe removal increased approximately two-fold, while the As removal rate increased no more than 1.6-fold (Fig. SI-1). As(V) removal was systematically more efficient than As(III) removal in all experiments (Fig. 3DE). Consequently, dissolved arsenic in the outlet consisted mainly of As(III) (Fig. 3CD).

3.4 Oxygen concentration profiles

The DO profile recorded in the water column at 4 mm water height in absence of a biogenic precipitate at the bottom of the channels (Fig. 4A) showed oxygen saturation concentrations in the whole water column. On the contrary, after biogenic precipitate had formed (profiles from Exp 1_(OA) and Exp 2_(4 mm)), oxygen was consumed in the water column. The DO profiles showed saturation at the water surface and gradual concentration decrease from 8.7 to 5.7 mg L⁻¹ within the first 1 mm depth, then a further decrease to ~ 2.0 mg L⁻¹ at the interface between the water column and the biogenic precipitate. The floating film limited oxygen concentration to less than 0.01 mg L⁻¹ for depths below 1 mm.

The DO profile at 25 mm water height showed a rapid decrease from saturation value (8.0 mg L⁻¹) at the water surface to less than 4.0 mg L⁻¹ below 20 mm, then relatively constant value at 3.9 ± 0.3 mg L⁻¹ near the biogenic precipitate. After aeration in Exp 2_(25 mm, Air), the DO concentration in the water column averaged 6 ± 1 mg L⁻¹, according to three measurements carried out at inlet, middle and outlet of the channel (micro-profiling was not possible due to turbulent conditions).

3.5 Arsenic speciation and mineralogy in the biogenic precipitates and floating film

The biogenic precipitates at the channel bottom contained ~35 wt. % Fe and ~7 wt. % As, with an As/Fe molar ratio ranging from 0.14 to 0.20 (Table 3). XRD analysis (Table 3, Fig. SI-2), showed that the nano-crystalline fraction of the solid phase was similar irrespective of the experiment conditions, consisting mainly of schwertmannite. Fe K-edge EXAFS data (Fig. 5, Table 3, Table SI-3 and Fig. SI-4) revealed the additional presence of 15-33 mol% amorphous ferric arsenate, in mixture with 67-85 mol% of schwertmannite. K-edge XANES indicated 16-52 % As(III)/Total As and 48-84 % As(V)/Total As in the biogenic precipitates (Table 3, data from Resongles et al. (2016), see Table SI-1 for equivalence of sample names).

K-edge EXAFS (Fig. 5, Table 3, Table SI-2 and Fig. SI-3) showed that As(III) and As(V) species were distributed among three different solid phases: schwertmannite with As(III) (16 to 51 mol%), schwertmannite with As(V) (20 to 38 mol%) and amorphous ferric arsenate (31 to 62 mol%). The floating-film collected in Exp 1_(FF) had similar composition as the precipitates collected at the bottom of the reactor in the same experiment (Table 3). SEM images showed that the nature of this floating film consisted of a mineralogical layer with numerous bacteria, some of which were encrusted by mineral precipitates (Fig SI-6A) with similar morphology as those observed on the vertical plastic film and at the bottom of the bioreactor channels (Fig SI-6BC).

The arsenic oxidation state in the biogenic precipitates varied significantly among the experiments. Indeed, the proportion of As(V) was higher in Exp 1 than in Exp 2 (Fig. 5, Table 3). In addition, increasing the water height in Exp 2 resulted in a higher proportion of As(V), mainly in the form of amorphous ferric arsenate in the precipitate (Fig. 5). The effect of the water height was confirmed by spatially resolved XANES and micro-X-ray fluorescence analyses of the precipitates deposited on the vertical plastic film in Exp 2 (Fig. 6 and Fig. SI-7). In Exp 2_(4 mm), the proportion of As(V) in the precipitate remained below 50% and decreased from ~50% at the bottom of the channel to ~30% at the water surface (Fig. 6A). In Exp 2_(25 mm), similar behaviour was observed up to 5 mm water height and the proportion of As(V) then largely increased, up to ~60-80%, at the top of the water column. Air bubbling in Exp 2_(25 mm Air) slightly decreased the proportion of As(V) in the precipitates that formed in the water column. These variations of the proportion of As(V) were positively correlated to the As/Fe molar ratio in the precipitates (Fig. 6B).

3.6 *Microbial characterization of the biogenic precipitates*

Bacterial biomass within the bottom biogenic precipitates remained in the same order of magnitude between the experiments, with an average of $6 \pm 4 \times 10^6$ bacterial cells g⁻¹ (dry weight) (Table 3).

The bacterial community in the biogenic precipitates differed widely from that in the corresponding feed waters (Fig. 7). Concerning the different experimental conditions, the structure of the community does not seem to have been affected notably by the presence of the floating film (Exp 1_(FF) and Exp 1_(OA)). Conversely, water height appeared as a potential factor influencing the bacterial community structure; indeed, the bacterial community from Exp 2_(25 mm) is well discriminated from all the communities obtained at 4 mm water height and from the community exposed to air bubbling (Exp 2_(25 mm, Air)). The variation in the bacterial community of the biogenic precipitates of the similar experiments Exp 1_(OA) and Exp 2_(4 mm) can be linked at least partially to the different feed waters. *aioA* genes were detected in the biogenic precipitates in all experiments. The proportion of bacteria possessing the genetic potential for As(III) oxidation averaged 25 % of the total community (mean *aioA*/16S ratio of 0.25, Table 3) .

4. Discussion

4.1 Fe(II) oxidation and precipitation in the channel bioreactor: influence of the microbial community

The channel bioreactor allowed to achieve Fe(II) oxidation and subsequent Fe precipitation, providing the basis for arsenic removal. In the setting up stage of the continuous flow experiments (Fig. 2), the gradual increase of the Fe(II) oxidation rate was concomitant to the microbial colonization of the bottom of the bioreactor channels. This highlighted the role of microorganisms developing within the biogenic precipitate as catalysts for Fe(II) oxidation.

The biogenic precipitates contained $3\text{-}9 \times 10^6$ bacterial cells g^{-1} dry wt., which was comparable to the Carnoulès sediment content (3×10^6 cells g^{-1} dry wt., Desoeuvre, personal communication) and to other TIFs, for which generally $10^6\text{-}10^7$ bacterial cells g^{-1} have been reported (Brown et al., 2011; Brantner et al., 2014).

Bacteria colonizing the biogenic precipitate in the channel bioreactor were inherited from the seed community present in AMD water from the Carnoulès mine. Fe(II)-oxidizing bacteria are likely to belong to the genera *Gallionella* and *Acidithiobacillus*, which are known to be permanent members of the Carnoulès AMD autochthonous community (Volant et al., 2014). In spite of the wide variability of the water bacterial community structure, relatively similar communities were selected within the biogenic precipitates originating from different waters (Fig. 7); these biogenic precipitates allowed to maintain over 50 % Fe(II) oxidation within a residence time of 300 min, in all the experiments conducted at 4 mm water height. This corroborated the findings of Sheng et al. (2016) who obtained efficient Fe(II) oxidizing activity with different seed AMD communities in chemostatic bioreactors treating AMD.

The average Fe(II) oxidation rate ($3.4 \pm 0.9 \times 10^{-7}$ mol L^{-1} s^{-1}) ranged within literature values for other natural or engineered TIFs (Larson et al., 2014, and references therein). However, Fe(II) oxidation rates differed slightly between Exp 1 (OA) and Exp 2 (4 mm) carried out in the same operating conditions (Fig. 3), with similar feed water physico-chemistry in term of pH, Fe(II) and As concentration (Table 1). This suggests that slight variations in the structure or the activity of the bacterial community may affect Fe(II) oxidation rates to some extent. Further insight into the taxonomic composition of the bacterial community would be required to check this hypothesis.

4.2 Influence of floating film on Fe(II) oxidation in the channel bioreactor

The floating film formed in these experiments consisted of schwertmannite and amorphous ferric arsenate associated with bacteria. Such mineralogy logically differed from that found in floating films from circum-neutral pH waters where ferrihydrite was dominant (Grathoff et al., 2007; Kleja et al., 2012). The identity of bacteria evidenced in this floating film is still unknown; elsewhere, the sheathed bacterium *Leptothrix* (van Veen et al., 1978; Robbins et al., 2000; Eggerichs et al., 2014) and other genera (Wilmes et al., 2009; Reina et al., 2015) have been identified.

To our knowledge, the effect of the floating film on the functioning of natural or engineered TIFs, regarding Fe(II) oxidation, had never been investigated before. In the kinetic study of Exp 1_(FF), delayed Fe(II) exhaustion observed for residence times below 150 minutes, was associated with anoxic conditions in the water column below 1 mm depth (Fig. 4A). Oxygen depletion had been evidenced below floating iron-oxide films that formed at circum-neutral pH in several groundwater seepage areas of the Doñana National Park (SW Spain) compared with other film-free wetland areas (Reina et al., 2015). Puyate and Rim-Rukeh (2008) suggested that in biofilms, bacteria embedded in a slime of extracellular polymeric substances used oxygen as a nutrient and depleted the DO concentration in the surrounding liquid medium. Thus, it was anticipated that faster rates of metabolic consumption, relative to the oxygen diffusion rate, induced a decline in concentrations of DO with increasing distance from the air–solution interface. In batch experiments carried out with AMD from Esperanza mine, the lack of oxygen resulting from standing conditions in a tank induced a slowdown in Fe(II) oxidation compared to aerated conditions, showing that oxygen transfer was a limiting factor (Sánchez-España et al., 2007). More specifically, studies dedicated to the Fe(II) oxidation kinetics by *A. ferrooxidans* have reported that the mesophilic oxidation rate of ferrous iron with air was limited by the oxygen transfer rate (Savić et al., 1998). According to Liu et al. (1988), a DO value below 0.29-0.7 mg L⁻¹ was limiting for *A. ferrooxidans*.

It can be hypothesized that the floating film, by limiting oxygen transfer rate at the air/water interface, lowered oxygen concentration in the water column, which had an adverse effect on the activity of iron oxidizing populations, such as *A. ferrooxidans*, and this effect was most pronounced at low residence times (< 150 min) in the bioreactor. However, the floating film had a limited influence on the whole bacterial community structure (Fig. 7).

4.3 Influence of water height on Fe(II) oxidation in the channel bioreactor

The increase of water height in Exp 2 was associated to several changes that may explain Fe(II) oxidation slowdown: i) the bacterial cell concentration in the biogenic precipitate was slightly lower at 25 mm ($4.4 \pm 0.6 \times 10^6$ cells g⁻¹ dry wt.) than at 4 mm ($9 \pm 5 \times 10^6$ cells g⁻¹ dry wt.), ii) the bacterial community structure was slightly different (Fig. 7), iii) oxygen concentration was depleted in the water column and iv) the ratio between the volume of water in the channel and the surface of the biogenic precipitate was ~ 6-fold higher in Exp 2_(25mm) compared to Exp 2_(4mm).

The key role of oxygen transfer was evidenced in Exp 2_(25 mm, Air), considering the 2-fold improvement of the Fe oxidation rate resulting from air bubbling. Turbulent flow conditions favour mass transfer and oxygen diffusion (Eberl et al., 2000; Wäsche et al., 2002; Beyenal and Lewandowski, 2002). Brown et al. (2011) hypothesized an effect of hydrodynamic conditions (e.g., sheet flow and turbulence) on Fe(II) oxidation rates and subsequent treatment system performance in Lower Red Eyes AMD (Pennsylvania). Chen and Jiang (2012) observed that AMD creek sections in Chinkuashih (Taiwan) exhibited Fe(II) oxidation rates that were one to two orders of magnitude slower than in waterfall sections, where Fe oxidation rates reached 6.1 to 6.7×10^{-6} mol L⁻¹ s⁻¹. They suggested that waterfall aeration was the main factor driving up the Fe(II) oxidation rate and pointed out the dynamic splashing effects on chemical transport.

The reactive surface area per volume of water is also a key parameter driving Fe(II) oxidation rate in fixed-film continuous flow bioreactors. As an example, a ~ 2 -fold increase of surface area allowed a ~ 2 -fold increase of Fe(II) oxidation rate in a packed bed reactor working with glass beads as a support matrix (Grishin and Tuovinen, 1988). It is therefore anticipated that lower Fe(II) oxidation rate in Exp 2 (25 mm) is partly related to higher water volume to reactive surface area ratio.

4.4 Arsenic oxidation and removal in the channel bioreactor

4.4.1 Arsenic removal

The removal of arsenic concomitantly to Fe, so called “natural attenuation”, has been described in several AMD throughout the world (e.g. Cheng et al., 2009; Rait et al., 2010; Paikaray, 2015). In AMD, arsenic is generally removed from the dissolved phase by sorption onto schwertmannite (Carlson et al., 2002; Fukushi et al., 2003; Ohnuki et al., 2004) or formation of amorphous ferric arsenate (Maillot et al., 2013). In addition, a rare ferric arsenite phase (tooeleite) may occasionally occur at high As concentration (Morin et al., 2003). The rate of this removal has rarely been determined either in treatment trials or in natural AMD. In a recent study with a fluidized bed reactor inoculated with an iron-oxidizing culture and added flocculant, Ahoranta et al. (2016) obtained an As removal rate of $1.4\text{--}1.7 \times 10^{-10} \text{ mol L}^{-1} \text{ s}^{-1}$, reaching 99.5 % As removal with a retention time of 342 min at pH 3. Asta et al. (2010b) measured an As(V) removal rate of $7\text{--}8 \times 10^{-10} \text{ mol L}^{-1} \text{ s}^{-1}$ in the Tinto Santa Rosa AMD. Chen and Jiang (2012) obtained $4.7\text{--}6.3 \times 10^{-9} \text{ mol L}^{-1} \text{ s}^{-1}$ for the As sorption rate in AMD waterfall section from the Chinkuashih area.

In the present study, arsenic was efficiently removed from the dissolved phase in the channel bioreactor when it operated at 4 mm in open air, reaching 65 % abatement within 500 minutes (Fig. 3). This corresponded to an average removal rate of $\sim 4 \times 10^{-8} \text{ mol L}^{-1} \text{ s}^{-1}$, one order of magnitude lower than in the field at Carnoulès ($3.58 \times 10^{-7} \text{ mol L}^{-1} \text{ s}^{-1}$; Egal et al., 2010).

The faster As removal in the field may be associated with faster Fe(II) oxidation rates ($3.8 \times 10^{-6} \text{ mol L}^{-1} \text{ s}^{-1}$) and subsequent precipitation, compared to the laboratory (Fe oxidation rate = $3.4 \pm 0.9 \times 10^{-7} \text{ mol L}^{-1} \text{ s}^{-1}$), which was also observed in other studies (Sánchez-España et al., 2007; Larson et al, 2014). Photochemical As(III) oxidation in addition to microbial As(III) oxidation, may also favour As removal (Asta et al., 2012), since As(V) is less soluble than As(III) at acidic pH (Burton et al., 2009).

The arsenic removal rate was ten-fold lower in Exp 2_(25 mm), compared to Exp 2_(4 mm) (Table 2). The higher outlet pH in Exp 2_(25 mm), compared to other experiments (Fig. 3F) should have favoured the sorption of As(III) on schwertmannite (Burton et al., 2009); however, it was limited by the lower amount of Fe(II) oxidized. Conversely, the higher pH was not expected to significantly affect As(V) sorption on schwertmannite, which is more readily sorbed than As(III) within this acid pH range (Burton et al., 2009). Similarly, there was no clear evidence for a retardation of the As removal by the floating film, in relation with the higher proportion of more readily precipitated As(V) in the feed water in Exp 1_(FF) (57 %) compared to Exp 1_(OA) (39 %).

4.4.2 Arsenic oxidation

According to mass balance calculations (SI-Experimental part), the amount of As(V) accumulated in the biogenic precipitate was higher than the amount of As(V) removed from the dissolved phase (Fig. SI-5), showing that arsenic oxidized to some extent in the bioreactor. In addition, the detection of *aioA* genes suggests that microbially mediated arsenic oxidation occurred within the bioreactor, in agreement with the sustained detection of an As-oxidizing activity in the Carnoulès AMD (Casiot et al., 2003), particularly attributable to *Thiomonas* spp. (Bruneel et al., 2003; Hovasse et al., 2016). The relative abundance of *aioA*-carrying bacteria in the biogenic precipitates was higher than in waters moderately contaminated with arsenic (Quéméneur et al., 2010) and in geothermal springs (Jiang et al.,

2014). Nevertheless, the abiotic oxidation of arsenic cannot be totally ruled out; indeed, Fe(III) originating from bacterial Fe(II) oxidation may oxidize As(III) in the presence of visible light (Bhandari et al., 2011; Asta et al., 2012). Furthermore, As(III) oxidation may occur prior to precipitation but also during ageing of the precipitate. In this respect, Ona-Nguema et al. (2010) showed that oxidation of Fe(II) can lead to the formation of reactive oxygen species able to oxidize As(III) to As(V) at the surface of iron oxides or oxyhydroxides at neutral pH, while Burton et al. (2009) indicated that As(III) sorbed on schwertmannite did not undergo abiotic oxidation in oxic conditions, within a period of 2.5 days.

Considering both As(III) concentration remaining in the dissolved phase (Fig. 3D) and the proportion of As(III) (16 – 52 %) in the final precipitate (Table 3), As(III) oxidation was not complete in the present experiments. The data indicates first order kinetics for As(III) exhaustion in Exp 1_(OA) (Fig. 3D), and assuming that all exhausted As(III) has been oxidized, a kinetic constant value $k = 0.0065 \text{ min}^{-1}$ and half-life value $t_{1/2} = 107 \text{ min}$ were determined. This is at least one order of magnitude lower than k values obtained in fixed-bed bioreactors working at higher pH, inoculated with the As-oxidizing bacteria *Thiomonas arsenivorans* ($k \approx 0.05 \text{ min}^{-1}$, $t_{1/2} = 13.6 \text{ min}$ in Wan et al., 2010) or a consortium ($k \approx 0.04 \text{ min}^{-1}$, $t_{1/2} = 16.5 \text{ min}$ in Michon et al., 2010) originating from a gold mine site, used for the treatment of As(III)-rich groundwater. Thus, although biogenic precipitates formed in the bioreactor contain a substantial proportion (average of 25 %) of As-oxidizing bacteria, the operating conditions tested in this study did not appear to favour their activity. A number of factors may influence their activity. Debiec et al. (2017) showed that pH, temperature and aeration impacted the rate of As(III) oxidation in cultures of As-oxidizing strain *Sinorhizobium* sp. M14, isolated from an arsenic-contaminated mine. In the present study, both a lower water height that favours oxygen diffusion to the biogenic precipitate, and air bubbling, increased the rate of As removal; however it did not increase arsenite oxidizer abundance (*aioA* gene), nor the

proportion of As(V) in the precipitate, which was even lower in Exp 2_(4 mm) than in Exp 2_(25 mm). This suggested that oxygen diffusion to this precipitate was not a key factor driving arsenite oxidation and removal from the water, contrary to Fe oxidation. Most probably, the rate of As(III) exhaustion and the proportion of As(III) and As(V) in the biogenic precipitate were controlled by the rate of Fe(II) oxidation and subsequent precipitation, as well as by the As(V) proportion in the inlet water. Higher Fe(II) oxidation and Fe precipitation rates in Exp 2_(4 mm), compared to Exp 2_(25 mm), favoured the rapid achievement of the schwertmannite solubility product, and subsequent sorption of As(III) and As(V), before appreciable As(III) oxidation took place. Another hypothesis could be that As-oxidizing bacteria may be more active or more numerous in the water column than Fe-oxidizing bacteria. In this respect, Michel et al. (2007) showed that the specific As(III)-oxidase activity of *Thiomonas arsenivorans* was higher (9-fold) for planktonic cells than for sessile ones. Similarly, in the deposits along the vertical plastic film, the higher proportion of As(V) and higher As/Fe molar ratio (Fig. 6), at greater distance from the biogenic precipitates, suggests the occurrence of vertical gradients of Fe(II) oxidation and As(III) oxidation within the water column. Further research is required to find out key factors able to influence the growth and As-oxidizing activity of microorganisms in the bioreactor. Nevertheless, since As(III) oxidation was limited in the bioreactor, the proportion of As(V) in the inlet water (Table 1) largely influenced the As removal rate, since As(V)-bearing precipitates are ten times less soluble than As(III) ones at acidic pH (Burton et al., 2009; Maillot et al., 2013).

4.4.3 Control of dissolved As(V) and Fe(III) on the mineralogy of the biogenic precipitates

The formation of amorphous ferric arsenate over schwertmannite is favoured by an increase in the As(V)/Fe(III) ratio in solution. Indeed, the high affinity of As(V) oxyanions for the complexation with Fe(III) has been shown to inhibit the nucleation of schwertmannite and to

favour the formation of amorphous ferric arsenate for dissolved As(V)/Fe(III) ratio above 0.15-0.2 (Carlson et al., 2002; Maillot et al., 2013). In the present study, for residence times between ~ 20-500 min, the range of dissolved As(V)/Fe(III) molar ratios removed from the dissolved phase (Fig. 3) increased in the order: Exp 2_(4 mm) (< 0.01-0.11) < Exp 1_(OA) (< 0.01-0.18) < Exp 2_(25 mm) (0.09-0.38) < Exp 1_(FF) (0.02-1.68). The proportion of ferric arsenate increased accordingly (Fig. 5), thus corroborating the influence of both dissolved As(V) proportion in the inlet and Fe(II) oxidation rate on the proportion of ferric arsenate in the biogenic precipitate.

5. Conclusions

Within the continuous flow bioreactor designed for this study more than 80 % of Fe(II) was oxidised and ~ 65 % of As was removed from As-rich (30-40 mg L⁻¹) AMD within 500 min. The treatment produced sludge with high As concentration (6.3-8 % dry wt.), mainly in the less toxic As(V) form (49-85 %). Distinct feed waters, in term of chemical and microbiological composition, led to the settlement of relatively constant bacterial community in the bioreactor capable to catalyse the Fe(II) oxidation and the As removal. Such resilience is promising for future *in situ* treatment of AMD.

This study contributes to a better understanding of the influence of operating conditions, i.e. water height and floating film, on Fe(II) oxidation rate, arsenic speciation and removal, which control natural attenuation process that takes place in many As-rich AMD. Such factors should be considered in future design of passive treatment systems. Water height appeared salient for controlling the Fe(II) oxidation rate, which is the basis of the natural attenuation of arsenic. The floating film that formed naturally under low flow regime limited the oxygen transfer to the biogenic precipitate, thus delaying Fe(II) oxidation. Water height also strongly affected the Fe(II) oxidation rate and subsequent As removal; a thin water layer running

across the biogenic precipitate maximized both oxygen diffusion and biofilm surface area per unit water volume, thus improving the Fe(II) oxidation rate. The initial arsenic oxidation state also affected the As removal and speciation in the neoformed solid phases; higher initial As(V) thus favoured As removal in the form of amorphous ferric arsenate. The stability of these phases upon long-term storage should be considered in a general waste management scenario. Such treatment constitutes a first step of a whole AMD treatment since acid pH remains to be neutralized and metal cations have to be removed before the effluent can be released in the environment. Calcite drains or biological treatment based on bacterial sulphate reduction might be promising as complementary processes.

Acknowledgements

The authors thank the IngECOST-DMA project (ANR-13-ECOT-0009), the OSU OREME and the Ecole Doctorale GAIA (PhD fellowship of Lidia Fernandez-Rojo) for the financial support. We thank Remi Freydier for ICP-MS analysis on the AETE-ISO platform (OSU OREME, University of Montpellier). Christophe Duperray and Yann Bordat from the Montpellier RIO Imaging microscopy platform are gratefully acknowledged for their kind assistance in cytometry. We also thank Luca Olivi from the XAFS beamline from the ELETTRA-synchrotron for his assistance and advices, as well as the staff of the SAMBA beamline at the SOLEIL synchrotron for their assistance in XAS experiments. Benoit Baptiste (IMPMC) is acknowledged for his help to achieve micro-XRF measurements. We thank Didier Cot, from the Institut Européen de Membranes for its assistance in the Scanning Electron Microscope. Blandine Savajols, from the University of Montpellier, is also thanked for her valuable contribution during their internships in the laboratory HydroSciences Montpellier.

References

- Ahoranta, S.H., Kokko, M.E., Papirio, S., Özkaya, B., Puhakka, J.A., 2016. Arsenic removal from acidic solutions with biogenic ferric precipitates. *Journal of Hazardous Materials* 306, 124–32. doi:10.1016/j.jhazmat.2015.12.012
- Asta, M.P., Ayora, C., Román-Ross, G., Cama, J., Acero, P., Gault, A.G., Charnock, J.M., Bardelli, F., 2010a. Natural attenuation of arsenic in the Tinto Santa Rosa acid stream (Iberian Pyritic Belt, SW Spain): The role of iron precipitates. *Chemical Geology* 271 (1–2), 1–12. doi:http://dx.doi.org/10.1016/j.chemgeo.2009.12.005
- Asta, M.P., Ayora, C., Acero, P., Cama, J., 2010b. Field rates for natural attenuation of arsenic in Tinto Santa Rosa acid mine drainage (SW Spain). *Journal of Hazardous Materials* 177 (1–3), 1102–1111. doi:10.1016/j.jhazmat.2010.01.034
- Asta, M.P., Kirk Nordstrom, D., Blaine McCleskey, R., 2012. Simultaneous oxidation of arsenic and antimony at low and circumneutral pH, with and without microbial catalysis. *Applied Geochemistry* 27 (1), 281–291. doi:http://dx.doi.org/10.1016/j.apgeochem.2011.09.002
- Battaglia-Brunet, F., Dictor, M.-C., Garrido, F., Crouzet, C., Morin, D., Dekeyser, K., Clarens, M., Baranger, P., 2002. An arsenic(III)-oxidizing bacterial population: selection, characterization, and performance in reactors. *Journal of Applied Microbiology* 93 (4), 656–667. doi:10.1046/j.1365-2672.2002.01726.x
- Battaglia-Brunet, F., Itard, Y., Garrido, F., Delorme, F., Crouzet, C., Greffie, C., Joulian, C., 2006. A simple biogeochemical process removing arsenic from a mine drainage water. *Geomicrobiology Journal* 23 (3–4), 201–211. doi:10.1080/01490450600724282
- Beyenal, H., Lewandowski, Z., 2002. Internal and external mass transfer in biofilms grown at various flow velocities. *Biotechnology Progress* 18 (1), 55–61. doi:10.1021/bp010129s
- Bhandari, N., Reeder, R.J., Strongin, D.R., 2011. Photoinduced oxidation of arsenite to arsenate on ferrihydrite. *Environmental Science & Technology* 45 (7), 2783–2789. doi:10.1021/es103793y
- Brantner, J.S., Haake, Z.J., Burwick, J.E., Menge, C.M., Hotchkiss, S.T., Senko, J.M., 2014. Depth-dependent geochemical and microbiological gradients in Fe(III) deposits resulting from coal mine-derived acid mine drainage. *Frontiers in Microbiology* 5 (215), 1–15. doi:10.3389/fmicb.2014.00215

- Brown, J.F., Jones, D.S., Mills, D.B., Macalady, J.L., Burgos, W.D., 2011. Application of a depositional facies model to an acid mine drainage site. *Applied and Environmental Microbiology* 77 (2), 545–554. doi:10.1128/aem.01550-10
- Bruneel, O., Personné, J.C., Casiot, C., Leblanc, M., Elbaz-Poulichet, F., Mahler, B.J., Le Flèche, A., Grimont, P.A.D., 2003. Mediation of arsenic oxidation by *Thiomonas* sp. in acid-mine drainage (Carnoulès, France). *Journal of Applied Microbiology* 95 (3), 492–499. doi:10.1046/j.1365-2672.2003.02004.x
- Burton, E.D., Bush, R.T., Johnston, S.G., Watling, K.M., Hocking, R.K., Sullivan, L.A., Parker, G.K., 2009. Sorption of arsenic(V) and arsenic(III) to schwertmannite. *Environmental Science & Technology* 43 (24), 9202–9207. doi: 10.1021/es902461x
- Carlson, L., Bigham, J.M., Schwertmann, U., Kyek, A., Wagner, F., 2002. Scavenging of As from acid mine drainage by schwertmannite and ferrihydrite: a comparison with synthetic analogues. *Environmental Science & Technology* 36 (8), 1712–1719.
- Casiot, C., Morin, G., Juillot, F., Bruneel, O., Personné, J.-C., Leblanc, M., Duquesne, K., Bonnefoy, V., Elbaz-Poulichet, F., 2003. Bacterial immobilization and oxidation of arsenic in acid mine drainage (Carnoulès creek, France). *Water Research* 37 (12), 2929–2936. doi:http://dx.doi.org/10.1016/S0043-1354(03)00080-0
- Chen, C.-J., Jiang, W.-T., 2012. Influence of waterfall aeration and seasonal temperature variation on the iron and arsenic attenuation rates in an acid mine drainage system. *Applied Geochemistry* 27 (10), 1966–1978. doi:http://dx.doi.org/10.1016/j.apgeochem.2012.06.003
- Cheng, H., Hu, Y., Luo, J., Xu, B., Zhao, J., 2009. Geochemical processes controlling fate and transport of arsenic in acid mine drainage (AMD) and natural systems. *Journal of Hazardous Materials* 165 (1–3), 13–26. doi:http://dx.doi.org/10.1016/j.jhazmat.2008.10.070
- Debiec, K., Krzysztoforski, J., Uhrynowski, W., Sklodowska, A., Drewniak, L., 2017. Kinetics of arsenite oxidation by *Sinorhizobium* sp. M14 under changing environmental conditions. *International Biodeterioration & Biodegradation* 119, 476–485. doi:10.1016/j.ibiod.2016.10.049
- DeSa, T. C., Brown, J.F., Burgos, W.D., 2010. Laboratory and field-scale evaluation of low-pH Fe(II) oxidation at Hughes Borehole, Portage, Pennsylvania. *Mine Water and the Environment* 29 (4), 239–247. doi:10.1007/s10230-010-0105-5

- Eberl, H.J., Picioreanu, C., Heijnen, J.J., van Loosdrecht, M.C.M., 2000. A three-dimensional numerical study on the correlation of spatial structure, hydrodynamic conditions, and mass transfer and conversion in biofilms. *Chemical Engineering Science* 55 (24), 6209–6222. doi:10.1016/S0009-2509(00)00169-X
- Egal, M., Casiot, C., Morin, G., Elbaz-Poulichet, F., Cordier, M.-A., Bruneel, O., 2010. An updated insight into the natural attenuation of As concentrations in Reigous Creek (southern France). *Applied Geochemistry* 25 (12), 1949–1957. doi:http://dx.doi.org/10.1016/j.apgeochem.2010.10.012
- Eggerichs, T., Opel, O., Otte, T., Ruck, W., 2014. Interdependencies between biotic and abiotic ferrous iron oxidation and influence of pH, oxygen and ferric iron deposits. *Geomicrobiology Journal* 31 (6), 461–472. doi:10.1080/01490451.2013.870620
- Elbaz-Poulichet, F., Bruneel, O., Casiot, C., 2006. The Carnoulès mine. Generation of As-rich acid mine drainage, natural attenuation processes and solutions for passive in-situ remediation, in: *Difpolmine (Diffuse Pollution from Mining Activities)*, Dec 2006, Montpellier, France. <hal-00184269>
- Fukushi, K., Sasaki, M., Sato, T., Yanase, N., Amano, H., Ikeda, H., 2003. A natural attenuation of arsenic in drainage from an abandoned arsenic mine dump. *Applied Geochemistry* 18 (8), 1267–1278. doi:http://dx.doi.org/10.1016/S0883-2927(03)00011-8
- Grathoff, G.H., Baham, J.E., Easterly, H.R., Gassman, P., Hugo, R.C., 2007. Mixed-valent Fe films (“Schwimmeisen”) on the surface of reduced ephemeral pools. *Clays and Clay Minerals* 55 (6), 635–643. doi:10.1346/CCMN.2007.0550610
- Grishin, S.I., Tuovinen, O.H., 1988. Fast kinetics of Fe^{2+} oxidation in packed-bed reactors. *Applied and Environmental Microbiology* 54 (12), 3092–3100.
- Hovasse, A., Bruneel, O., Casiot, C., Desoeuvre, A., Farasin, J., Héry, M., Van Dorsselaer, A., Carapito, C., Arsène-Ploetze, F., 2016. Spatio-temporal detection of the *Thiomonas* population and the *Thiomonas* arsenite oxidase involved in natural arsenite attenuation processes in the Carnoulès acid mine drainage. *Frontiers in Cell and Developmental Biology* 4 (3), 1–14. doi:10.3389/fcell.2016.00003
- Hug, S.J., Leupin, O., 2003. Iron-catalyzed oxidation of arsenic(III) by oxygen and by hydrogen peroxide: pH-dependent formation of oxidants in the Fenton reaction. *Environmental Science & Technology* 37 (12), 2734–2742. doi:10.1021/es026208x

- Jiang, Z., Li, P., Jiang, D., Wu, G., Dong, H., Wang, Y., Li, B., Wang, Y., Guo, Q., 2014. Diversity and abundance of the arsenite oxidase gene *aoxA* in geothermal areas of Tengchong, Yunnan, China. *Extremophiles* 18 (1), 161–170. doi:10.1007/s00792-013-0608-7
- Kim, J.-Y., Davis, A.P., Kim, K.-W., 2003. Stabilization of available arsenic in highly contaminated mine tailings using iron. *Environmental Science & Technology* 37 (1), 189–195. doi:10.1021/es020799+
- Kleja, D.B., van Schaik, J.W.J., Persson, I., Gustafsson, J.P., 2012. Characterization of iron in floating surface films of some natural waters using EXAFS. *Chemical Geology* 326, 19–26. doi:10.1016/j.chemgeo.2012.06.012
- Larson, L.N., Sánchez-España, J., Kaley, B., Sheng, Y., Bibby, K., Burgos, W.D., 2014. Thermodynamic controls on the kinetics of microbial low-pH Fe(II) Oxidation. *Environmental Science & Technology* 48 (16), 9246–9254. doi:10.1021/es501322d
- Lawrence, R.W., Higgs, T.W., 1999. Removing and stabilizing As in acid mine water. *Journal of The Minerals, Metals & Materials Society (TMS)* 51 (9), 27–29. doi:10.1007/s11837-999-0154-z
- Leblanc, M., Achard, B., Ben Othman, D., Luck, J.M., Bertrand-Sarfati, J., Personné, J.C., 1996. Accumulation of arsenic from acidic mine waters by ferruginous bacterial accretions (stromatolites). *Applied Geochemistry* 11 (4), 541–554. doi:http://dx.doi.org/10.1016/0883-2927(96)00010-8
- Liu, M.S., Branion, R.M.R., Duncan, D.W., 1988. The effects of ferrous iron, dissolved oxygen, and inert solids concentrations on the growth of *Thiobacillus ferrooxidans*. *The Canadian Journal of Chemical Engineering* 66 (3), 445–451. doi:10.1002/cjce.5450660315
- Macías, F., Caraballo, M.A., Nieto, J.M., Rötting, T.S., Ayora, C., 2012. Natural pretreatment and passive remediation of highly polluted acid mine drainage. *Journal of Environmental Management* 104, 93–100. doi:http://dx.doi.org/10.1016/j.jenvman.2012.03.027
- Maillot, F., Morin, G., Juillot, F., Bruneel, O., Casiot, C., Ona-Nguema, G., Wang, Y., Lebrun, S., Aubry, E., Vlaic, G., Brown Jr, G.E., 2013. Structure and reactivity of As(III)- and As(V)-rich schwertmannites and amorphous ferric arsenate sulfate from the Carnoulès acid mine drainage, France: Comparison with biotic and abiotic model compounds and implications for As remediation. *Geochimica et Cosmochimica Acta* 104, 310–329. doi:http://dx.doi.org/10.1016/j.gca.2012.11.016

- Michel, C., Jean, M., Coulon, S., Dictor, M.-C., Delorme, F., Morin, D., Garrido, F., 2007. Biofilms of As(III)-oxidising bacteria: formation and activity studies for bioremediation process development. *Applied Microbiology and Biotechnology* 77 (2), 457–467. doi:10.1007/s00253-007-1169-4
- Michon, J., Dagot, C., Deluchat, V., Dictor, M.-C., Battaglia-Brunet, F., Baudu, M., 2010. As(III) biological oxidation by CAsO1 consortium in fixed-bed reactors. *Process Biochemistry* 45 (2), 171–178. doi:10.1016/j.procbio.2009.09.003
- Morin, G., Juillot, F., Casiot, C., Bruneel, O., Personné, J.-C., Elbaz-Poulichet, F., Leblanc, M., Ildefonse, P., Calas, G., 2003. Bacterial formation of tooeleite and mixed arsenic(III) or arsenic(V)–iron(III) gels in the Carnoulès acid mine drainage, France. A XANES, XRD, and SEM study. *Environmental Science & Technology* 37 (9), 1705–1712. doi:10.1021/es025688p
- Ohnuki, T., Sakamoto, F., Kozai, N., Ozaki, T., Yoshida, T., Narumi, I., Wakai, E., Sakai, T., Francis, A.J., 2004. Mechanisms of arsenic immobilization in a biomat from mine discharge water. *Chemical Geology* 212 (3–4), 279–290. doi:10.1016/j.chemgeo.2004.08.018
- Ona-Nguema, G., Morin, G., Wang, Y., Foster, A.L., Juillot, F., Calas, G., Brown, G.E., 2010. XANES evidence for rapid arsenic(III) oxidation at magnetite and ferrihydrite surfaces by dissolved O_2 via Fe^{2+} -mediated reactions. *Environmental Science & Technology* 44 (14), 5416–5422. doi:10.1021/es1000616
- Paikaray, S., 2015. Arsenic geochemistry of acid mine drainage. *Mine Water and the Environment* 34 (2), 181–196. doi:10.1007/s10230-014-0286-4
- Puyate, Y., Rim-Rukeh, A., 2008. Some physico-chemical and biological characteristics of soil and water samples of part of the Niger Delta area, Nigeria. *Journal of Applied Sciences and Environmental Management* 12 (2), 135–141.
- Quéméneur, M., Cébron, A., Billard, P., Battaglia-Brunet, F., Garrido, F., Leyval, C., Joulian, C., 2010. Population structure and abundance of arsenite-oxidizing bacteria along an arsenic pollution gradient in waters of the upper isle River Basin, France. *Applied and Environmental Microbiology*. 76 (13), 4566–4570. doi:10.1128/AEM.03104-09
- Rait, R., Trumm, D., Pope, J., Craw, D., Newman, N., MacKenzie, H., 2010. Adsorption of arsenic by iron rich precipitates from two coal mine drainage sites on the West Coast of New Zealand. *New Zealand Journal of Geology and Geophysics* 53 (2–3), 177–193.

- Reina, M., Portillo, M.C., Serrano Martín, L., Lucassen, E.C.H.E.T., Roelofs, J.G.M., Romero, A., González, J.M., 2015. The interplay of hydrological, chemical and microbial processes in the formation of iron-rich floating films in aquatic environments at a circumneutral pH. *Limnetica* 34 (2), 365–380.
- Resongles, E., Le Pape, P., Fernandez-Rojo, L., Morin, G., Brest, J., Guo, S., Casiot, C., 2016. Routine determination of inorganic arsenic speciation in precipitates from acid mine drainage using orthophosphoric acid extraction followed by HPLC-ICP-MS. *Analytical Methods* 8, 7420–7426. doi:10.1039/c6ay02084d
- Robbins, E.I., Rodgers, T.M., Alpers, C.N., Nordstrom, D.K., 2000. Ecogeochemistry of the subsurface food web at pH 0–2.5 in Iron Mountain, California, U.S.A. *Hydrobiologia* 433 (1/3), 15–23. doi:10.1023/A:1004050216537
- Sánchez España, J., López Pamo, E., Santofimia Pastor, E., 2007. The oxidation of ferrous iron in acidic mine effluents from the Iberian Pyrite Belt (Odiel Basin, Huelva, Spain): Field and laboratory rates. *Journal of Geochemical Exploration* 92 (2–3), 120–132. doi:http://dx.doi.org/10.1016/j.gexplo.2006.08.010
- Savić, D.S., Veljković, V.B., Lazić, M.L., Vrvic, M.M., Vučetić, J.I., 1998. Effects of the oxygen transfer rate on ferrous iron oxidation by *Thiobacillus ferrooxidans*. *Enzyme and Microbial Technology* 23 (7), 427–431. doi:10.1016/S0141-0229(98)00071-4
- Sheng, Y., Bibby, K., Grettenberger, C., Kaley, B., Macalady, J.L., Wang, G., Burgos, W.D., 2016. Geochemical and temporal influences on the enrichment of acidophilic iron-oxidizing bacterial communities. *Applied and Environmental Microbiology* 82 (12), 3611–3621. doi:10.1128/AEM.00917-16
- van Veen, W.L., Mulder, E.G., Deinema, M.H., 1978. The *Sphaerotilus-Leptothrix* group of bacteria. *Microbiological Reviews* 42 (2), 329–356.
- Volant, A., Bruneel, O., Desoeuvre, A., Héry, M., Casiot, C., Bru, N., Delpoux, S., Fahy, A., Javerliat, F., Bouchez, O., Duran, R., Bertin, P.N., Elbaz-Poulichet, F., Lauga, B., 2014. Diversity and spatiotemporal dynamics of bacterial communities: physicochemical and other drivers along an acid mine drainage. *FEMS Microbiology Ecology* 90 (1), 247–263. doi:10.1111/1574-6941.12394
- Wan, J., Klein, J., Simon, S., Joulain, C., Dictor, M.-C., Deluchat, V., Dagot, C., 2010. As^{III} oxidation by *Thiomonas arsenivorans* in up-flow fixed-bed reactors coupled to As sequestration onto zero-valent iron-coated sand. *Water Research* 44 (17), 5098–5108. doi:10.1016/j.watres.2010.08.044

Wäsche, S., Horn, H., Hempel, D.C., 2002. Influence of growth conditions on biofilm development and mass transfer at the bulk/biofilm interface. *Water Research* 36 (19), 4775–4784. doi:10.1016/S0043-1354(02)00215-4

Williams, M., 2001. Arsenic in mine waters: an international study. *Environmental Geology* 40 (3), 267–278. doi:10.1007/s002540000162

Wilmes, P., Remis, J.P., Hwang, M., Auer, M., Thelen, M.P., Banfield, J.F., 2009. Natural acidophilic biofilm communities reflect distinct organismal and functional organization. *The ISME Journal* 3 (2), 266–270. doi:10.1038/ismej.2008.90

TABLES

Table 1. Inlet water chemistry for the whole duration of the experiments. In Exp 1_(OA) the channels were fed for 17 days with the water collected on 20/01/2015, followed by 14 days with the water collected on 12/02/2015. SD = standard deviation, n= number of samples. Fe(II), Total Fe, As(III), As(V), Total As are concentrations in the dissolved phase.

Code	Experimental conditions	Date of water collection	Experiment duration (days)	pH	T (°C)	EC (mS cm ⁻¹)	Eh (mV)	Fe(II) (mg L ⁻¹)	Total Fe (mg L ⁻¹)	As(III) (mg L ⁻¹)	As(V) (mg L ⁻¹)	Total As (mg L ⁻¹)	SO ₄ ²⁻ (g L ⁻¹)
Exp 1 _(FF)	Water height: 4 mm	20/01/2015	17	Average	3.0	20	3.21	535	439	13	17	30	1.8
	Floating film: Yes			SD	0.1	1	0.06	39	30	4	6	9	0.1
	Air bubbling: No			SD (%)	2	3	2	7	7	32	36	31	5
				n	20	19	8	8	20	8	8	8	8
Exp 1 _(OA)	Water height: 4 mm	20/01/2015 + 12/02/2015	34	Average	3.1	19.7	3.0	547	432	19	12	31	1.8
	Floating film: No			SD	0.2	0.5	0.2	34	29	8	7	7	0.1
	Air bubbling: No			SD (%)	5	3	7	6	7	40	54	22	4
				n	27	25	15	15	27	15	15	15	15
Exp 2 _(4 mm)	Water height: 4 mm	23/03/2015	35	Average	3.4	20.0	2.89	499	480	30	6	36	1.9
	Floating film: No			SD	0.3	0.8	0.06	45	25	10	2	10	0.1
	Air bubbling: No			SD (%)	9	4	2	9	5	34	42	29	6
				n	35	35	22	23	35	18	18	18	15
Exp 2 _(25 mm)	Water height: 4 mm (22 days) + 25 mm (13 days)	23/03/2015	35	Average	3.4	19.8	2.89	494	476	33	6	39	1.9
	Floating film: No			SD	0.3	0.8	0.06	46	29	12	2	11	0.1
	Air bubbling: No			SD (%)	10	4	2	9	6	37	40	30	3
				n	28	28	16	16	28	11	11	11	8
Exp 2 _(25 mm, Air)	Water height: 4 mm (22 days) + 25 mm (13 days)	23/03/2015	35	Average	3.4	19.8	2.87	492	472	33	5	38	1.9
	Floating film: No			SD	0.3	0.8	0.07	48	29	12	2	12	0.1
	Air bubbling: last 6 days			SD (%)	10	4	2	10	6	37	41	31	4
				n	28	28	16	16	28	11	11	11	8

Table 2. Average Fe(II) oxidation rate, total Fe precipitation rate, and total As, As(III) and As(V) removal rates calculated within the range of residence times 20-500 minutes during kinetic studies.

	Fe(II) oxidation rate (mol L ⁻¹ s ⁻¹)	Total Fe precipitation rate (mol L ⁻¹ s ⁻¹)	Total As removal rate (mol L ⁻¹ s ⁻¹)	As(III) removal rate (mol L ⁻¹ s ⁻¹)	As(V) removal rate (mol L ⁻¹ s ⁻¹)
Exp 1 (FF)	3 ± 2 x 10 ⁻⁷	1.2 ± 0.9 x 10 ⁻⁷	5 ± 4 x 10 ⁻⁸	8 ± 6 x 10 ⁻⁹	5 ± 4 x 10 ⁻⁸
Exp 1 (OA)	4.4 ± 0.6 x 10 ⁻⁷	2.4 ± 0.5 x 10 ⁻⁷	5 ± 2 x 10 ⁻⁸	3 ± 2 x 10 ⁻⁸	1.2 ± 0.9 x 10 ⁻⁸
Exp 2 (4 mm)	2.7 ± 0.5 x 10 ⁻⁷	1.3 ± 0.2 x 10 ⁻⁷	2 ± 2 x 10 ⁻⁸	2 ± 1 x 10 ⁻⁸	7 ± 6 x 10 ⁻⁹
Exp 2 (25 mm)	4 ± 3 x 10 ⁻⁸	1.8 ± 0.9 x 10 ⁻⁸	5 ± 3 x 10 ⁻⁹	2 ± 2 x 10 ⁻⁹	3 ± 2 x 10 ⁻⁹

Table 3. Characterization of the biogenic precipitates recovered from the channel bottom at the end of experiments and of the floating film from Exp 1_(FF). SD = standard deviation, n= number of samples, Schwert. = Schwertmannite. Exp 1_(FF), Exp 1_(OA), and Exp 2_(4 mm) have two experiment replicates (Referred as channel 1 and channel 2 in figures in the SI). Exp 2_(25 mm, Air) have been conducted in a single channel. Results from As K-edge XANES (Resongles et al., 2016), As K-edge EXAFS and Fe K-edge EXAFS are normalized to 100%. Non-normalized results are reported in Table SI-2 and SI-3, together with uncertainties on the proportion of each component.

Flow cytometry			qPCR	Acid Digestion		XRD	As K-edge XANES		As bearing phases (As K-edge EXAFS)		Fe bearing phases (Fe K-edge EXAFS)		
	Cells g ⁻¹ (dry wt.)	<i>aiiA</i> /16S rRNA gene ratio	Total As % (dry wt.)	Total Fe % (dry wt.)	As/Fe molar ratio	Mineralogy	As(III) %	As(V) %	Schwert.-As (III) %	Schwert.-As(V) %	Amorphous ferric arsenate %	Schwert. %	Amorphous ferric arsenate %
Exp 1 (FF) Biogenic precipitate	Average	8 x 10 ⁶	8	35	0.18	Schwert.	16	84	16	22	62	67	33
	SD	2 x 10 ^{6 a}	1	0	0.01		2 ^c	2 ^c	2 ^c	7 ^c	65 ^c	13 ^c	5 ^c
Floating film	6	5	2	2	2	2	1	1	1	1	1	1	1
	Average	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27	22	51	78	22
Exp 1 (OA) Biogenic precipitate	SD	n	n	n	n	n	3 ^c	10 ^c	3 ^c	10 ^c	9 ^c	12 ^c	5 ^c
	n	1	1	1	1	1	1	1	1	1	1	1	1
Exp 2 (4 mm) Biogenic precipitate	Average	3 x 10 ⁶	8	34	0.18	Schwert.	23	77	23	38	39	77	23
	SD	1 x 10 ^{6 a}	1	0	0.00		2	2	3	17	20	7	3
Exp 2 (25 mm) Biogenic precipitate	6	6	2	2	2	2	2	2	2	2	2	2	2
	Average	9 x 10 ⁶	6.3	36	0.14	Schwert.	52	48	51	18	31	85	15
SD	5 x 10 ^{6 a}	0.4	0	0.00	3		3	4	4	0	0	11 ^c	3 ^c
Exp 2 (25 mm) Biogenic precipitate	n	6	2	2	2	2	2	2	2	2	2	1	1
	Average	4.4 x 10 ⁶	8	35	0.20	Schwert.	31	69	29	16	55	68	32
SD	0.6 x 10 ^{6 a}	1 ^b	0 ^b	0.00 ^b	2 ^c		2 ^c	2 ^c	2 ^c	2 ^c	7 ^c	5 ^c	3 ^c
Exp 2 (25 mm, Air) Biogenic precipitate	3	1	1	1	1	1	1	1	1	1	1	1	1
	Average	3 x 10 ⁶	8	36	0.16	Schwert.	46	54	44	20	36	81	19
SD	1 x 10 ^{6 a}	1 ^b	0 ^b	0.00 ^b	2 ^c		2 ^c	2 ^c	2 ^c	7 ^c	6 ^c	11 ^c	3 ^c
Biogenic precipitate	n	3	1	1	1	1	1	1	1	1	1	1	1
	SD	n	n	n	n	n	n	n	n	n	n	n	n

n.d.= not determined

a. calculated from DNA extraction replicates and from experimental replicates

b. analytical uncertainty

c. SD of the LCF obtained with the Athena Software, multiplied by a factor of 3 (see SI-Experimental part for details)

Other SD values were calculated from experimental replicates

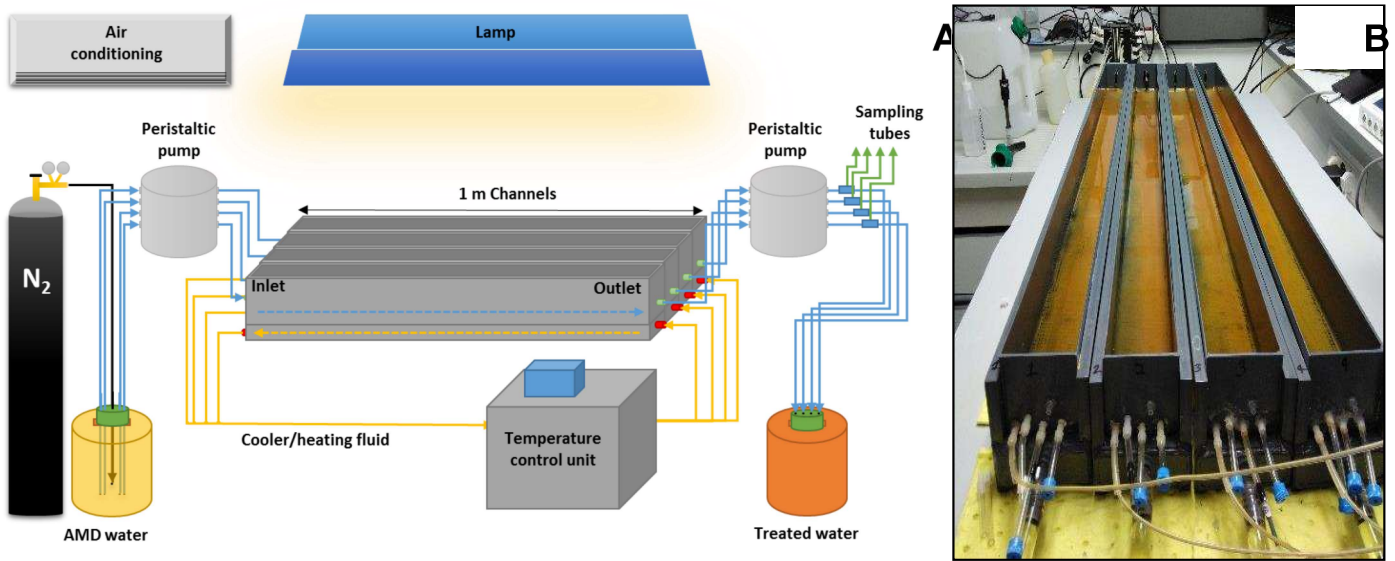


Figure 1. Schematic representation of the continuous flow bioreactor simulating natural attenuation (A). The four channels fed with AMD and covered with the Fe-As biogenic precipitates at the bottom (B).

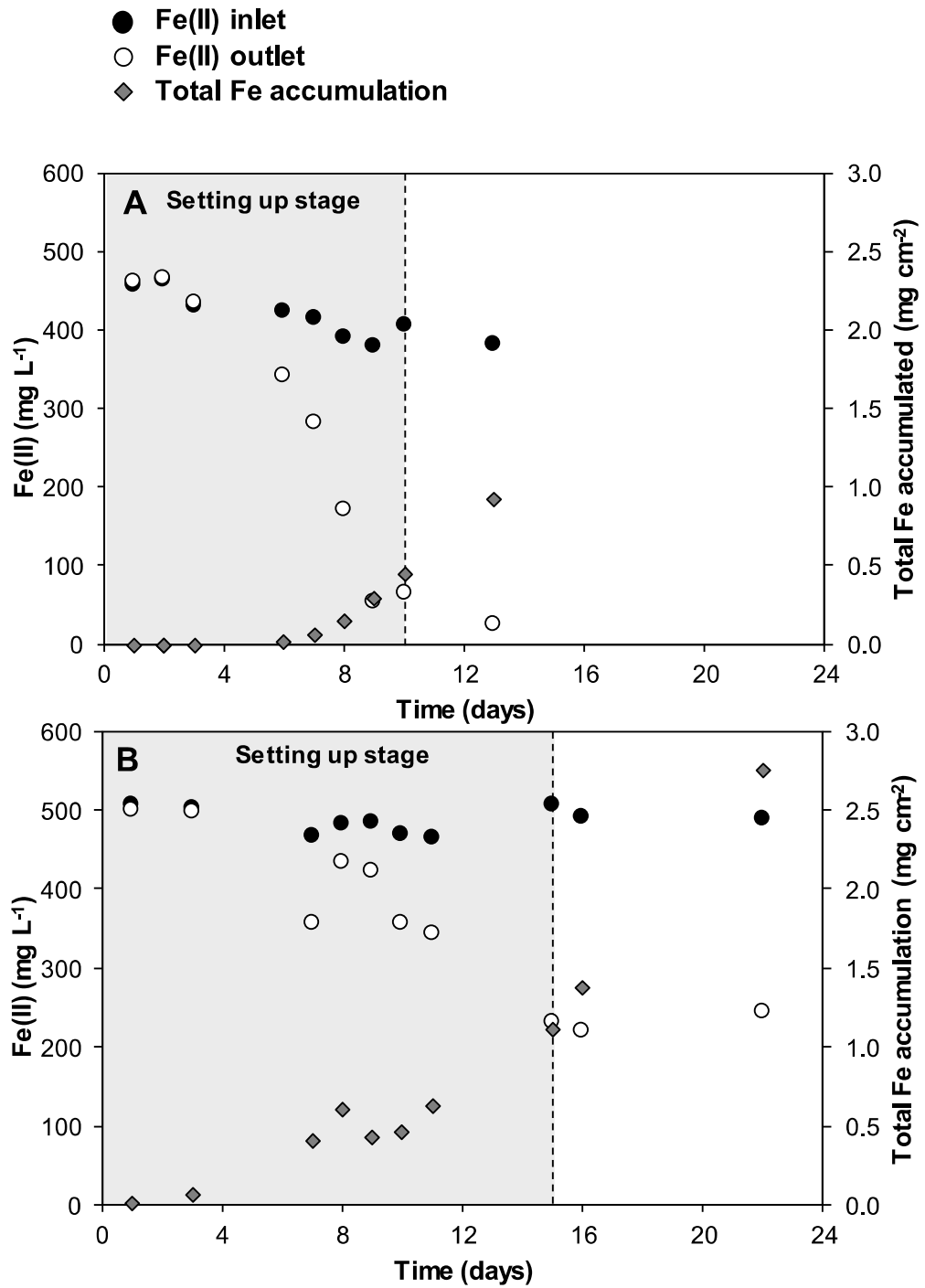


Figure 2. Dissolved Fe(II) concentration in the inlet and outlet waters and cumulated precipitated Fe (expressed as mass of Fe precipitated per surface area of the channel bottom) during setting up stage of experiments (A) Exp 1 and (B) Exp 2. Dashed line represents the period after which the steady-state was reached regarding outlet Fe(II) concentration : ~10 days in Exp 1 and ~15 days in Exp 2.

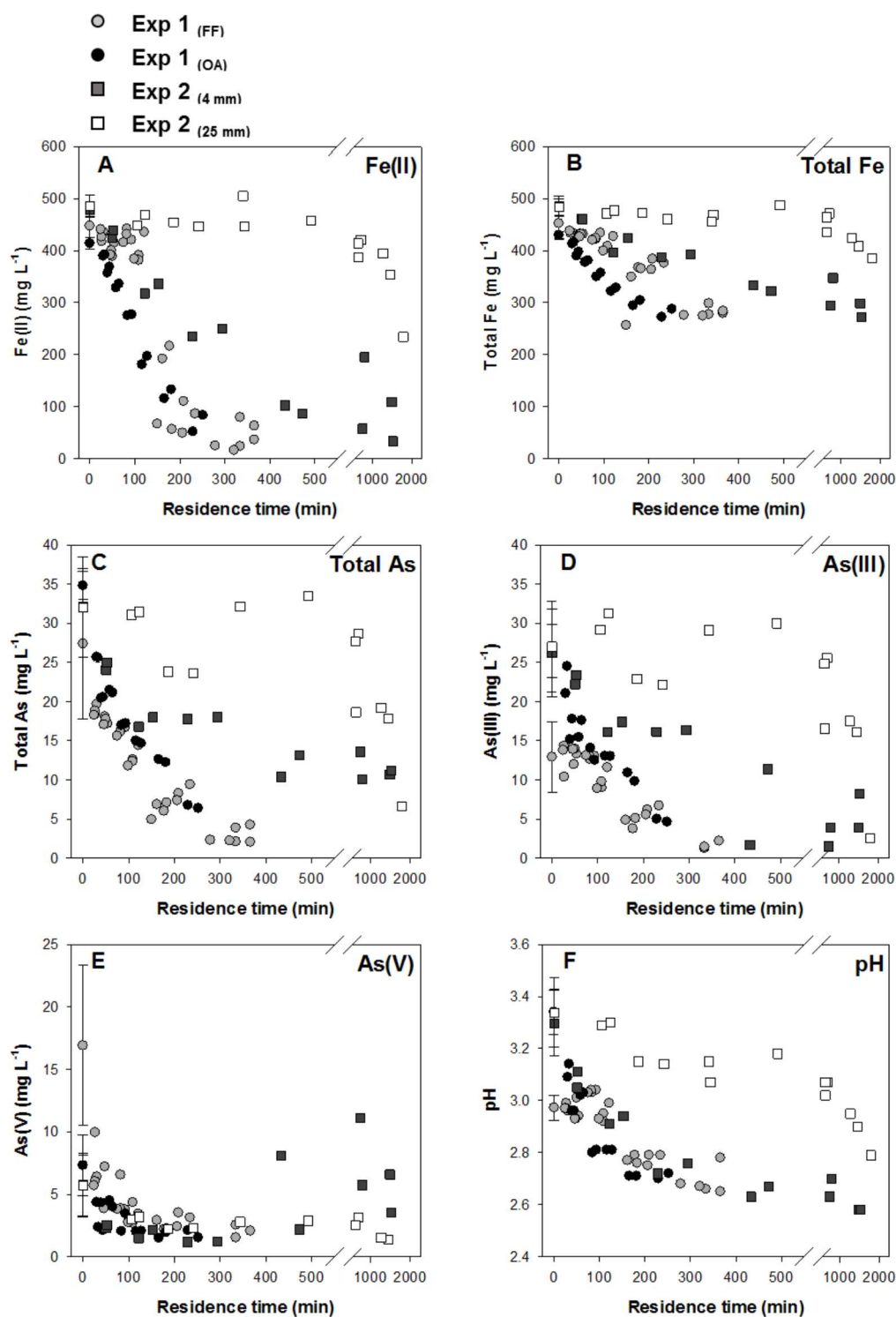


Figure 3. Water composition at the outlet of the flow reactor as a function of residence time during kinetic studies. Variation of dissolved Fe(II) (A), total dissolved Fe (B), total dissolved As (C), dissolved As(III) (D), dissolved As(V) (E) and pH (F) as a function of residence time during kinetic studies, carried out once steady-state condition was reached. Exp 1_(FF) (four replicate channels), Exp 1_(OA) (two replicate channels), and Exp 2 (Exp 2_(4 mm) and Exp 2_(25 mm), two replicate channels for each water height) were carried out with water from the Carnoulès AMD collected on 20th January, 12th February, and 23th March respectively. The corresponding values of Fe(II) oxidation rate, Fe and As

precipitation rates, As(III) and As(V) removal rates within the range of residence times 30 – 500 minutes are stated in Table 2.

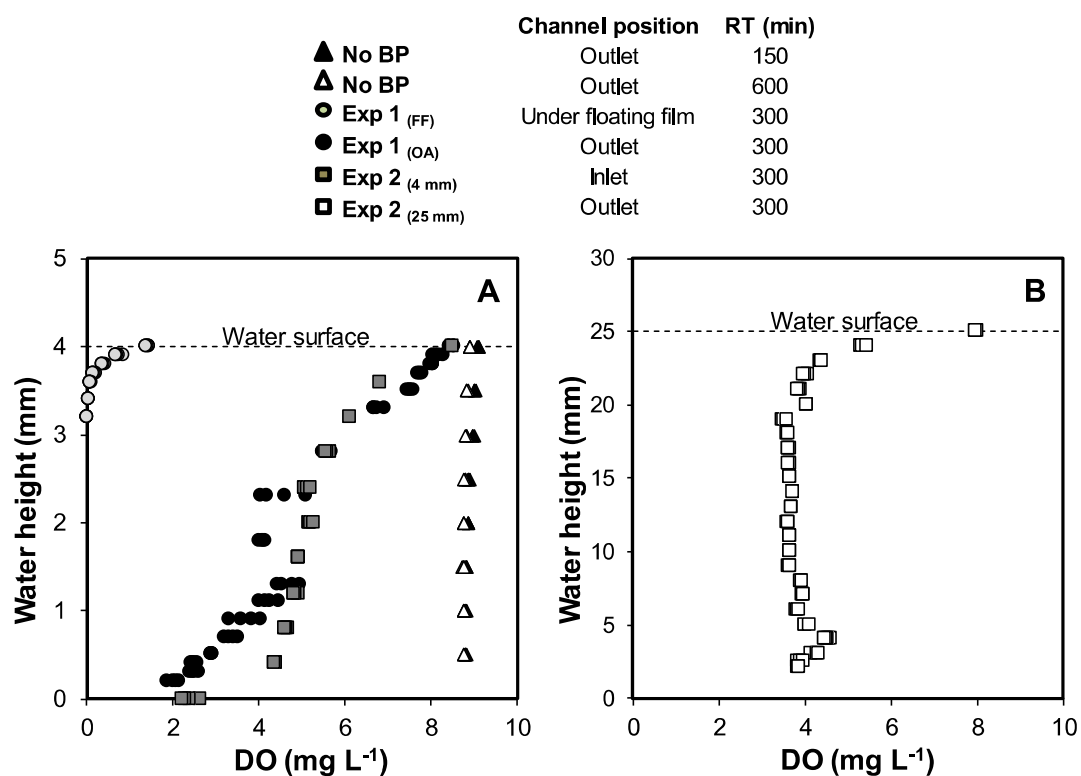


Figure 4. DO concentration profiles recorded in experiments conducted at 4 mm (A) and at 25 mm (B). BP = biogenic precipitate.

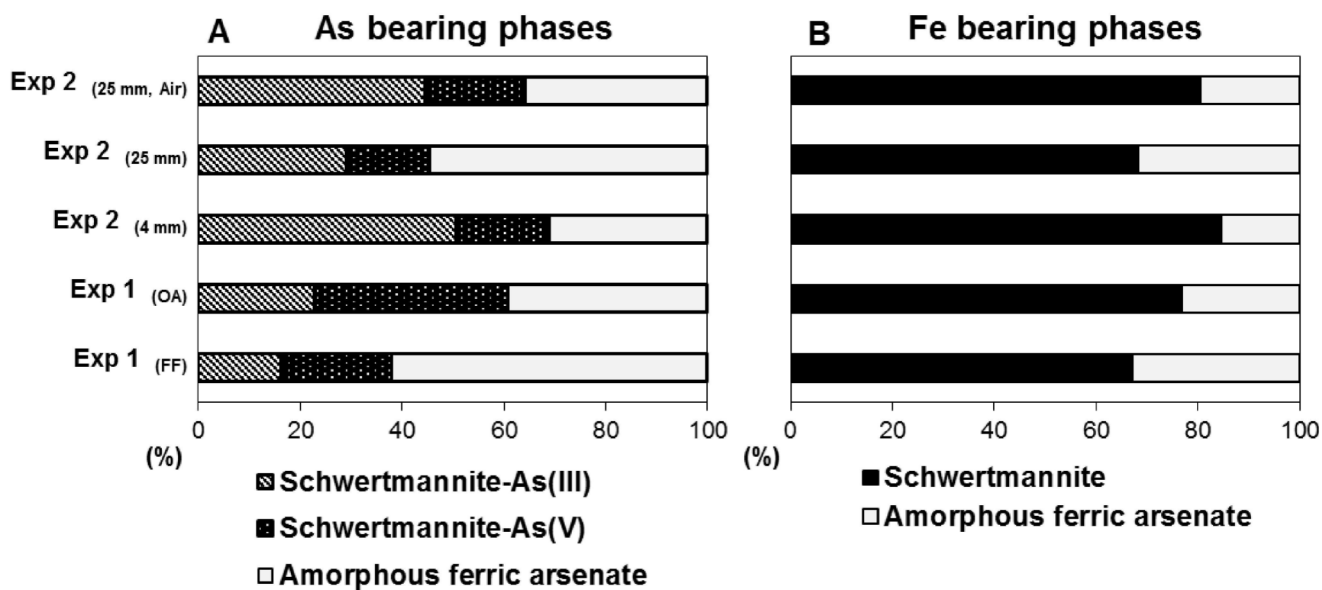


Figure 5. Arsenic speciation and mineralogy in the biogenic precipitates determined by LCF analysis of As (A) and Fe K-edge (B) EXAFS data, respectively. Corresponding spectra are displayed in Figures SI-3 and SI-4, respectively. LCF results are reported in Tables SI-2 and SI-3, respectively.

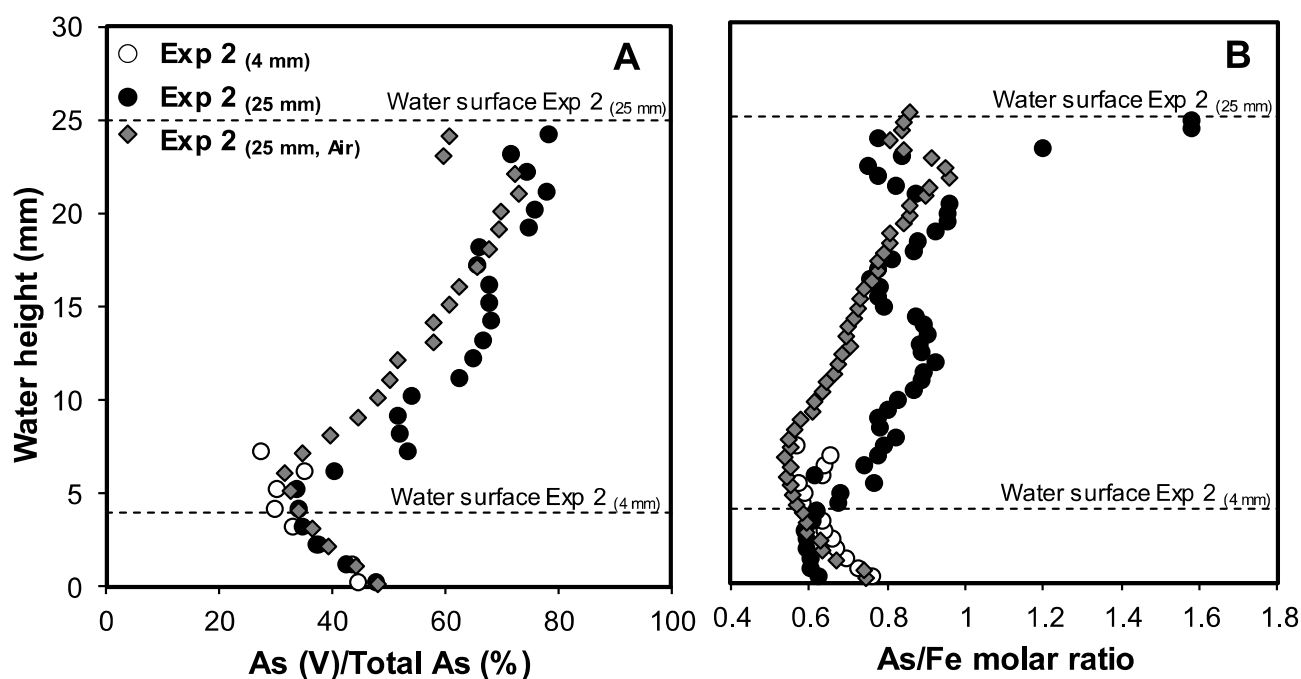


Figure 6. Normalized proportion of As(V)/Total As obtained by micro-XANES (A) and molar ratio of As/Fe obtained by and micro-XRF (B) in the precipitate deposited on the plastic film inserted vertically in the water column, in Exp 2 (4 mm)-channel 2, Exp 2 (25 mm) and Exp 2 (25 mm, Air).

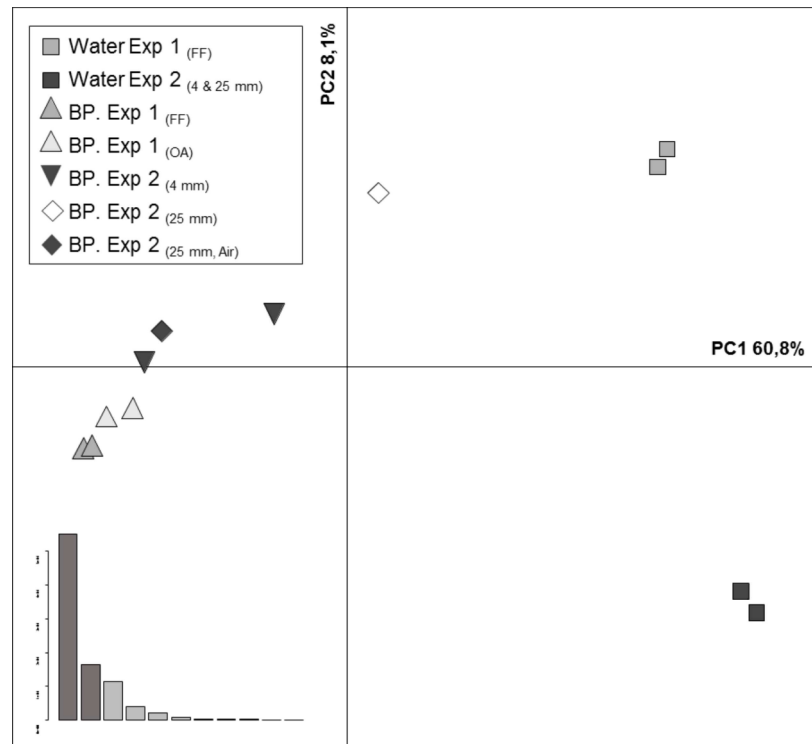


Figure 7. Principal component analysis plot generated from ARISA profiles obtained from the bacterial communities of water used in Exp 1_(FF) and in Exp 2 (4 mm & 25 mm), and those of the biogenic precipitates (BP) formed at the bottom of the channels recovered at the end of the experiments: Exp 1_(FF), Exp 1_(OA), Exp 2 (4 mm), Exp 2 (25 mm), and Exp 2 (25 mm, Air). Legend is shown in the upper left corner. Percentages of variance of all axes are shown in the lower left corner. The first component (PC1) represented 60.8 % of the bacterial community variability and indicates that the bacterial community in the biogenic precipitates differed widely from the bacterial community in the corresponding feed water. The second component (PC2) represented 8.1 %, of the bacterial community variability and highlights the variability between the feed waters.

SUPPORTING INFORMATION FILE

Biological attenuation of arsenic and iron in Acid Mine Drainage (AMD) in a continuous flow reactor

Fernandez-Rojo L.¹, Héry M.¹, Le Pape P.², Braungardt C.^{3,1}, Desoeuvre A.¹, Torres E.¹, Tardy V.¹, Resongles E.¹, Laroche E.¹, Delpoux S.¹, Jouliau C.⁴, Battaglia-Brunet F.⁴, Boisson J.⁵, Grapin G.⁶, Morin G.², Casiot C.¹

¹ Hydrosiences Montpellier, UMR 5569 CNRS-IRD-UM, CC57, 163, rue Auguste Broussonet, 34090, Montpellier, France

² Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), UMR 7590 CNRS-UPMC-IRD-MNHN, 4 place Jussieu, 75252 Paris cedex 05, France

³ School of Geography, Earth and Environmental Sciences (Faculty of Science & Engineering), Plymouth University, United Kingdom

⁴ French Geological survey (BRGM), 3 avenue Claude Guillemin, 45060, BP 36009, Orléans Cedex, France

⁵ IRH Ingénieur Conseil, 197 avenue de Fronton, 31200, Toulouse, France

⁶ IRH Ingénieur Conseil, 427 rue Lavoisier - CS 50155, 54714, Ludres Cedex, France

1. SI-EXPERIMENTAL PART

2. Chemical analysis

2.1 Water chemistry analysis

Each water sample collected in the bioreactor was divided in two sub-samples. In one sub-sample, pH, conductivity and redox potential were determined with a multiparameter analyzer (Ultrameter™ Model 6P) equipped with the specific electrodes. DO and temperature were measured directly within the channel. An oxygen microoptode (50 μm tip diameter, Unisense, DL = 0.01 mg L^{-1}), coupled to a microoptode-meter and fixed in a micromanipulator, was used to record dissolved oxygen depth profiles in the water column. The second sub-sample was immediately filtered using syringes (20 mL, Low Density Polyethylene, Codan) and disposable syringe filters (cellulose acetate, pore size 0.2 μm). The filtrate was distributed between four polypropylene tubes of 10 mL and preserved according to the procedures adapted to each analyte of interest (Fe(II), sulphate, total Fe and As, and As speciation) (Casiot *et al.*, 2003; Héry *et al.*, 2014; Resongles *et al.*, 2014), as described below.

All the reagents used were Merck Suprapur® quality. For total Fe and As analyses, a 2.5 mL aliquot was acidified with HNO_3 (Suprapur) to pH 1 and stored at 4 °C. For Fe(II) determination, a 50 μL aliquot was buffered to pH 4.5 with 0.5 mL of ammonium acetate/acetic acid buffer and the Fe (II) was complexed with 1 mL of 0.5 % (w/w) 1,10-phenantroline chloride solution (Rodier *et al.*, 1996). The tube was completed with 8.45 mL of double deionized water (DDW), and stored at 4 °C in the dark. For sulphate determination, a 100 μL aliquot was stabilized with 200 μL of HCl 10 % (v/v). Two millimetres of BaCl_2 solution were added and made up to 10 mL with DDW. For As speciation, 25 μL of EDTA (0.13 M) and 25 μL of acetic acid (8.7 M) were added to 2.5 mL sample and stored at 4 °C until analysis within less than 8 weeks.

For each analysis, the detection limit was calculated by multiplying by three the standard deviation of the blank, and the uncertainty was checked by repeating the measure of the Certified Reference Material (CRM) ten times and by calculating the relative standard deviation (RSD). Analysis of total Fe and As in water were carried out by inductively coupled plasma-mass spectrometer (ICP-MS; iCAP™ Q, Thermo Scientific, As detection limit = 0.03 $\mu\text{g L}^{-1}$, Fe detection limit = 0.3 $\mu\text{g L}^{-1}$, uncertainty = ± 1 %), after preparing the appropriate dilutions. The certified reference water SLRS-5 (CNRC Canada) was used to check the analytical accuracy and the RSD was always lower than 5 % with respect to the certified value. Spectrophotometric methods (SECOMAN S250) were used to determine Fe(II) (wave length (λ) = 510 nm, detection limit = 88 $\mu\text{g L}^{-1}$, uncertainty = ± 5 %) and sulfate (λ = 650 nm, detection limit = 1000 $\mu\text{g L}^{-1}$, uncertainty = ± 5 %). Fe and SO_4^{2-} standards (1000 mg L^{-1} , SCP Science), respectively, were used for calibrations. Samples were diluted for As speciation analysis and analysed using high performance liquid chromatography (HPLC)-ICP-MS with an anion exchange column (25 cm \times 4.1 mm i.d. Hamilton PRP-X100) coupled to the ICP-MS (XSERIES II, Thermo Scientific, detection limit = 0.2 $\mu\text{g L}^{-1}$ for As(III) and 0.4 $\mu\text{g L}^{-1}$ for As(V), uncertainty = ± 5 %). The certified reference water NIST1643e was used to check analytical accuracy for total As concentration and the RSD was always lower than 5 % with respect to the certified value.

2.2 *Sediment acid digestion*

All reagents were of analytical grade and all material was decontaminated with HNO₃ (10 %) before starting. Solid samples were dried under vacuum at ambient temperature. One hundred milligrams of dried sample were introduced in 30 mL Teflon reactors placed on hot-plates. Samples were first attacked for 24 h with 3 mL H₂O₂ (30 % w/w) and then with aqua regia (3 mL HCl (35 % w/w), 1 mL HNO₃ (67 % w/w)) for 24 h more. After each attack, the solution was evaporated until the sample was dry. Subsequently, trace elements were dissolved in 3 mL HNO₃ (67 % w/w) and 27 mL of DDW. If necessary, appropriate dilutions with HNO₃ (2% (v/v)) were carried out before analysis with ICP-MS (same procedure as described above in “water chemistry analysis”). A certified reference material (Stream sediments LGC6189 from United Kingdom Accreditation Service and NCS DC70317 from LGC Standards, number of extractions = 3) was used to assess the extraction quality with recoveries of 108 ± 5 % for As (Fe concentration not referenced). Two blanks were prepared in each digestion and its concentration represented always less than 1 % with respect to Fe and As concentrations from samples.

2.3 *Arsenic mass balance*

At the end of each experiment, a mass balance was calculated in an attempt to determine in a qualitative way whether As(III) oxidation took place in the channels during the whole experiment. The trapezoidal rule was applied to calculate the defined integrals of inlet and outlet As(III), As(V) and total As load (mg s^{-1}) over the duration of the experiment. The difference between inlet and outlet load provided the masses of As(III) and As(V) removed from the dissolved phase during the whole experiment, which were compared with the masses of As(III) and As(V) determined in the precipitates at the end of the experiments.

3. Mineralogical analysis

Before mineralogical analysis, the aliquots of the biogenic precipitate samples recovered in the channels were deposited on silicon single-crystal low-background sample holders and dried under anoxic atmosphere in a Jacomex[®] anaerobic chamber filled with argon to avoid As(III) oxidation.

3.1 X-Ray diffraction (XRD)

XRD measurements were performed using the Co K α radiation of a Panalytical®X'Pert Pro diffractometer. Data were then collected in continuous mode between 5 and 100°2 θ with a 0.033°2 θ step, counting around 4 h per sample.

3.2 Extended X-ray absorption fine structure (EXAFS) spectroscopy

Both, As and Fe K-edge EXAFS spectra for the biogenic precipitate samples recovered in the channels of the experimental bioreactor system were recorded at 80 K in transmission mode on the XAFS beamline (ELETTRA, Trieste, Italy) using a Si(111) double-crystal monochromator. To preserve the As redox, status samples were mounted on the cryostat sample rod within a glovebox next to the beamline and quickly transferred into the cryostat. The incident beam energy was calibrated by setting to 11947 eV the energy position of the absorption maximum in the L_{III}-edge of an Au foil recorded in double-transmission for As, and by setting to 7112 eV the energy position of the absorption maximum in the K-edge of a Fe(0) foil for Fe. Up to two scans were recorded for each sample depending on As and Fe concentrations, i.e. on signal to noise ratio.

Scans were averaged, normalized and background subtracted over the 0–15 Å⁻¹ k-range for As and over the 0–14 Å⁻¹ k-range for Fe using the Athena Software (Ravel and Newville, 2005). Linear combination fitting (LCF) of EXAFS data was performed on k³-weighted curves over the 3–15 Å⁻¹ k-range for As and the 2–14 Å⁻¹ k-range for Fe.

EXAFS data at both the As and Fe K-edges were interpreted by comparison with a large set of iron arsenic hydroxysulfate amorphous and nano-crystalline phases already analysed in detail by Maillot *et al.* (2013). These model compounds includes biotic and abiotic sorption and coprecipitation samples for As(III) and As(V) with various As/Fe molar ratios. Experimental spectra of three of these compounds, namely: As(III)-coprecipitated schwertmannite with As/Fe = 0.2 mol/mol (As3_02), As(V)-sorbed schwertmannite with As/Fe = 0.01 mol/mol (As5_ads) and Amorphous Ferric Arsenate with As/Fe = 0.8 mol/mol (As5_0.8) were used as fitting components for LCF analysis of the As K-edge EXAFS spectra of the biogenic precipitates. Indeed, these compounds reliably represent the variety of local structures encountered in such AMD precipitates and that can be distinguished by EXAFS at the As K-edge (Maillot *et al.* 2013). In the same way, As-free schwertmannite (As Free), As(III)-coprecipitated schwertmannite with As/Fe = 0.2 mol/mol (As3_02), and amorphous ferric arsenate with As/Fe = 0.8 mol/mol (As5_0.8) were used as fitting components for LCF analysis of the Fe K-edge EXAFS spectra.

3.3 Spatially resolved X-ray absorption near fine structure (XANES) measurements on plastic films

As K-edge XANES spectra on mineralized plastic films recovered in the channels of the experimental bioreactor system were collected at 15 K in fluorescence mode on the SAMBA beamline (SOLEIL, Saint-Aubin, France) using a Si(220) double-crystal monochromator and a 30 element Ge detector. XANES measurements were collected from the bottom to the top of the plastic films with a 1 mm step with a sample holder specially designed for this purpose. Beam size for the XANES measurements was approximately 0.25 mm vertically and 0.5 mm horizontally.

XANES linear combination fits (LCF) using As(III) and As(V) model compounds were performed using a in-house program based on a Levenberg–Marquardt algorithm. Model compounds used to fit the experimental spectra were As(III) and As(V) coprecipitated schwertmannites. Details about those compounds and data processing procedures can be found in Resongles *et al.* (2016).

3.4 Micro-X-ray fluorescence on plastic films

Micro X-ray fluorescence measurements were performed using a RIGAKU® Mo K α rotating anode source collimated to $50 \times 70 \mu\text{m}^2$. For this experiment, the vertical step size was approximately 0.1 mm, and measurements were performed with steps of 0.5 mm from the bottom to the top of the plastic films.

After obtaining the X-ray emission spectra, As/Fe molar ratio for each point on the plastic films was calculated using a homemade program that calculates and divides As area measured at the As K α emission line by the Fe area measured at the Fe K α emission line. Then the area ratio is weighted by the ratio of the absorption factors corresponding to these emission lines.

4. Microbiological analysis

4.1 *Bacterial quantification by flow cytometry*

4.1.1 Bacterial quantification in water

Bacterial cells were pelleted by centrifugation (15 min at 6000 g, VWR® Mini Centrifuge) and resuspended into 50 µL of the same water. Bacterial cells were then stained (LIVE/DEAD® BacLigh Bacterial Viability Kits, Life Technologies) according to the manufacturer recommendations. Stained cells were counted automatically using a Gallios™ flow cytometer (Beckman Coulter) and data were analyzed with the Gallios software. More than 20,000 analytical events were counted, in triplicate, for each sample.

4.1.2 Bacterial quantification in the biogenic precipitate

Bacteria from recovered biogenic precipitates were detached from their particle-association according to the procedure described by Lunau *et al.* (2005) with some modifications. Briefly, the biogenic precipitate was sonicated (15 min, Transsonic 275 Prolabo) with 10 % methanol and centrifuged (1 min at 190 g, VWR® Mini Centrifuge) to remove detrital and inorganic particles. The supernatant containing suspended bacterial cells was centrifuged (15 min at 6000 g) and the resulting pellet was washed with 1 mL of sterile water, centrifuged again (15 min at 6000 g) and the supernatant discarded. Bacteria were stained and directly counted as described in the previous section “bacterial quantification in water”.

4.2 Bacterial community structure analysis by automated ribosomal intergenic spacer analysis (ARISA)

4.2.1 Bacterial community structure analysis in water

Total DNA was extracted from two of the three filters using the Power water® DNA Isolation kit (Mobio Laboratories) according to the manufacturer recommendations. The bacterial ribosomal intergenic regions were amplified by polymerase chain reaction (PCR) from the DNA extracts using primers ITSF and ITSr according to Cardinale *et al.* (2004). ARISA was performed on an Agilent 2100 Bioanalyzer using the 7500 DNA kit (Agilent Technologies). Principal component analysis (PCA) was performed from the ARISA profiles with the R free software (<http://www.r-project.org/>) and ade4 package.

4.2.2 Bacterial community structure analysis in the biogenic precipitate

Because sample acidity can interfere with the DNA extraction protocol, aliquots of the biogenic precipitate recovered from the bottom of the channels (~300 mg) were washed with 1 mL Tris-HCl (1 M, pH 8) to increase the pH of the sample. Then DNA was extracted using an UltraClean Soil DNA Isolation Kit (MOBIO Laboratories), according to the manufacturer's instructions. Up to three DNA replicates extractions were made on one sample. The bacterial community structures (ARISA profiles) were determined as described above in "bacterial community analysis in water".

4.3 16S rRNA and *aioA* genes quantification in the biogenic precipitate

The abundance of *aioA* genes, encoding the large catalytic subunit of the As(III) oxidase, was determined by quantitative *real-time* PCR (qPCR) in each DNA replicate extract of samples recovered from the biogenic precipitates (DNA extraction protocol described in “bacterial community structure analysis in the biogenic precipitate”). The reverse primer aoxBM2-1R (5'-GGAGTTGTAGGCGGGCCKRTRTGDAT-3') (Quéméneur *et al.*, 2010) was combined with the forward primer aoxBM4-1F (5'-TTCTGCATCGTGGGCTGYGGNTAYMA-3') to target a 110 bp fragment of the *aioA* gene (Quéméneur, 2008). Each primer was used at a concentration of 0.3 μ M, with 100 ng of T4GP32 (MP Biomedicals), in 1X IQ SYBR Green Supermix (BioRad) and a final volume of 20 μ L. Two microliters of DNA, diluted at 1 ng μ L⁻¹ when appropriate, were used as template. The program was run in a CFX Connect (BioRad) and consisted in an initial denaturation at 95 °C for 3 min, followed by 45 cycles of 95 °C for 10 s, 54 °C for 20 s, 72 °C for 10 s, and a data acquisition step at 80 °C for 10 s. At the end, a melting curve analysis was performed through measurement of the SYBR Green I signal intensities during a 0.5 °C temperature increment every 10 s, from 65 °C to 95 °C. Negative controls received 2 μ L of ultra-pure water (MP Biomedicals) instead of DNA. Total *Bacteria* abundance was measured by qPCR on the 16S rRNA gene using universal bacterial primers 341F (5'-CCTACGGGAGGCAGCAG-3') and 534R (5'-ATTACCGCGGCTGCTGGCA-3'), as previously described (López-Gutiérrez *et al.*, 2004). Six-point serial decimal dilution of a linearized plasmid carrying the target gene (*aioA* or 16S rRNA gene) was used to generate a linear calibration curve of threshold cycle versus a number of gene copies ranging from 10² to 10⁷. All measurements were run in duplicates.

5. TABLES

Table SI-1. Equivalence of samples names in this paper and in Resongles *et al.*, (2016), which includes the methodology and results of the As K-edge XANES data, also presented in this study.

Resongles et al., (2016)	This study
1A-G4	Exp 1 _(FF) - Channel 2
1B-G1	Exp 1 _(OA) - Channel 1
1B-G2	Exp 1 _(OA) - Channel 2
2A-G2	Exp 2 _(4 mm) - Channel 1
2A-G4	Exp 2 _(4 mm) - Channel 2
2A-G3	Exp 2 _(25 mm)
2A-G1	Exp 2 _(25 mm, Air)

Table SI-2. Results of the linear combination fitting (LCF) procedure (non-normalized data) applied to As K-edge EXAFS of the biogenic precipitate and floating film samples. LCF were performed using the Athena Software on the 3–15 Å⁻¹ k-range. The fitted contributions of the As species are reported in percentages, without normalizing the sum to 100%. The model compounds used for the LCF procedure consisted of As(III)-coprecipitated schwertmannite “As3_0.2”, As(V)-sorbed schwertmannite “As5_ads”, and ferric arsenate “As5_0.8” reported by Maillot *et al.* (2013). R-factor (R_f), chi-square (χ^2) and reduced chi-square (Red- χ^2) are given as classical estimators of the goodness of fit. Uncertainties on the contributions are given in parentheses and refer as 3 standard deviation (σ) obtained with the Athena Software. Experimental replicates are referred as Channel 1 and Channel 2.

Sample s	As3_0.2	As5_ads	As5_0.8	Rf	χ^2	Red- χ^2
Exp 1 (FF)- Channel 1	—	—	—	—	—	—
Exp 1 (FF)- Channel 2	17 (2)	23 (7)	65 (6)	0.0038	20.6	0.086
Exp 1 (FF)- Floating film	28 (3)	22 (10)	53 (9)	0.0129	53.8	0.226
Exp 1 (OA)- Channel 1	22 (2)	27 (7)	55 (7)	0.0051	24.5	0.103
Exp 1 (OA)- Channel 2	26 (2)	53 (8)	27 (8)	0.0065	28.8	0.121
Exp 2 (4 mm)- Channel 1	54 (2)	16 (9)	32 (9)	0.0146	36.7	0.158
Exp 2 (4 mm)- Channel 2	49 (2)	22 (7)	32 (6)	0.0076	21.1	0.089
Exp 2 (25 mm)	30 (2)	17 (2)	56 (7)	0.0055	21.9	0.092
Exp 2 (25 mm, Air)	44 (2)	20 (7)	36 (6)	0.0073	20.7	0.087

Table SI-3. Results of the linear combination fitting (LCF) procedure (non-normalized data) applied to Fe K-edge EXAFS of the biogenic precipitate and floating film samples. LCF were performed using the Athena Software on the 2–14 Å⁻¹ k-range. The fitted contributions of the Fe species are reported in percentages, without normalizing the sum to 100%. The model compound spectra used in the LCF procedure correspond to the schwertmannite samples “As free”, “As3_0.6”, and the amorphous ferric arsenate sample “As5_0.8” (Maillot *et al.*, 2013). R-factor (R_f), chi-square (χ^2) and reduced chi-square (Red- χ^2) are given as classical estimators of the goodness of fit. Uncertainties on the contributions are given in parentheses and refer as 3 standard deviation (σ) obtained with the Athena Software. Experimental replicates are referred as Channel 1 and Channel 2.

Samples	As free	As3_0.6	As5_0.8	Rf	χ^2	Red- χ^2
Exp 1 (FF)- Channel 1	—	—	—	—	—	—
Exp 1 (FF)- Channel 2	28 (8)	34 (10)	31 (5)	0.0084	9.8	0.045
Exp 1 (FF)- Floating film	14 (7)	56 (10)	19 (5)	0.0091	9.2	0.042
Exp 1 (OA)- Channel 1	32 (9)	38 (12)	23 (5)	0.0126	14.5	0.067
Exp 1 (OA)- Channel 2	27 (9)	46 (12)	20 (5)	0.0140	15.8	0.073
Exp 2 (4 mm)- Channel 1	—	—	—	—	—	—
Exp 2 (4 mm)- Channel 2	22 (6)	57 (9)	14 (3)	0.0069	7.5	0.035
Exp 2 (25 mm)	0 (0)	64 (5)	30 (3)	0.0081	9.3	0.042
Exp 2 (25 mm, Air)	19 (6)	56 (9)	18 (3)	0.0072	8.0	0.037

Table SI-4. Micro-X-ray fluorescence data for the biogenic precipitate encrusting the plastic film inserted vertically in the water column of in Exp 2 (4 mm)-channel 2, Exp 2 (25 mm) and Exp 2 (25 mm, Air).

Height (mm)**	As/Fe molar ratio*		
	Exp 2 (4mm) - C2	Exp 2 (25 mm)	Exp 2 (25 mm, Air)
0.2	0.76	0.63	0.75
0.7	0.73	0.61	0.74
1.2	0.70	0.61	0.67
1.7	0.68	0.60	0.64
2.2	0.66	0.60	0.63
2.7	0.65	0.59	0.59
3.2	0.64	0.61	0.59
3.7	0.62	0.62	0.58
4.2	0.59	0.68	0.57
4.7	0.59	0.68	0.56
5.2	0.58	0.77	0.55
5.7	0.64	0.62	0.54
6.2	0.64	0.74	0.55
6.7	0.66	0.78	0.54
7.2	0.57	0.80	0.55
7.7	0.59	0.83	0.55
8.2	0.63	0.79	0.56
8.7	0.50	0.78	0.58
9.2	-	0.81	0.61
9.7	-	0.83	0.61
10.2	-	0.87	0.64
10.7	-	0.89	0.65
11.2	-	0.90	0.67
11.7	-	0.93	0.68
12.2	-	0.89	0.68
12.7	-	0.89	0.70
13.2	-	0.91	0.70
13.7	-	0.90	0.70
14.2	-	0.88	0.71
14.7	-	0.80	0.72
15.2	-	0.78	0.73
15.7	-	0.79	0.74
16.2	-	0.76	0.76
16.7	-	0.78	0.78
17.2	-	0.82	0.78
17.7	-	0.87	0.79
18.2	-	0.88	0.81
18.7	-	0.93	0.81
19.2	-	0.96	0.84
19.7	-	0.96	0.86
20.2	-	0.97	0.86
20.7	-	0.88	0.90
21.2	-	0.83	0.91
21.7	-	0.78	0.96
22.2	-	0.76	0.95
22.7	-	0.84	0.91
23.2	-	1.21	0.84
23.7	-	0.78	0.80
24.2	-	1.58	0.84
24.7	-	1.58	0.84
25.2	-	-	0.86

* As/Fe ratio is calculated by dividing the integrated areas under the K α peaks of As and Fe, and taking into account As and Fe fluorescence absorption coefficients

**Height is the height of analysis on the plastic films; 0 stands for the bottom of the channel

Table SI-5. Results of the LCF procedure (non-normalized data) applied to micro-XANES. Reduced χ^2 is given as an estimator of the goodness of fit.

Height (mm)*	Exp 2 (4 mm)- Channel 2 - XANES LCF			Exp 2 (25 mm) - XANES LCF			Exp 2 (25 mm, Air) - XANES LCF		
	As(V) \pm 3 %	As(III) \pm 3 %	Reduced χ^2 ($\times 10^{-2}$)	As(V) \pm 3 %	As(III) \pm 3 %	Reduced χ^2 ($\times 10^{-2}$)	As(V) \pm 3 %	As(III) \pm 3 %	Reduced χ^2 ($\times 10^{-2}$)
0.1	45	56	1.46	50	54	1.33	50	53	1.35
1.1	44	57	1.42	44	59	1.48	46	58	1.46
2.1	38	62	1.42	39	64	1.40	41	63	1.36
3.1	34	67	1.29	36	67	1.43	38	66	1.28
4.1	30	71	1.23	35	68	1.18	36	69	1.38
5.1	31	71	1.22	35	68	1.21	34	71	1.31
6.1	36	65	1.27	42	61	1.30	33	72	1.25
7.1	28	73	1.16	56	48	1.19	36	68	1.31
8.1	24	78	1.20	54	49	1.18	41	63	1.47
9.1	37	65	3.68	54	50	1.14	46	57	1.51
10.1	-	-	-	56	47	1.20	49	53	1.39
11.1	19	81	1.60	65	39	1.03	52	51	1.39
12.1	31	70	1.31	68	36	1.09	53	50	1.34
13.1	-	-	-	70	34	1.17	59	43	1.30
14.1	-	-	-	71	33	2.42	59	43	1.39
15.1	-	-	-	71	33	0.99	62	40	1.32
16.1	-	-	-	71	33	9.35	63	38	1.30
17.1	-	-	-	68	36	1.03	66	35	1.30
18.1	-	-	-	69	35	0.94	68	33	1.01
19.1	-	-	-	78	26	0.82	70	31	1.01
20.1	-	-	-	79	25	0.96	70	30	1.41
21.1	-	-	-	82	23	0.89	73	27	1.37
22.1	-	-	-	78	26	0.94	72	28	1.70
23.1	-	-	-	75	29	1.05	60	41	4.11
24.1	-	-	-	82	22	0.90	61	39	3.15

* Height is the height of analysis on the plastic film; 0 stands for the bottom of the channel

6. FIGURES

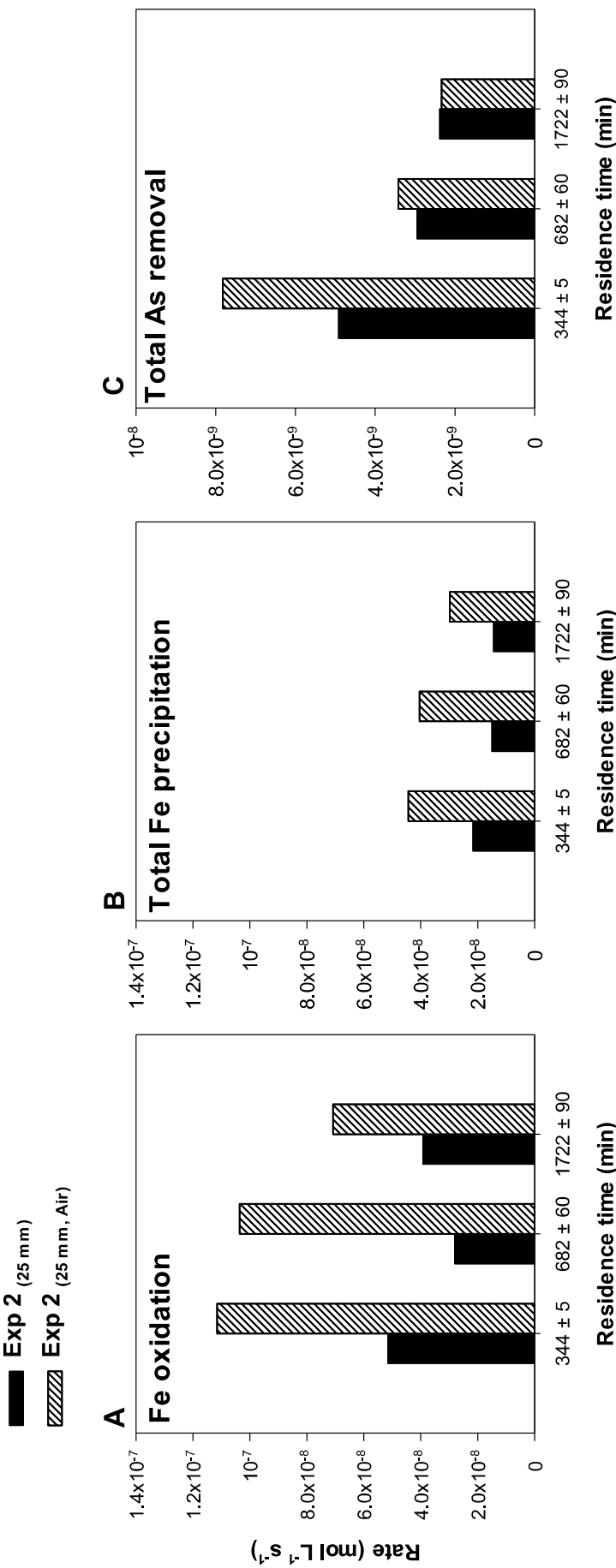


Figure SI-1. Fe oxidation (A), total Fe precipitation (B) and total As removal (C) in Exp 2 (25 mm) and Exp 2 (25 mm, Air) determined at three different residence times.

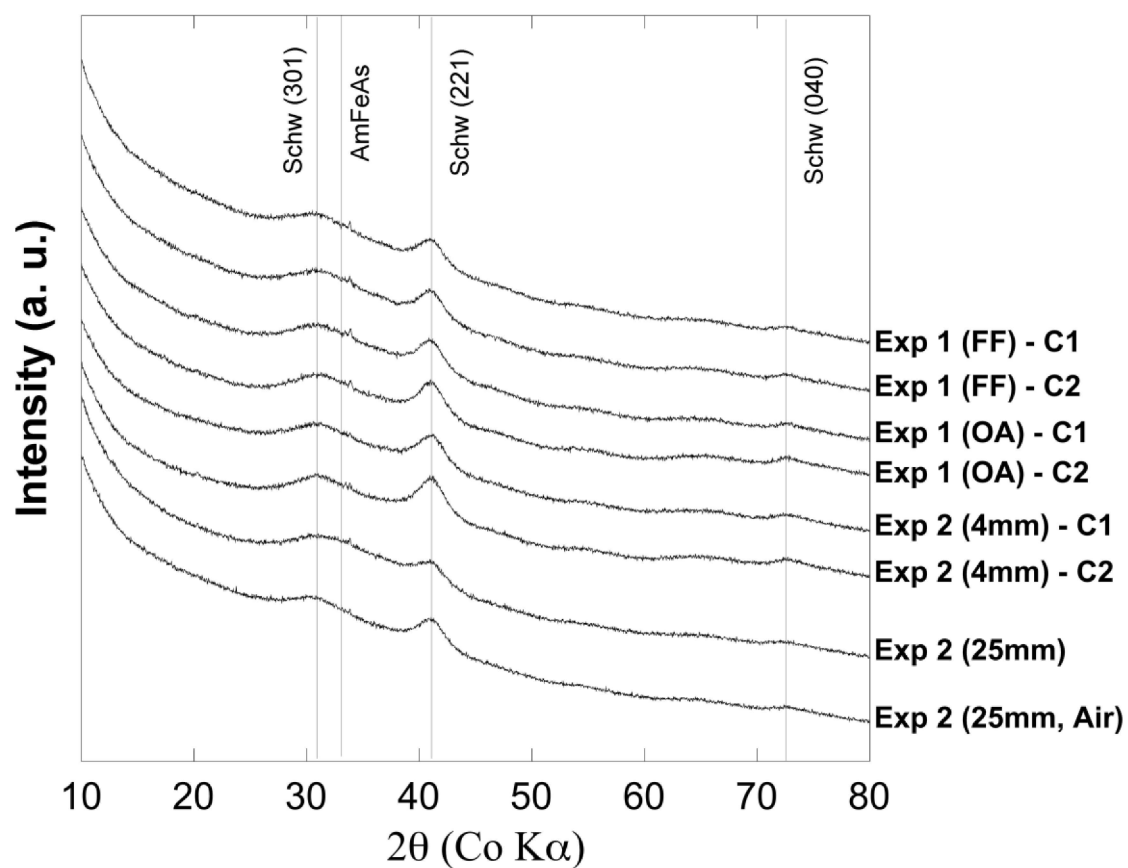


Figure SI-2. Powder X-Ray diffraction patterns of the biogenic precipitates. Experimental replicates are referred as Channel 1 (C1) and Channel 2 (C2)

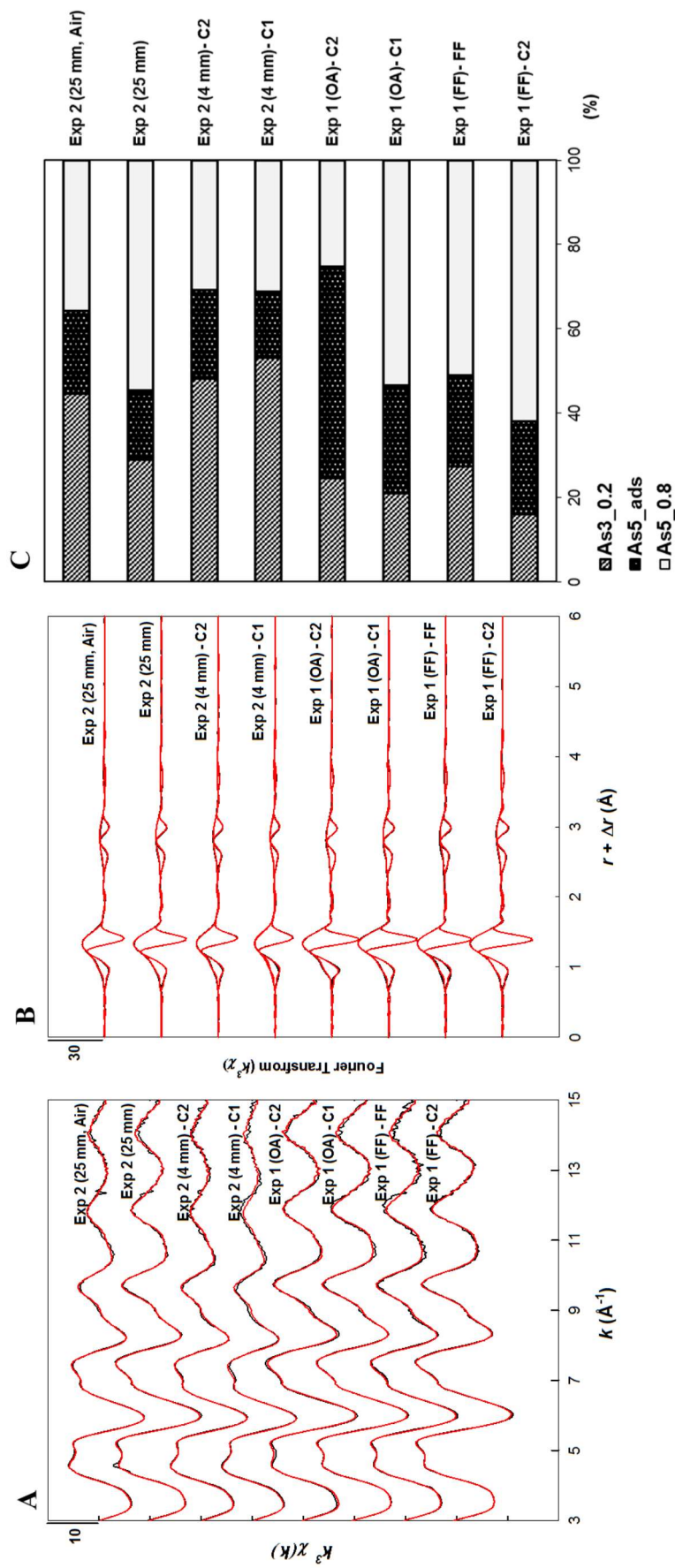


Figure SI-3. LCF of As K-edge EXAFS data of the biogenic precipitate and floating film samples. Experimental and fit curves are displayed in black and red lines, respectively. (A) k^3 -weighted EXAFS and (B) their Fast Fourier transforms. (C) Normalized molar proportions of the LCF components in each sample, with respect to total arsenic. The three fitting components, described in detail by Maillot *et al.* (2013), are the following: As(III)-coprecipitated schwertmannite with As/Fe = 0.2 mol/mol (As3_02), As(V)-sorbed schwertmannite with As/Fe = 0.01 mol/mol (As5_ads) and Amorphous Ferric Arsenate with As/Fe = 0.8 mol/mol (As5_0.8). Redox state of arsenic determined from LCF analysis of the XANES region of the same data are given in Resongles *et al.* (2016) (see Table SI-1), and fall within the standard deviation obtained from the present EXAFS LCF results, as given in Table SI-2. Experimental replicates are referred as Channel 1 (C1) and Channel 2 (C2).

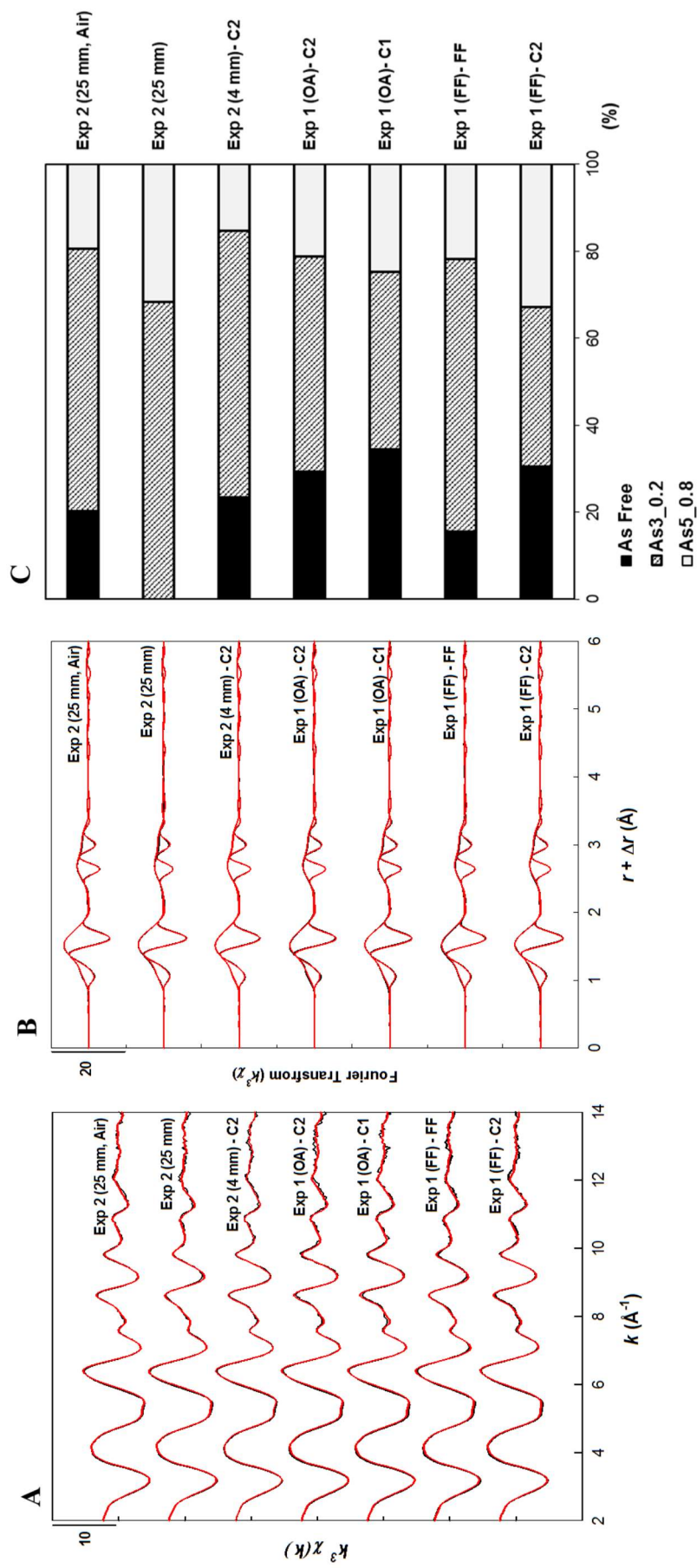


Figure SI-4. LCF of Fe K-edge EXAFS data of the biogenic precipitate and floating film samples. Experimental and fit curves are displayed in black and red lines, respectively. (A) k^3 -weighted EXAFS and (B) their Fast Fourier transforms. (C) Normalized molar proportions of the LCF components in each sample, with respect to total iron. The three fitting components, described in detail by Maillot *et al.* (2013), are the following: As-free schwertmannite (As Free), As(III)-coprecipitated schwertmannite with As/Fe = 0.2 mol/mol (As3_02), and Amorphous Ferric Arsenate with As/Fe = 0.8 mol/mol (As5_0.8). Results of the LCF are reported in Table SI-3. Experimental replicates are referred as Channel 1 (C1) and Channel 2 (C2).

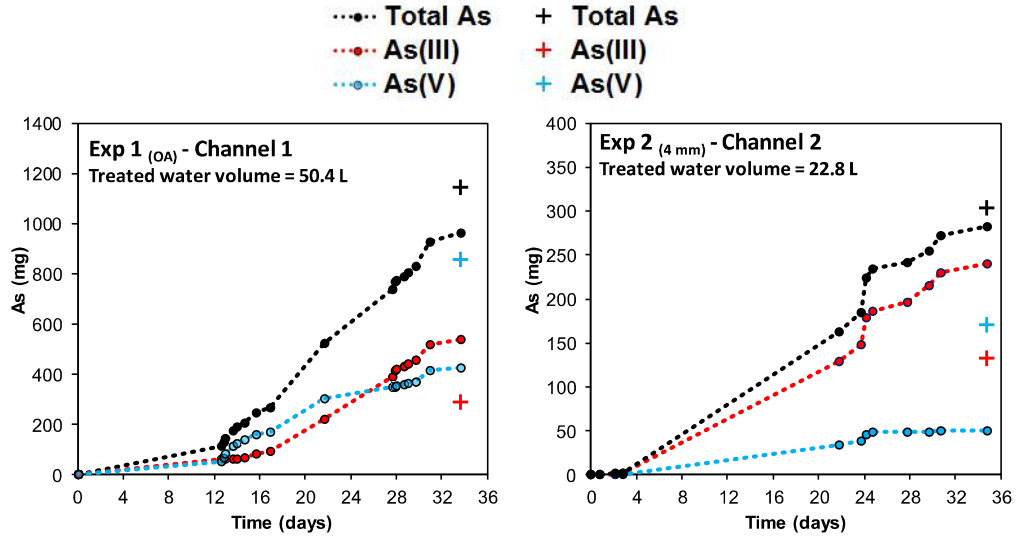


Figure SI-5. Accumulation of arsenic mass (Total As, As(III), As(V)) removed from the dissolved phase as a function of time (dots) in Exp 1_(OA) and Exp 2_(4 mm), and arsenic mass measured in the biogenic precipitate at the end of each experiment (crosses). This figure only includes the treatment replicates whose estimations have an error lower than 16 % between the calculated Total As removed from the dissolved phase and the Total As found in the biogenic precipitate. For the rest of the experiments, calculations did not match the measured arsenic in the biogenic precipitate because of the lack of sampling points (Exp 1_(FF)), or the higher treated water volume (Exp 2_(25 mm)) which does not provide a good extrapolation from few points. The other treatment replicate from Exp 1_(OA) and Exp 2_(4 mm) has an error of 19 % and 21 % respectively.

Equations used to calculate the As mass accumulated at time " t_i ":

$$As\ load_{t_i} = As_{t_i} (mg\ L^{-1}) * Q_{t_i} (L\ s^{-1})$$

$$As\ mass_{t_i} = \int_{t_{i-1}}^{t_i} As\ load(t) dt = \left[\frac{As\ load_{t_i} + As\ load_{t_{i-1}}}{2} \right] \times (t_i - t_{i-1})$$

$$As\ mass\ accumulated_{t_i} = (As\ mass\ inlet_{t_i} - As\ mass\ outlet_{t_i}) + As\ mass\ accumulated_{t_{i-1}}$$

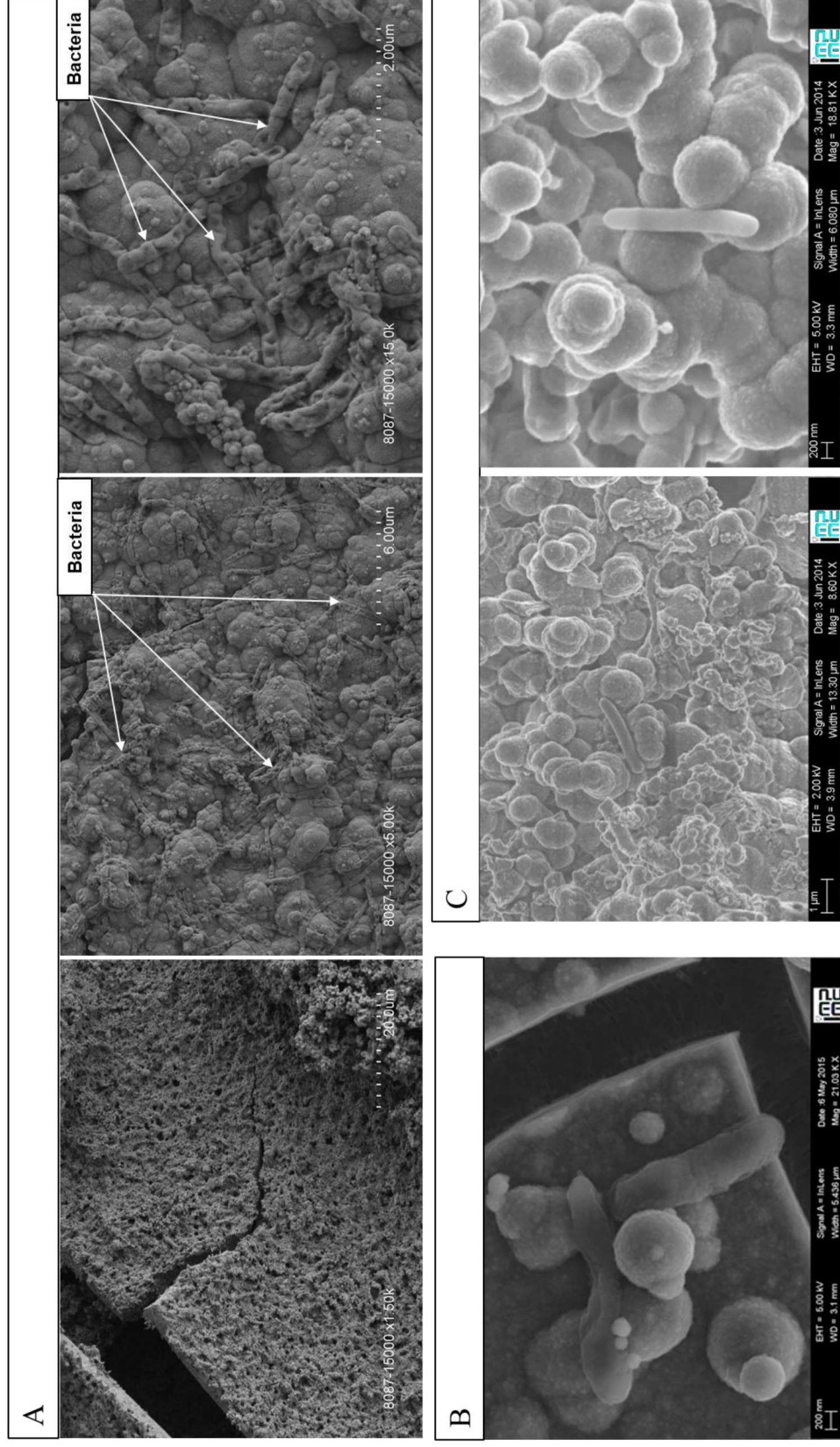


Figure SI-6. SEM images at different scales of (A) the floating film, (B) the precipitates deposited on the plastic films inserted in Exp 2, (C) typical precipitates collected at the bottom of the bioreactor. The mineral spheroids with stitched surface exhibit characteristic morphology of As-rich schwertmannite.

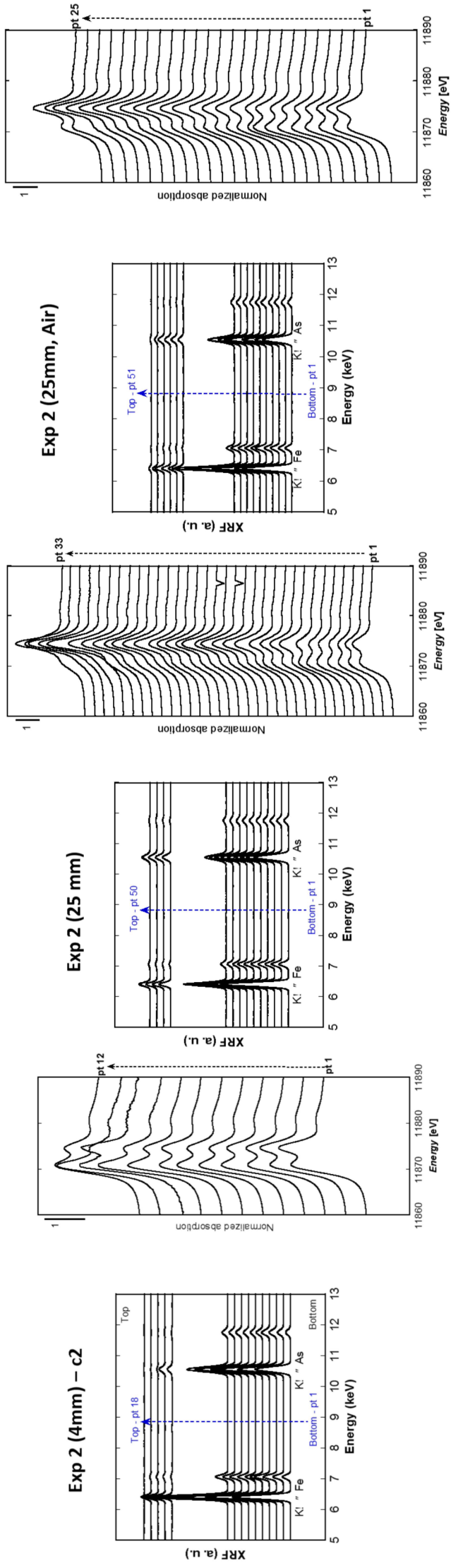


Figure SI-7. Micro XRF bulk spectra and spatially resolved XANES bulk spectra of the results presented in Figure 6. The molar As/Fe and As(III)/As(V) ratio values calculated using those spectra are given in Tables SI-4 and SI-5.

7. REFERENCES

- Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C., Daffonchio, D., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. *Applied and Environmental Microbiology* 70 (10), 6147–6156. doi:10.1128/AEM.70.10.6147-6156.2004
- Casiot, C., Morin, G., Juillot, F., Bruneel, O., Personné, J.-C., Leblanc, M., Duquesne, K., Bonnefoy, V., Elbaz-Poulichet, F., 2003. Bacterial immobilization and oxidation of arsenic in acid mine drainage (Carnoulès creek, France). *Water Research* 37 (12), 2929–2936. doi:http://dx.doi.org/10.1016/S0043-1354(03)00080-0
- Héry, M., Casiot, C., Resongles, E., Gallice, Z., Bruneel, O., Desoeuvre, A., Delpoux, S., 2014. Release of arsenite, arsenate and methyl-arsenic species from streambed sediment affected by acid mine drainage: a microcosm study. *Environmental Chemistry* 11 (5), 514. doi:10.1071/EN13225
- López-Gutiérrez, J.C., Henry, S., Hallet, S., Martin-Laurent, F., Catroux, G., Philippo, L., 2004. Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR. *Journal of Microbiological Methods* 57 (3), 399–407. doi: 10.1016/j.mimet.2004.02.009
- Lunau, M., Lemke, A., Walther, K., Martens-Habbena, W., Simon, M., 2005. An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environmental Microbiology* 7 (7), 961–968. doi:10.1111/j.1462-2920.2005.00767.x
- Maillot, F., Morin, G., Juillot, F., Bruneel, O., Casiot, C., Ona-Nguema, G., Wang, Y., Lebrun, S., Aubry, E., Vlaic, G., Brown Jr, G.E., 2013. Structure and reactivity of As(III)- and As(V)-rich schwertmannites and amorphous ferric arsenate sulfate from the Carnoulès acid mine drainage, France: Comparison with biotic and abiotic model compounds and implications for As remediation. *Geochimica et Cosmochimica Acta* 104, 310–329. doi:http://dx.doi.org/10.1016/j.gca.2012.11.016
- Quéméneur, M., 2008. Les processus biogéochimiques impliqués dans la mobilité de l'arsenic: recherche de bioindicateurs. PhD thesis, Nancy University, 21st November 2008.
- Quéméneur, M., Cébron, A., Billard, P., Battaglia-Brunet, F., Garrido, F., Leyval, C., Joulain, C., 2010. Population structure and abundance of arsenite-oxidizing bacteria along an arsenic pollution gradient in waters of the Upper Isle River basin, France. *Applied Environmental Microbiology* 76 (13), 4566–4570.
- Ravel B. and Newville M. (2005) ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *Journal of Synchrotron Radiation* 12 (4), 537–541.
- Resongles, E., Casiot, C., Freydier, R., Dezileau, L., Viers, J., Elbaz-Poulichet, F., 2014. Persisting impact of historical mining activity to metal (Pb, Zn, Cd, Tl, Hg) and

metalloid (As, Sb) enrichment in sediments of the Gardon River, Southern France. *Science of the Total Environment* 481, 509–521. doi:10.1016/j.scitotenv.2014.02.078

Resongles, E., Le Pape, P., Fernandez-Rojo, L., Morin, G., Brest, J., Guo, S., Casiot, C., 2016. Routine determination of inorganic arsenic speciation in precipitates from acid mine drainage using orthophosphoric acid extraction followed by HPLC-ICP-MS. *Analytical Methods* 8, 7420–7426. doi:10.1039/c6ay02084d

Rodier, J., Broutin, J., Chambon, P., Champsaur, H., Rodi, L., 1996. *L'analyse de l'eau*. Paris: Dunod, 1383 p.