Laboratory-Scale Bio-Treatment of Real Arsenic-Rich Acid Mine Drainage

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Abstract

Acid mine drainage (AMD) still represents a huge environmental problem. Technical and scientific breakthroughs are still needed to decrease environmental damages, treatment cost and waste production associated with AMD. The feasibility of combining continuously fed anaerobic sulfate-reducing bioreactor with downstream iron oxidation step was tested, at laboratory scale, with two types of real arsenic rich AMD waters from the site of Carnoulès (France), that differed in acidity (pH 3.3 and 4.0), arsenic (As, 18 and 174 mg/L) and metals concentrations. Iron remained in solution while up to 99% of As was precipitated as amorphous orpiment in the anaerobic sulfate-reducing bioreactor. Zinc (Zn) precipitation was also

observed, up to 99%, however the efficiency of Zn precipitation was less stable than that of As. The anaerobic bioreactor presented a stable bacterial community including a *Desulfosporosinus*-related sulfate-reducer. When the effluents from the anaerobic process step were treated in a laboratory aerobic bioreactor, iron was oxidized efficiently. The feasibility of efficient orpiment bio-precipitation coupled with downstream iron oxidation was shown, thus opening the perspective of low-cost combination of treatment steps for the removal of arsenic, zinc and iron in As-rich AMDs.

Key words arsenic, acid mine drainage, bioreactors, sulfate-reduction, bio-oxidation

1 Introduction

Acid mine drainage (AMD) causes extensive damage to the aquatic environment all around the world (Simate and Ndlovu, 2014). This phenomenon affects many abandoned and remote sites, where the application of the classical active lime treatment is technically and economically problematic. Passive or semi-passive technologies have been developed, the global remediation strategy generally involving several biological or physic-chemical treatment steps (Johnson and Hallberg, 2005; Nairn et al., 2010; Mattes et al., 2011). Oxidation steps can be efficient to remove iron (Fe), manganese (Mn), or co-precipitate Fe and arsenic (As) (Casiot et al., 2003; Battaglia-Brunet et al., 2006; Wang et al., 2006; De Sa et al., 2010; Nairn et al., 2010; Fernandez-Rojo et al., 2017, 2019). However, such aerobic processes are unsuitable for the removal of divalent metal cations due to their poor sorption onto positively charged surface of Fe-oxyhydroxides in acid conditions (Lee et al., 2002). On a complementary way, biological reduction of sulfate efficiently removes metals such as zinc (Zn), copper (Cu), and lead (Pb) as metallic sulfide precipitates and induces an increase of alkalinity and pH (Kaksonen and Puhakka, 2007, Touzé et al., 2008). This treatment is also efficient to remove As (Mattes et al.,

2011), provided that low pH and/or low dissolved sulfide concentrations maintain sulfide amounts sufficiently low (< 1 $mg.L^{-1}$) to avoid the formation of soluble thioarsenic species (Smieja and Wilkin, 2003). Sulfate reduction needs a costly supply in electron donor, such as hydrogen or organic molecules. To limit such costs, passive anaerobic bioreactors are generally filled with raw organic materials, such as agricultural, wood or food processing wastes which degradation provides electron donors for sulfate reduction (Parvis and Younger, 1999; Gibert et al., 2004; Mattes et al., 2011). However, in these systems, the sulfate-reducing activity cannot be regulated, all metals being precipitated as a bulk without selectivity. It would be interesting to regulate the activity of sulfate-reducing bacteria so as to remove only metals that cannot be eliminated, or only partially removed, in the aerobic steps. In this respect, iron sulfide (FeS) precipitation should be avoided since Fe can be efficiently treated in an oxidizing process (Fernandez-Rojo et al., 2017). Allocate the sulfate-reducing activity to the precipitation of a selection of metals will thus save organic substrate costs. Considering that FeS precipitates at pH higher than 5 (Kontopoulos, 1997), the sulfate-reducing bioreactor should be able to perform efficiently at lower pH. Sulfate reduction by acidophilic or acido-tolerant sulfate-reducing bacteria (SRB) has been reported as a promising way to remediate acid mine water while selectively precipitating metals as sulfides (Kimura et al., 2006; Johnson et al., 2009; Nancucheo and Johnson, 2012; Nancucheo et al., 2014; Sanchez-Andrea et al., 2015; Gonzales et al., 2019). The feasibility of As sulfide biosynthesis was demonstrated in batch laboratory bioreactors (Serrano and Leiva, 2017; Le Pape et al., 2017) or continuously fed with acidic (pH 2.5 to 4, Battaglia-Brunet et al., 2012; Altun et al., 2014) or mild acidic synthetic (pH 6-7, Rodriguez-Freire et al., 2014, 2015) solutions. Growth of SRB inducing precipitation of the orpiment and realgar, together with ZnS nanoparticles, has been proven in the real arsenic-rich acid mine water from Carnoulès site (France) in batch experiments (Le Pape et al., 2017).

Here, the two steps of a semi-passive process configuration including complementary anaerobic and aerobic reactions were separately tested with the Carnoulès AMD in continuous laboratory-scale bioreactors. These two complementary processes should allow removal of toxic metals and high As contents from the AMD. Three main issues were addressed: (i) the feasibility of orpiment precipitation from real arsenic-rich acidic mine water in an anaerobic SRB bioreator, (ii) the separation of Fe, remaining in solution, from Zn and As, precipitated as sulfides, and (iii) the aerobic bio-oxidation of remaining Fe present in the effluent from the anaerobic bioreactor, and subsequent As removal.

2 Materials and Methods

2.1 Mine waters

AMD water was collected in July 2014 at the Carnoulès mine (Gard, France) from two different springs: S1 and SSR, filtrated at 0.45 µm in a glovebox and stored at 5°C under nitrogen. NaOH solution (1 M) was added to the water in a glovebox when pH adjustment was needed. Characteristics of the water samples are given in Table 1. Among all metals and metalloids present in these mine waters, As is particularly toxic, Zn is the most concentrated divalent metal, and Fe is present at high level, close to 1 $g.L^{-1}$ (le Pape et al., 2017).

2.2 Sulfate-reducing bioreactor

2.2.1 Experimental device

The sulfate-reducing bioreactor consisted of a 320 mm high and 35 mm internal diameter glass column, equipped with a water jacket for temperature regulation. The bioreactor filling method was conceived to: (1) test the passive distribution of nutrients to the bacteria, and (2) try to establish different levels of SRB activities in the bottom and the top zones based on the stimulation of SRB growth by yeast extract, thus testing the possibility to precipitate As in the top layer (lower sulfate-reduction activity) and Zn in the bottom layer. The bioreactor (Fig. 1) was filled in two distinct layers. For the top half of the bioreactor, pozzolana was mixed with hot liquid agar (25 $g.L^{-1}$) prepared with deionized water acidified at pH 4.5 with H_2SO_4, containing 5 $g.L^{-1}$ glycerol and 0.5 $g.L^{-1}$ K_2HPO_4. After solidification of the agar, pozzolana grains were separated using a spatula and mixed with a carrier biomaterial named "biocompounds", provided by Vertum GmbH (Walter et al., 2009) and composed of polyhydroxybutyrate and polycaprolactone. The proportions of components were 160 g of pozzolana / 100 g of biocompounds / 200 mL of agar with nutrients. The bottom filling material had same composition except for the nutritive agar, supplemented with 1 $g.L^{-1}$ yeast extract. Sampling ports equipped with septa allowed liquid sampling from the middle, bottom and top levels.

The bioreactor was inoculated with a sulfate-reducing microbial consortium, enriched from sediments of the Carnoulès site. A basal medium (SM1) prepared at pH 3.5 and 4.5 was supplemented with 327 $mg.L^{-1}$ Zn and 75 $mg.L^{-1}$ As(V) (using respectively 16 $g.L^{-1}$ $ZnSO_4$ and 10 $g.L^{-1}$ As(V) anaerobic stock solutions). Active cultures precipitating yellow-white precipitates were sub-cultured in 40 mL basal medium supplemented with As and major metals

present in Carnoulès water: 2 mL of Fe(II) solution (final concentration 1 $g.L^{-1}$), 2 mL of metals solution, 0.5 mL of As(III) solution (final concentration 100 $mg.L^{-1}$) and 0.2 mL of As(V) solution (final concentration 40 $mg.L^{-1}$). Concentrated Fe(II) and metals solutions were prepared under N_2 atmosphere, with oxygen-free ultrapure water acidified at pH 4.5 with H_2SO_4. Fe(II) solution contained, per litre, 99.2 g of $FeSO_4.7H_2O$. Metals solution contained, per litre: $Al_2(SO_4)_3.18H_2O$, 13.6 g ; $MnSO_4.H_2O$, 368 mg; $NiSO_4.6\ H_2O$, 44 mg ; $ZnSO_4.7H_2O$, 1.77 g; $CuSO_4.5\ H_2O$, 32 mg. Concentrated As(III) and As(V) solutions (10 $g.L^{-1}$ As) were prepared as described in Battaglia-Brunet et al. (2002). Two sulfate-reducing cultures were obtained after five sub-cultures at pH 3.5 and 4.5; they were mixed to obtain the sulfate-reducing microbial consortium used to inoculate the bioreactor. A pellet of this inoculum was stored at -20°C for DNA extraction and bacterial taxonomic identification.

2.2.2 Operating conditions

The bioreactor first worked in passive down-flow condition for 199 days, during which the microbial activity was exclusively supported by the nutrients present in the solid filling materials (Battaglia-Brunet et al., 2016). During this phase, the feed solution was a synthetic mine water prepared adding the following components to 1 L deionized water whose pH was adjusted with concentrated H_2SO_4: 2.3 g Na_2SO_4, 50 mL of Fe(II) solution, 50 mL of metals solution, 10 mL of As(III) solution and 2.5 mL of As(V) solution. The pH dropped from 4.5 to 3.0 in 60 days. The present experiment started at Day 200, after this passive phase, using real spring S1 mine water (pH 3.3) amended with 250 $mg.L^{-1}$ of glycerol as feed. The bioreactor was down-flow fed during 26 days. Down-flow configuration consumes less energy than up-flow however it increases the phenomena of preferential flow paths and clogging. Then, down-flow was changed to up-flow feeding (day 226). At last, from day 315, the column was up-flow

fed with the spring SSR (pH 4.0) mine water complemented with 50 $mg.L^{-1}$ glycerol. The temperature was maintained constant at 25°C.

2.2.3 Monitoring of the bioreactor

The outlet solution was collected and maintained under N_2 atmosphere. Once a day, the outlet bottle was weighted to measure flow-rate. pH was measured and 5 mL of bioreactor outlet were filtrated at 0.45 µm, acidified with nitric acid and stored at 5°C until As, Fe and Zn analyzes. The remaining effluent was stored at 5°C under N_2 atmosphere until treatment in the aerobic bioreactor. Feed was sampled once a week to check pH and chemical composition. Total As was analyzed by oven AAS (Varian SpectrAA 220Z), Zn and Fe by flame AAS (VARIAN SpectrAA 300). Total organic carbon was quantified by hot oxidation with sodium persulfate (method NF EN 1484) and acetate was analyzed by ionic chromatography. Punctually (on days 201, 219, 288, 309 and 367), 5 mL of slurry were collected from top, middle and bottom septa with a sterile syringe, filtrated on a sterile 0.22 µm cellulose acetate filter and the filters were stored at -20°C for biomolecular analyzes. At the end of the experiment, the column was opened in a glovebox under N_2 atmosphere. Samples of the filling material were stored at -20 °C for biomolecular analyzes, and other samples were stored under N_2 for mineralogical analyzes. After the bioreactor dismantling, "biocompound" particles were recovered, rinsed with deionized water and dried at 40°C for 24 h then weighted to estimate the amount of consumed material.

2.2.4 Composition of the inoculated sulfate-reducing consortium and evolution of the bacterial community structure in the column

Microbial DNA was extracted from the -20°C stored samples, *i.e.* pellet of the inoculum, frozen filters and filling materials (sampled at the end of the experiment), with the FastDNA Spin Kit for soil applying a mechanical lysis at speed 5 during 30 s. Bacteria composing the inoculum were identified by cloning and sequencing of the 16S rRNA gene. Briefly, about 1400 bp of the gene were amplified from the extracted microbial DNA with primers 8F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1406R (5'-GACGGGCGGTGTGTRCA-3'), and the purified PCR product was cloned into a PCR®4-TOPO vector, according to the manufacturer instructions (Invitrogen). 32 plasmids carrying correct-length insert were sequenced by the GATC company with primers T7 end T3 targeting the vector. Consensus sequences were manually verified and aligned with ClustalW, and distances on unambiguous nucleotide sequences were determined by DNADist, using applications implemented in the BioEdit software (Hall, 1999). Operating taxon units (OTU) were built with retrieved sequences sharing more than 98% identities. Distances between most related Genbank 16S rRNA gene sequences of known bacteria, obtained by Blastn (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), and defined OTU sequences were determined by DNADist on 1372 aligned and unambiguous nucleotides. In addition, about 1.9 kb of the *dsr*AB gene encoding the dissimilatory bisulfite reductase involved in reduction of sulfate, were amplified with primers DSR1F (5'-ACSCAYTGGAARCACG-3') and DSR42R (5'-GTGTARCAGTTDCCRCA-3'), cloned and sequenced as described. Consensus *dsr*AB gene sequences sharing more than 98% identities by DNADist were grouped in OTU and compared to Genbank reference sequences by Blastn. Most related reference and OTU *dsr*AB gene sequences were translated into amino acids sequences, aligned and distances were calculated by ProtDist (implemented in BioEdit software) on 552 unambiguous amino acids. Sequences reported in this study are available under GenBank accession numbers MZ476566-MZ476597 (16S rRNA gene sequences) and MZ497397 to MZ497410 (*dsr*AB gene sequences).

The evolution of the structure of the bacterial community in the bioreactor was monitored by 16S rRNA gene CE-SSCP genetic fingerprints. About 200 bp of the V3 region of the bacterial 16S rRNA gene were amplified from DNAs (column samples and clones representing each OTU from the inoculum) with the reverse primer w34 (5′-TTACCGCGGCTGCTGGCAC-3′), 5′ end-labelled with the fluorescent dye FAM, and the forward primer w49 (5′-ACGGTCCAGACTCCTACGGG-3′). Diluted PCR products (100 to 200 fold, to avoid saturation of the fluorescent signal) were heat-denaturated (95°C, 10 min) in HiDi Formamide (Applied BioSystems) and submitted to a non-denaturating electrophoresis using the polymer CAP and an ABI 310 genetic analyzer (Life Technologies), as already described (Battaglia-Brunet et al., 2012). Internal DNA standard (Genescan 600-LYZ, Life Technologies) was used to calibrate the runs. DNA fragments of 200 bp from columns' samples and inoculum clones were run concomitantly in the same CE-SSCP capillary electrophoresis. Matching migration pattern was used to assign a clone to a signal of the community patterns and determine if OTU maintained or not in the column. Fingerprint profiles were aligned and compared with BioNumerics version 7.6 software (Applied Maths).

2.2.5 Characterization of the precipitates

Black pouzzolana and yellow precipitate samples were analyzed using XRD. XRD measurements were performed using a specifically designed anoxic cell with CoKa radiation using a Panalytical X'Pert Pro diffractometer. Samples were grinded and suspended in ethanol within an anaerobic chamber before being deposited on a Si single-crystal low-background sample holder. Data were collected between 5 and 80°2 θwith a 0.033°2 step, counting 2 hour per sample. The yellow precipitate around pozzolana grains was observed using electron microscopy associated with microanalysis (SEM-EDXS analysis) on a GEMINI ZEISS Ultra55

Field Emission Gun Scanning Electron Microscope operating at 15 kV. Observations and microanalysis were performed using backscattering electron signal and a Bruker QUANTAX Energy dispersive X-ray spectrometer, respectively. For synchrotron based X-ray absorption spectroscopy, the set of As-sulfide references considered in this study is detailed in Le Pape et al. (2017). Briefly, experimental XAS spectra obtained on the column samples were compared to the ones of amorphous orpiment (am_As_2S_3), crystalline orpiment (As_2S_3), and realgar (AsS), minerals that are representative of an S-bound local molecular environment. As(V)- and As(III)-sorbed ferrihydrites were used as reference compounds for O-bound local molecular environment. As K-edge XANES and EXAFS spectra were collected at 15 K (He crysostat) in both transmission and fluorescence mode on the SAMBA beamline (SOLEIL, France) using a Si(220) double-crystal monochromator. Samples were always kept under inert atmosphere to avoid oxidation. The incident energy was calibrated by measuring the LIII-edge of an Au foil recorded in double transmission. Scans were averaged, normalized and background substracted using the Athena software. Linear combination fitting of XANES spectra were performed over the -20 to 60 eV energy range.

2.3 Aerobic step

Effluents from the anaerobic bioreactor were treated in an aerobic bioreactor whose design was described in Fernandez-Rojo et al. (2017b). Briefly, it was composed of 4 rectangular polyvinyl chloride channels (1 m length × 0.06 m width × 0.06 m depth) equipped with a double envelope for temperature regulation (20 ± 0.8 °C). One channel was used for the present experiment. A canvas was placed on the bottom of the channel to provide a rough surface for the adhesion of the biogenic precipitate. A peristaltic pump (Gilson, Minipuls 3) supplied the channel inlet with

AMD at a flow rate that determined the water residence time, here ~300 min. A second peristaltic pump was necessary to pump the fluid out of the channel and to maintain the water level at specific height (4 mm). A valve was positioned at the end of outlet tube for sample collection. Prior to the treatment, a "conditioning" step was required to allow the development of an active Fe-oxidizing biofilm. During this step, the aerobic bioreactor was fed with 0.5 $mL.min^{-1}$ of water S1, corresponding to a residence time (RT) of 324 ± 30 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. After steady-state achievement (19 days), the channel was successively fed with (1) the anaerobically-treated spring S1 water and (2) the anaerobically-treated spring SSR water. Efficiency of the aerobic step was assessed by monitoring the inlet and outlet water chemistry. Analyzes included measurement of pH, the determination of total concentrations of dissolved Fe(II) using spectrophotometry, and total dissolved Fe, Zn and As using ICP-MS, after 0.2 µm filtration. Arsenic redox speciation was determined using HPLC-ICP-MS. Details of these analytical procedures are reported in Fernandez-Rojo et al. (2017).

3 Results

3.1 Anaerobic bioreactor

3.1.1 Evolution of physic-chemical parameters

During the down-flow feeding phase with spring S1, As removal efficiency averaged 64 ± 4 % (157 $mg.L^{-1}$ As in the feed, 46 to 71 mg $.L^{-1}$ in the outlet) for a measured residence time of 298 ± 26 h (Fig. 2A and 2B) whereas within the same time, Zn removal (Fig. 2C) varied from 2 to

25% (24 mg.L^{-1} Zn in the feed, 16 to 23 mg .L^{-1} in the outlet) and Fe removal (Fig. 2D) from 10 to 30% (1270 mg.L^{-1} Fe in the feed, 84 to 1146 mg .L^{-1} in the outlet).

Substantial increase of Zn (Fig. 2C) concentration in the effluent after 208 days occurred concomitantly to effluent pH decrease (Fig. 2E), consequence of clogging of the filling material that resulted in disconnections of feeding pipe. The change (day 226) from down-flow to up-flow configuration improved the efficiency of the bioreactor for As removal, that was higher than 85% from day 245 to day 290 (Fig. 2B). As removal declined slightly with the decrease of residence time from 300 h to 250 h after day 245 (event 1 on Fig. 2) but recovered at day 260. The decrease of the mine water pH from 3.1 to 2.9 after day 270 (event 2 on Fig. 2) also induced transient rise of As concentration in the outlet. A delay of 4-5 days was still observed between the source of disturbance and the rise of As concentration in the outlet because of the high residence time. After day 290, a new decrease in residence time from 250 h to 70 h (event 3 on Fig. 2) induced a worsening of As removal that stabilized around 65%. Zn concentration followed the same evolution as As but with variations of higher amplitude. The events that affected As removal by 15% affected Zn removal by 90%. After day 290, the decrease of residence time resulted in a very low level of Zn removal (less than 10%). During all the up-flow feeding period, Fe was not removed (Fig. 2D). The pH always increased: while fed with real mine water at pH close to 3, the pH in the outlet ranged from 4.3 to 4.8 (Fig. 2E).

Changing fed water from S1 to SSR corresponded to a decrease in Fe, Zn and As concentrations and higher pH of the feed (event 4 on Fig. 2). After a transient period during which parameters were influenced by the change of feed water quality, from day 312 to day 330, complete removal of As and Zn was obtained. When the residence time in the bioreactor was

progressively decreased from 70 to 30 h (Fig. 1A), As and Zn were always almost entirely removed from SSR water (Fig. 2B and 2C) and Fe was not precipitated (Fig. 2D).

Most of both As and Zn was precipitated in the bottom half of the column (Fig. SM2), so the separation of As and Zn inside the column using different filling compositions was not achieved. Acetate was always found in the outlet during the monitoring, however acetate concentration in the outlet was 50% to 99% lower than the theoretical concentration that would result from the incomplete oxidation of glycerol (Table SM3), except at the end of experiment with spring SSR water, when the bioreactor was in a phase of degraded efficiency. At the end of experiment, the visual appearance of the biocompounds and their weight did not differ significantly from those of fresh biocompounds, suggesting they have not been biodegraded in the bioreactor.

3.1.2 Composition of the inoculum and evolution of the bacterial community in the column

The 32 sequences of the 16S rRNA gene retrieved from the sulfate-reducing consortium enriched from the Carnoulès site were classified into three OTU. The dominant OTU (90.6 % of the sequences) shared 99.1 % identity on the 16S rRNA gene sequence with the acidophilic sulfate-reducing bacterium *Desulfosporosinus* sp. PFB (Sen and Johnson, 1999). Another *Desulfosporosinus*-related OTU represented 6.3 % of the sequences and showed more than 3 % differences on the 16S rRNA gene with the dominant OTU; it shared 97.7 % and 97.8 % 16S rRNA gene identities with *Desulfosporosinus* sp. OK and *Desulfosporosinus* sp. OL isolated from an AMD (sequences available in Genbank under numbers MT359229 and MT359230). The closest described *Desulfosporosinus* species most related (96-97 % identity) to these two OTU were *D. lacus, D. meridiei, D. orientis, D. fructosivorans* and *D. auripigmenti*. The third OTU grouping 3.1 % of the sequences was related (99.7 % 16S rRNA gene identity) to the

318 *Propionibacteraceae* strain H7p isolated from Carnoulès acid mine drainage (Delavat et al.,
319 2012); except for its tolerance to low pH, no data is currently available on its metabolism.

320 Sixteen dissimilatory bisulfite reductase (DsrAB) sequences retrieved from the inoculum
321 formed two related OTU sharing 97.4 % identity on their DsrAB aminoacids sequences. The
322 closely related (85.6 % identity) DsrAB sequence to both OTU belonged to the acidophilic
323 *Desulfosporosinus* sp. DB isolated from an AMD (Karnachuk et al., 2009), and the closest
324 described species were *Desulfosporosinus adidiphilus*, *D. acididurans* and *D. meridiei* with
325 respectively 76.5 %, 75.7 % and 72.3 % identities. These results are in line with those obtained
326 from 16S rRNA gene sequence analyzes, and confirm that sulfate-reducing strains belonging
327 to the *Desulfosporosinus* genus are present in the sulfate-reducing inoculum enriched from
328 Carnoulès site.

329 The evolution of fingerprints of the bacterial community sampled during time and location in
330 the column are presented in Fig. 3. Fingerprints were quite similar at the top, middle and bottom
331 of the column, showing a homogeneous distribution of the bacteria in the bioreactor. At days
332 288 and 309, the structure of the community has evolved and only two of the peaks detected at
333 day 219 remained largely dominant. Changes in operating conditions, *i.e.* upflow fed and pH
334 decrease from 3.1 to 2.9, may have favored the corresponding bacteria. The change from spring
335 S1 to SSR site water from day 309 to the end of the monitoring (day 367) did not affect much
336 the structure of the community at the top and bottom location, while more peaks were detected
337 at the middle location. The signal corresponding to the *Desulfosporosinus* OTU initially
338 dominant in the sulfate-reducing inoculum, which was also present during the previous stage
339 of feeding with synthetic mine water (Battaglia-Brunet et al., 2016), was one of the dominant
340 throughout the monitoring. Its presence regardless of the condition applied to the column
341 emphasizes its ability to maintain itself under the different conditions applied to the column, in
342 particular a pH of 3 of the feed water. It was also found on the filling material sampled at the

343 end of the monitoring (data not shown), confirming its presence in the biofilm attached on the
344 solid support. No signal corresponding to the *Propionibacteraceae* bacterium found in the
345 inoculum was evidenced, indicating that its proportion amongst other bacteria represented, if
346 present, only as a small fraction of the total community.

347 3.1.3 Characterization of the precipitates

348 In the samples from the column, *i.e.* black pouzzolana and yellow precipitate, analyzed using
349 XRD (Fig. SM4-A), no As-bearing phase was observed. The composition is in line with usual
350 mineral compounds used in pouzzolana, *e.g.* feldspars. In the yellow precipitate recovered
351 around the pouzzolana grains, only gypsum was observed as crystalline material. However,
352 very low amplitude reflexions as well as large bumps above the background suggest the
353 presence of amorphous or nanocrystalline materials.
354
355 Both XANES and EXAFS spectra obtained at the As K-edge for the different samples collected
356 in the column were compared to reference materials representative for specific As local
357 molecular environments. Realgar (AsS) and amorphous orpiment (am_As_2S_3) were used as
358 references for sulfur bound As, and As(III)-sorbed ferrihydrite and As(V)-sorbed ferrihydrite
359 were used as proxies of oxygen bound As. Linear combination fitting analysis performed on As
360 K-edge XANES spectra shows that the samples mainly contained amorphous orpiment (89 to
361 100 %) and a minor amount of oxygen bound As(III) (0 to 13 %) (Fig. SM4-B and Table SM4-
362 C). Even considering the low quality of EXAFS signal for some of the samples (Fig. SM4-D),
363 both EXAFS oscillations and Fast Fourier Transform confirm the major occurrence of
364 amorphous orpiment in the samples recovered from the column experiment (Fig. 4). As both
365 XANES and EXAFS spectra of thiol-bound As(III) are difficult to differ from the one of
366 amorphous orpiment, the presence of such As species cannot be excluded in the samples. In

addition, the presence of minor amount of oxygen bound As(V) and realgar (< 5%) cannot be discarded considering the standard uncertainty of XAS-LCF analysis.

In Fig. 4, the image of yellow precipitate was taken in backscattering electron mode, thus the brightest spots indicate the presence of the heaviest elements. In this particular case, the brightness of particles is related to the As content. Both encrusted and non-encrusted bacteria are observed, as well as submicrometric aggregates of spherical particles connected or not with bacteria. All the particles probed using EDXS (1 to 6, Fig. 4) showed a major signal of As and S, which is consistent with arsenic sulfides as the main products of the sulfate-reducing activity of the biofilm. Only traces of Fe and Zn were detected in the EDXS spectra, confirming that As was the main element to precipitate as biogenic sulfide in the bioreactor. In addition to the large occurrence of aggregates of spherical nanoparticles in the biofilm, nanospheres can be observed on the surface of bacterial cells (Fig. 4A). This observation would be consistent with those of Newmann (1997) who observed the extracellular precipitation of orpiment nanoparticles on sulfate-reducing bacteria mineralizing arsenic.

3.2 Coupling with aerobic step

The aerobic step was tested as a preliminary evaluation of the feasibility of biological oxidation of Fe(II) and increase of As removal in the sulfate-reduction bioreactor effluent, that contained also organic substances, potentially inhibitors of Fe(II) and As(III) bio-oxidation. This treatment induced a decrease of pH and Fe concentration: 7% (S1) and 9% (SSR) removal were obtained within 300 minutes (Fig. 5). Concerning As, for spring S1, nearly 90% removal was obtained in the anaerobic bioreactor, and the aerobic step improved the overall removal, decreasing both As(III) and As(V) concentrations by 33% and 44%, respectively. These results

represent a gain of 6% removal for As(III) and 4% for As(V) considering the initial composition of the mine water. Conversely, with spring SSR, the aerobic step did not induce any As removal. Surprisingly, a release of As(III) was observed in these conditions. The aerobic step had no effect on Zn concentration.

4 Discussion

Up to now, laboratory continuous experiments dedicated to the biological precipitation of arsenic sulfide were fed with synthetic solutions (Battaglia-Brunet et al., 2012; Altun et al., 2014; Rodriguez-Freire et al., 2014, 2015). Here, a real As-rich acidic mine water, of complex composition, was used to confirm the feasibility of continuous anaerobic precipitation of orpiment at low pH, in accordance with batch experiment data (Le Pape et al., 2017). The present experiment with real mine water and glycerol supply started on day 200, after the bioreactor was fed with synthetic mine water during 199 days under entirely passive conditions (Battaglia-Brunet et al., 2016). During this previous experimental phase, a progressive decrease of the bioreactor efficiency had been observed, linked to the exhausting of substrates initially placed into the column. Semi-passive sulfate-reducing bioreactors are increasingly seen as a viable option for highly contaminated AMD (Lounate et al., 2020). One of the objectives of the present study was to evaluate the precipitation of As and Zn in the sulfate-reducing bioreactor working in semi-passive condition, while Fe remained in solution, knowing that: (1) As is toxic and cannot be valorized, thus should be precipitated in the smallest volume of waste as possible, (2) Zn must be removed during the sulfate-reducing step because the oxidation step will not remove it, (3) Fe can be efficiently removed downstream by passive microbial or chemical oxidation step, co-precipitating the residual As. Furthermore, as Fe is the most concentrated

metal in Carnoulès AMD, avoiding its precipitation in the anaerobic bioreactor will save organic substrates and prevent rapid clogging.

The theoretical mass balance comparing our process configuration with a classical lime treatment directly applied to the spring S1 AMD (Fig. 6) suggests that the amount of As-contaminated solid waste could be drastically decreased applying a sulfate-reduction step followed by aerobic oxidation. Moreover, the classical lime application could not remove Zn from water unless reaching pH 9.5 (Murdock et al., 1993), thus implying a supplementary final step of acid addition.

The direct feeding of sulfate-reducing bacteria with the acid mine waters S1 et SSR was possible, in spite of the low pH, because iron, although present in high concentration, is mainly under the Fe(II) form in Carnoulès water (Casiot et al., 2003). The sulfate reduction induced an increase of one unit pH along the experiment. However, the pH in the outlet remained acidic, contrary to previous studies (Battaglia-Brunet et al., 2012; Altun et al., 2014; Rodriguez-Freire et al., 2014, 2015), where the electron donor was not limiting, supporting an extended sulfate-reduction activity and resulting in a pH value higher than pH 5-6 in the outlet. Here, the moderate pH increase, linked to the limited substrate supply, allowed separating Fe, that remained in solution, from Zn and As that were precipitated together during periods of efficient microbial activity. However, the anaerobic bioreactor was more efficient to remove As than Zn: the weakening of bacterial activity always affected Zn precipitation more than As removal during the experiment with spring S1. Precipitation of amorphous orpiment is favored at low pH (Eary, 1992): the more acid the medium is, the more orpiment precipitation is efficient. With spring S1 water, le Pape et al. (2017) already showed that As precipitation was more rapid than that of Zn. With spring SSR, the feed pH (pH 4) was already higher than the value necessary for ZnS precipitation, that could explain the efficient removal of both Zn and As from this mine water. Mattes et al. (2011) already reported efficient removal of both As and Zn from

441 a smelter landfill seepage (pH 5.9), through a real scale anaerobic passive bioreactor filled with
442 a mixture of limestone, quartz sand and organic waste.
443 Here, considering that production of sulfide was mainly coupled to incomplete degradation of
444 glycerol (reaction 1), and consumption of sulfide occurred according to reactions (2) and (3),
445 the total amount of glycerol necessary to precipitate all As (2 mM) and Zn (0.3 mM) present in
446 S1 should be 4.4 mM, *i.e.* 405 mg L^{-1}. Yet, the bioreactor feed contained only 250 mg L^{-1}
447 glycerol, a limiting concentration that should not have been sufficient for the precipitation of
448 both As and Zn, whereas the complete removal of these two elements was observed during
449 some periods of the experiment.
450

451 $$C_3H_8O_3 + 0.75\ SO_4^{2-} + 1.5\ H^+ \longrightarrow C_2H_4O_2 + CO_2 + 0.75\ H_2S + 2\ H_2O \quad (1)$$

452

453 $$2\ H_3AsO_3 + 3\ H_2S \longrightarrow \mathbf{As_2S_3} + 6\ H_2O \quad (2)$$

454

455 $$Zn^{2+} + S^{2-} \longrightarrow ZnS \quad (3)$$

456 This result may be explained by the complete oxidation of glycerol, according to reaction (4),
457 that would produce 2.3 times more H_2S than reaction (1). As a fact, acetate concentration in the
458 bioreactor outlet was lower than the theoretical concentration corresponding to the incomplete
459 oxidation of all the glycerol supplied with the feed water. Nancucheo and Johnson (2014)
460 already observed a decrease of acetate concentration in a sulfate-reducing bioreactor fed with
461 glycerol, when the working pH was decreased from 4.5 to 3.0. The switch from incomplete to
462 complete oxidation of glycerol was accompanied by a change in the bacterial community
463 structure. The solid filling material, initially loaded with agar, yeast extract and glycerol, could
464 also slowly release organic molecules that played the role of complementary electron donors.

$$4\ C_3H_8O_3 + 7\ SO_4^{2-} + 14\ H^+ \longrightarrow 12\ CO_2 + 7\ H_2S + 16\ H_2O \quad (4)$$

Initially, the bioreactor was inoculated with a sulfate-reducing consortium mainly composed SRB affiliated to the *Desulfosporosinus* genus. Two distinct OTU were retrieved; they showed less than 97 % sequence identity on their 16S rRNA genes, and 96-97 % identities with described *Desulfosporosinus* species (*D. lacus D. meridiei*, *D. orientis*, *D. fructosivorans* and *D. auripigmenti*). If we consider a cut-off value at the species level of 98.7% or above on the 16S rRNA gene (Rossi-Tamisier et al., 2015), the two-retrieved OTU may represent new acidophilic species of the *Desulfosporosinus* genus. The OTU sequences had higher identity values with yet undescribed acidophilic isolates of the genus: 99 % between the dominant OTU and *Desulfosporosinus* sp. PFB (Sen and Johnson, 1999) and 98 % between the second SRB OTU and *Desulfosporosinus* sp. OK and OL (MT359229 and MT359230 Genbank accession numbers). The *Desulfosporosinus* genus comprises several members known to reduce sulfate, in acidic conditions, and often isolated from mining sites (*e.g.* Sen and Johnson, 1999; Karnachuk et al., 2009; Alazard et al., 2012; Sánchez-Andrea et al., 2015). All these *Desulfosporosinus* strains are incomplete oxidizers using glycerol to reduce sulfate and produce acetate. Based on community fingerprints, one of the SRB OTU was among the dominant microorganism in the bioreactor, all along the experiment. *Desulfosporosimus*-like 16S sequences were already detected in bioreactors treating As-containing acid solutions (Battaglia-Brunet et al., 2012) and Gonzales et al. (2019) observed a SRB affiliated to *Desulfosporosinus acididurans* becoming dominant in their bioreactor treating a synthetic AMD in continuous mode. Moreover, analysis of microbial communities revealed the widespread presence of *Desulfosporosinus*-related sulfate reducers in diverse SRB reactors treating AMD from laboratory to real scale (Habe et al., 2020).

In the present study, mineralogical observations of the precipitates formed in the bioreactor were globally in line with those obtained from batch experiments, with AMD from the same

site, in comparable conditions (Le Pape et al., 2017). However, if the major occurrence of amorphous orpiment is strongly suspected from SEM-EDXS and confirmed by XANES and EXAFS spectroscopy, no realgar was detected in the form of nanowires in the biofilm observed by SEM-EDXS. Thus, the processes occurring within the continuous column experiment would be representative of the early stages of As reduction and precipitation processes identified in the batch experiment system, before reduction of orpiment into realgar (Le Pape et al., 2017).

When the outlet water from the anaerobic reactor was treated in aerobic condition, partial oxidation of iron and a decrease of pH were observed. The iron removal rate with S1 and SSR waters were 1.1 x 10^{-7} and 0.9 x 10^{-7} $mol.L^{-1}.s^{-1}$ respectively, that is of the same range but lower than the iron removal rates previously reported during direct aerobic treatment of this AMD in the same reactor (1.3 – 2.4 x 10^{-7} $mol.L^{-1}.s^{-1}$, Fernandez-Rojo et al., 2017). The hydraulic residence time was only 300 min, so largely lower than that applied in the anaerobic step. The conditions of this aerobic step remain to be optimized. Gonzales et al. (2019) observed the complete oxidation of the ferrous iron present in the outlet of a SRB bioreactor, corresponding to nearly 1 mM, in 1 h aeration. Here, some As was removed from S1 water previously treated anaerobically, however with SSR water, some release of As(III) occurred. This phenomenon may be linked to the sharp shift from S1 to SSR water compositions, inducing a decrease of As concentration in the feed (17 to 3 mg L^{-1} respectively), and might be linked to the sorption-desorption equilibrium of As(III) associated with the iron precipitates, or to the solubility of As(III)-shwermannite accumulated in the reactor during the previous step. A decrease of As(III)-oxidation activity might also have occurred. The activity of As(III)-oxidizing bacteria may be influenced by the residual organic molecules from the anaerobic reactor. As(III)-oxidizing bacteria can grow heterotrophically or autotrophically (Santini et al., 2000; Battaglia-Brunet et al., 2006; Garcia-Dominguez et al., 2008). In particular, strains of *Thiomonas*, the

515 main As(III)-oxidizing genus found in Carnoulès AMD (Hovasse et al., 2016), are facultative
516 chemolithoautotrophs that grow optimally in mixotrophic media containing reduced inorganic
517 sulfur compounds and organic supplements (Bryan et al., 2009; Slyemi et al., 2011). Tardy et
518 al. (2018) observed a stimulation of As(III)-oxidation by yeast extract, in the AMD of Carnoulès
519 mine. However, the effect of organic substrates might depend on the nature of the supplied
520 molecules: how the bacterial communities of the biofilms inside the aerobic channel are
521 impacted by the anaerobic effluent would require further analyzes.

522 **5 Conclusions**

523 The feasibility of arsenic precipitation as orpiment in a sulfate-reducing bioreactor fed with two
524 types of real As-rich mine drainage waters was demonstrated at laboratory scale. Limitation of
525 sulfate-reduction by low supply of carbon and energy source allowed the removal of most
526 arsenic while iron remained in the water. Precipitation of zinc in the sulfate-reducing reactor
527 was efficient with the low-concentrated AMD, whereas optimization is still needed in order to
528 stabilize this process with the most concentrated AMD. The separation of arsenic from iron in
529 such type of mine water may be beneficial in terms of most hazardous waste mass, as the main
530 part of the arsenic would be concentrated as As_2S_3. The net consumption of glycerol for a given
531 As and Zn removal could be lower than initially expected because this substrate can be entirely
532 and not incompletely degraded. Downstream oxidation and precipitation of iron, besides
533 additional As removal, was proven to occur in an aerobic laboratory bioreactor, although not
534 optimized in the present study. Whereas the two treatment steps studied here allowed the
535 removal of 70 to 95% of As, in two different AMD streams, a down-stream lime drain would
536 be necessary in order to increase the final pH of the treated water, and remove the residual As
537 and Fe by co-precipitation. Results support the feasibility of applying simple combination of
538 low-cost treatment options, including semi-passive SRB bioreactor, for the remediation of As-
539 rich AMDs.

Declarations

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Conflict of Interest The authors declare they have no conflict of interest.

Availability of data and material All data are available on request to the corresponding author (e.mail: f.battaglia@brgm.fr)

Authors contribution F. Battaglia-Brunet: Conceptualization, Writing, Supervision; L. Fernandez-Rojo: Investigation, Editing; M. Hery: Editing; C. Casiot: Conceptualization, Methodology, Supervision, Writing, Editing; P. Le Pape: Investigation, Writing, Editing; H. Tris: Investigation; S. Touzé: Conceptualization; G. Morin: Supervision, Editing; C. Joulian: Investigation, Supervision, Writing, Editing.

Figure captions

Figure 1. Bioreactor filling and feeding modes, down-flow (A), up-flow (B), and pictures taken just after filling (C) and at the end of experiment (D).

Figure 2. Evolution of parameters in the bioreactor fed with springs S1 then SSR mine water. A: residence time (h), B: arsenic concentration ($mg.L^{-1}$), C: zinc concentration ($mg.L^{-1}$), D: iron concentration ($mg.L^{-1}$), E: pH. Numbered vertical lines show specific events: (1) and (3) decrease of residence time; (2) decrease in feed pH, (4) change from spring S1 to spring SSR.

561 The pH value increased from 4.0 in the feed to 4.5-5.0 in the outlet, except during the last days
562 of experiment, probably because of the decrease of residence time from 40 to 30 h (Fig. 2E).

563

564 **Figure 3.** Evolution of 16S rRNA gene CE-SSCP fingerprints at the bottom (B), mid (M) and
565 top (T) of the column. *: signal matching with the signal of the *Desulfosporosinus* OTU
566 originally dominant in the inoculated bacterial mixed culture. Horizontal scale: migration
567 position of detected signals determined from electrophoretic scans.

568

569 **Figure 4.** Analysis of As mineralogy and speciation in yellow deposits that precipitated at the
570 surface of pouzzolana grains in the column experiment. (A) SEM image in backscattering
571 electron mode showing the biofilm morphology. Plain orange arrows indicate mineralized
572 encrusted bacterial cells; dashed orange arrows indicate non-mineralized cells; Blue arrows
573 indicate aggregates of spherical nanoparticles. (B) Microanalyses performed using Energy
574 dispersive X-Ray spectroscopy (EDXS) at different spots of approximately 1µm^3 on the biofilm
575 sample (spots 1 to 6 in red on the image). The emission spectra 1 to 6 show the presence of As
576 and S as main constitutive elements (C) XANES spectra and (D) EXAFS spectra of the bulk
577 sample (black) and of amorphous orpiment reference sample (red). Bulk solid speciation of As
578 in the biofilm is similar to that of amorphous orpiment (E) Fast Fourier transform of the EXAFS
579 signal. Additional data relative to XAS analysis for different samples of the column are
580 available in SM4 (Figures SM4B and SM4D, and Table SM4C).

581

582

583

584 **Figure 5.** Evolution of S1 (A) and SSR (B) water parameters before treatment, after the
585 anaerobic step (SRB) and in the outlet of the aerobic step (OX).
586
587 **Figure 6.** Theoretical mass balance for the treatment of spring S1 AMD by a SRB bioreactor
588 coupled with acidic aerobic step followed by lime addition, compared with simple one-step
589 lime treatment. The percentage of Fe removal as schwertmannite by acidic oxidation is inspired
590 by previous results of Fernandez-Rojo et al. (2017); (a) analytical quantification limit; (b)
591 formula according to Garcia-Rios et al. (2021); (c) solubility of gypsum according to Lebedev
592 and Kosorukov (2017).
593

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741

742

743 **Table 1.** Characteristics of the two water samples treated in this study

Water sample	As $mg.L^{-1}$	Zn $mg.L^{-1}$	Fe $mg.L^{-1}$	SO_4 $mg.L^{-1}$	pH
Spring S1	174	26.0	1345	3700	3.29
Spring SSR	18	4.4	307	1500	4.02

744

745

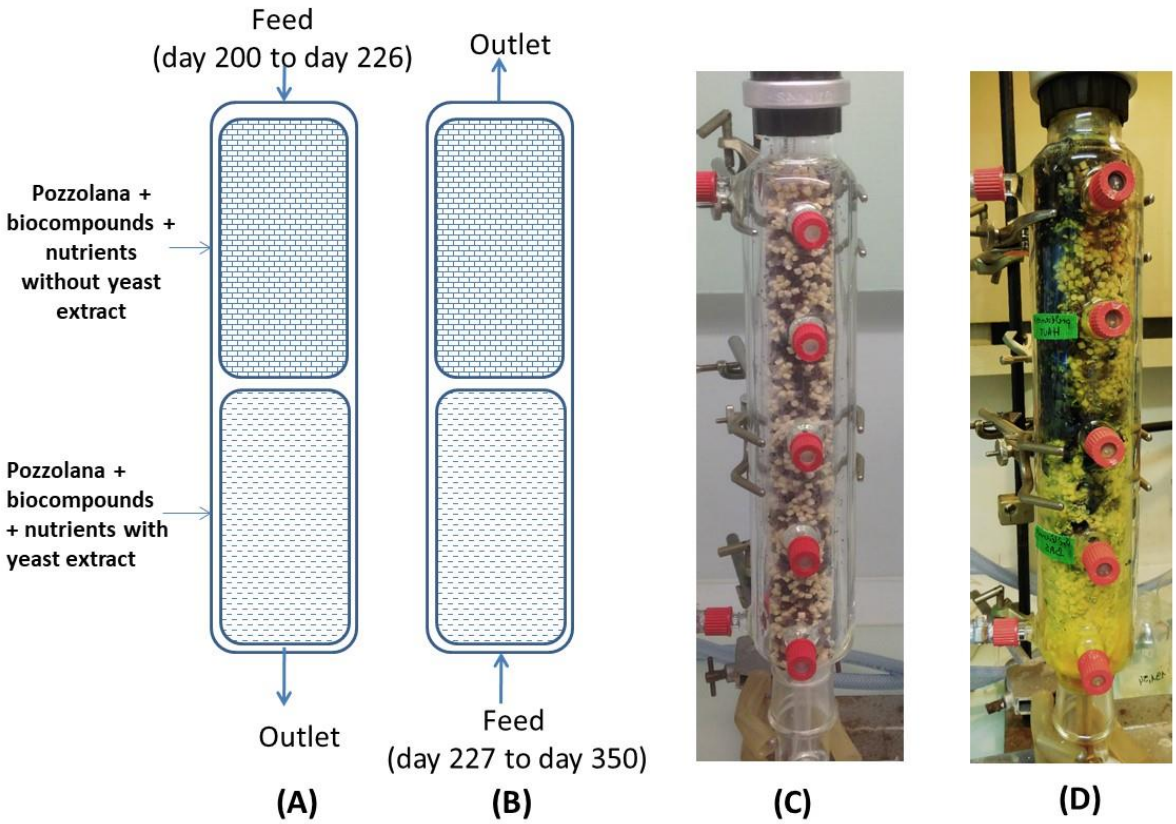


Figure 1

746

747

748

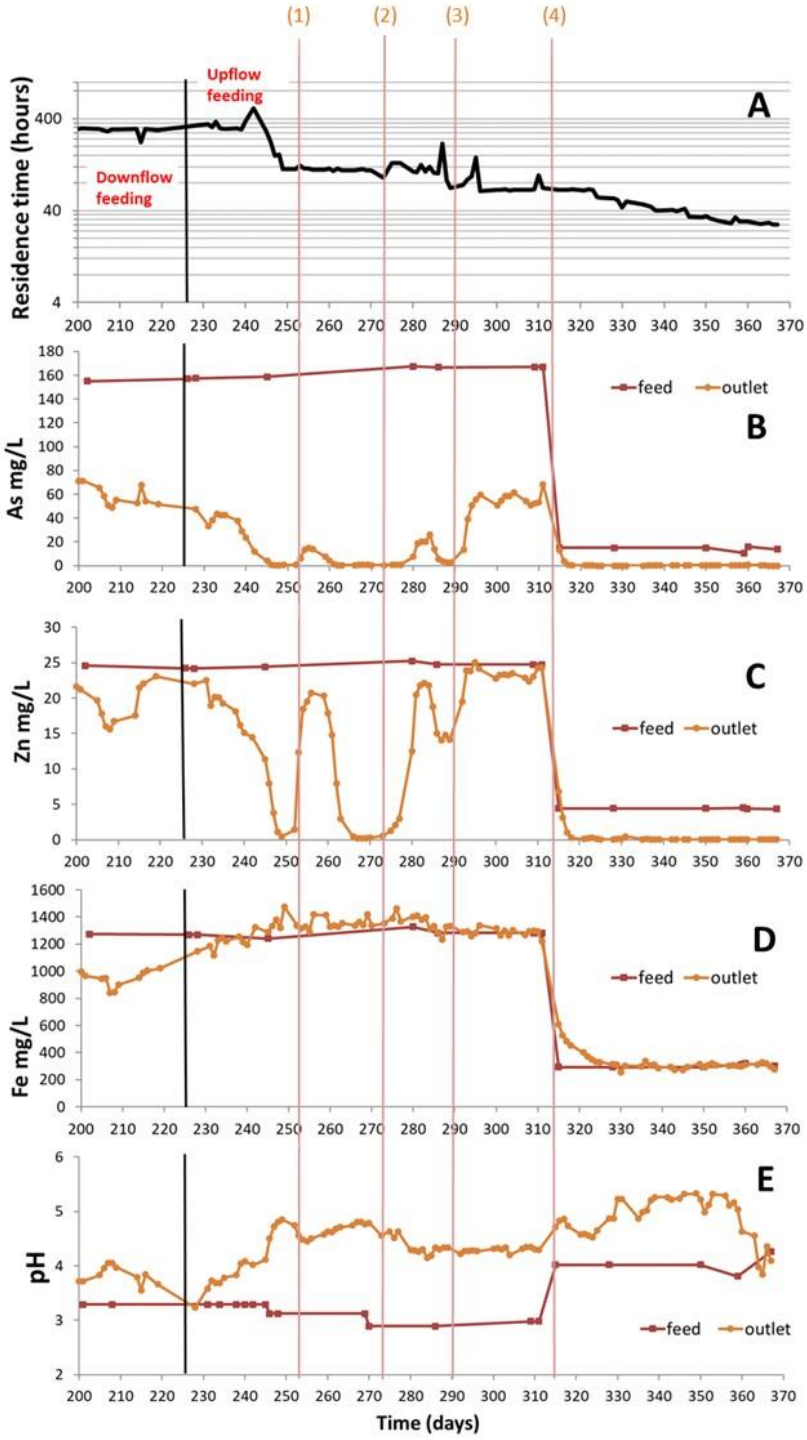

(1)
(2)
(3)
(4)
Upflow feeding
Downflow feeding
A
Residence time (hours)
400
40
4
200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370
B
As mg/L
feed
outlet
C
Zn mg/L
D
Fe mg/L
E
pH
Time (days)

Figure 2

752

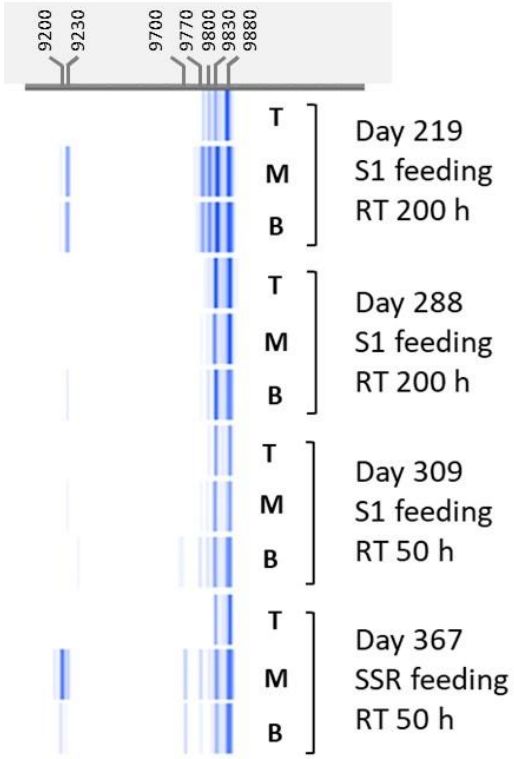


Figure 3

753

754

755

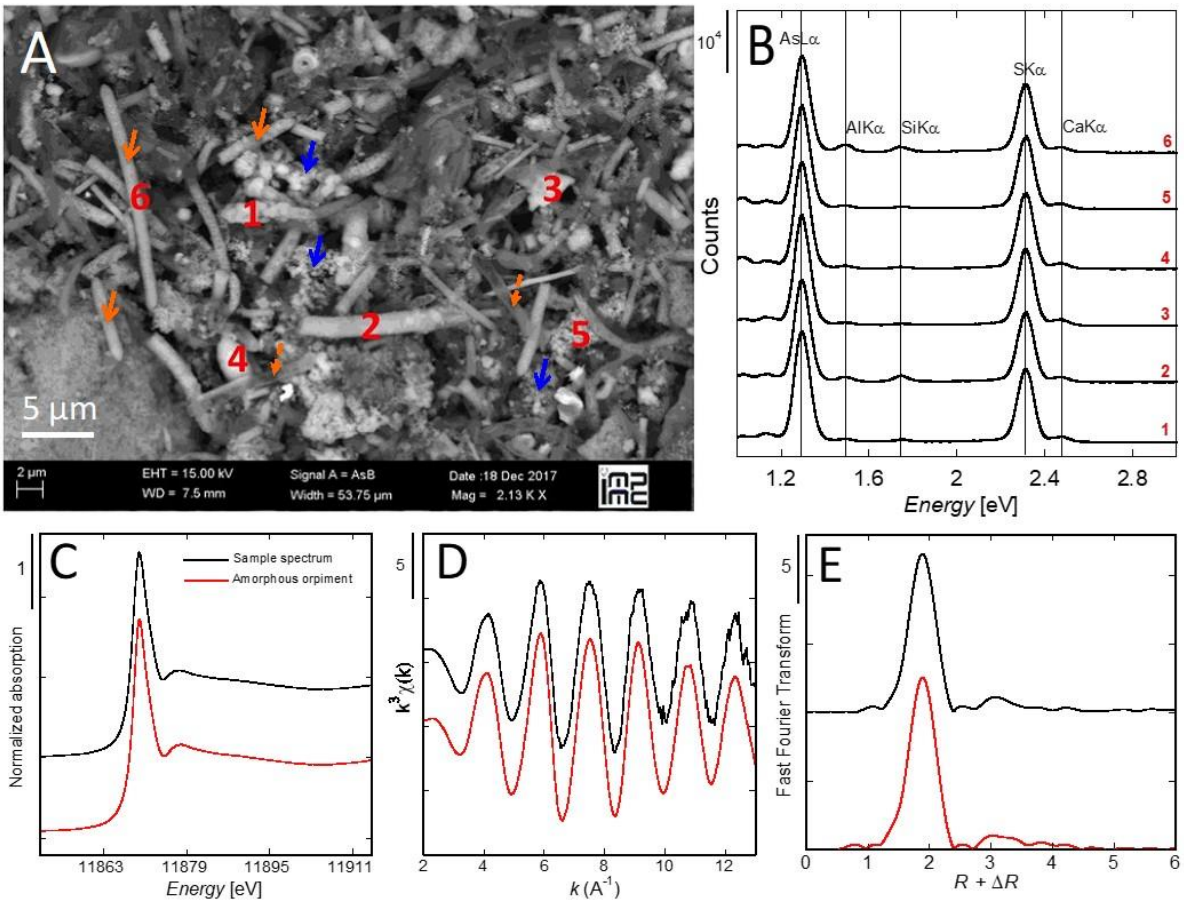

A
5 µm
2 µm
EHT = 15.00 kV
WD = 7.5 mm
Signal A = AsB
Width = 53.75 µm
Date :18 Dec 2017
Mag = 2.13 K X
B
AsLα
AlKα
SiKα
SKα
CaKα
Counts
Energy [eV]
C
Sample spectrum
Amorphous orpiment
Normalized absorption
Energy [eV]
D
k³χ(k)
k (A⁻¹)
E
Fast Fourier Transform
R + ΔR

Figure 4

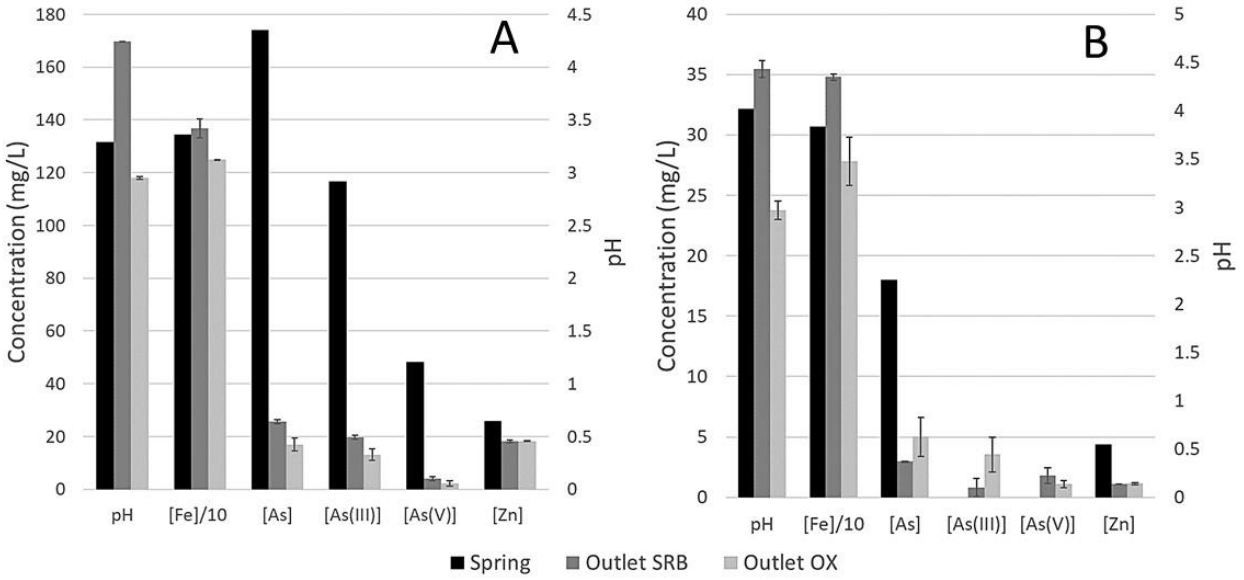


Figure 5

759

760

761

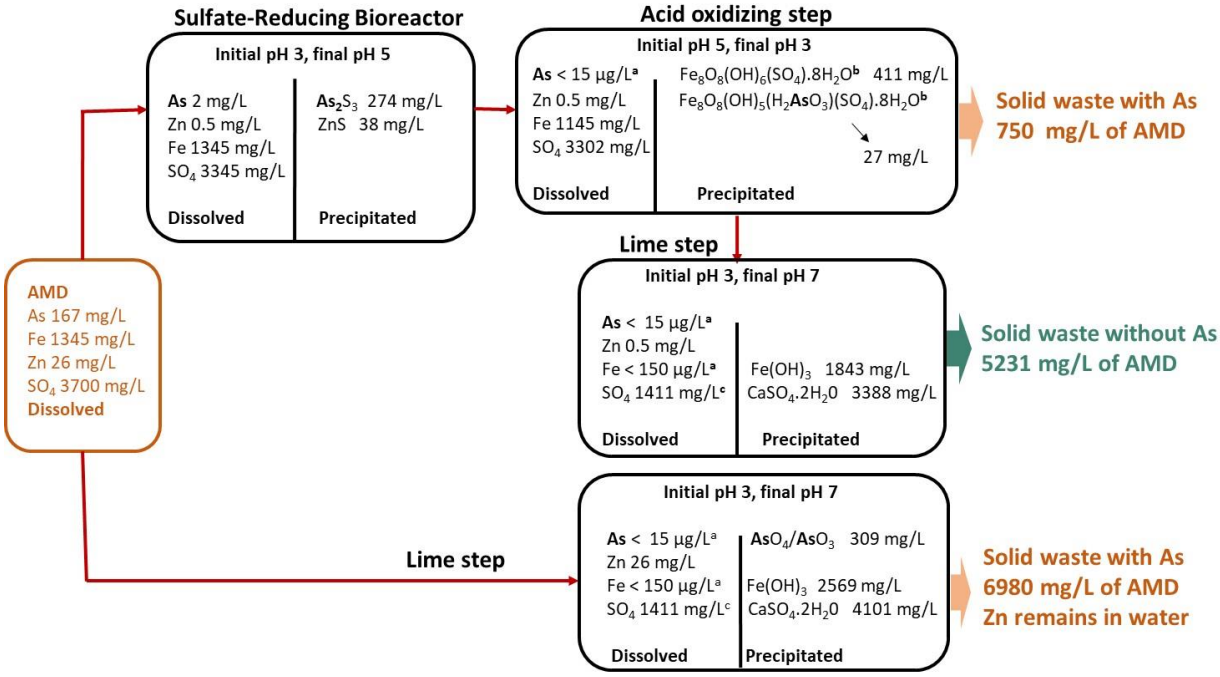

Sulfate-Reducing Bioreactor
Initial pH 3, final pH 5
As 2 mg/L
Zn 0.5 mg/L
Fe 1345 mg/L
SO4 3345 mg/L
Dissolved
As2S3 274 mg/L
ZnS 38 mg/L
Precipitated
Acid oxidizing step
Initial pH 5, final pH 3
As < 15 µg/La
Zn 0.5 mg/L
Fe 1145 mg/L
SO4 3302 mg/L
Dissolved
Fe8O8(OH)6(SO4).8H2Ob 411 mg/L
Fe8O8(OH)5(H2AsO3)(SO4).8H2Ob
27 mg/L
Precipitated
Solid waste with As
750 mg/L of AMD
Lime step
Initial pH 3, final pH 7
As < 15 µg/La
Zn 0.5 mg/L
Fe < 150 µg/La
SO4 1411 mg/Lc
Dissolved
Fe(OH)3 1843 mg/L
CaSO4.2H20 3388 mg/L
Precipitated
Solid waste without As
5231 mg/L of AMD
AMD
As 167 mg/L
Fe 1345 mg/L
Zn 26 mg/L
SO4 3700 mg/L
Dissolved
Lime step
Initial pH 3, final pH 7
As < 15 µg/La
Zn 26 mg/L
Fe < 150 µg/La
SO4 1411 mg/Lc
Dissolved
AsO4/AsO3 309 mg/L
Fe(OH)3 2569 mg/L
CaSO4.2H20 4101 mg/L
Precipitated
Solid waste with As
6980 mg/L of AMD
Zn remains in water

Figure 6

Laboratory-scale bio-treatment of real arsenic-rich acid mine drainage

Battaglia-Brunet Fabienne, Casiot Corinne, Fernandez-Rojo Lidia, Hery Marina, Le Pape Pierre, Tris Hafida, Morin Guillaume, Touzé Solène, Joulian Catherine

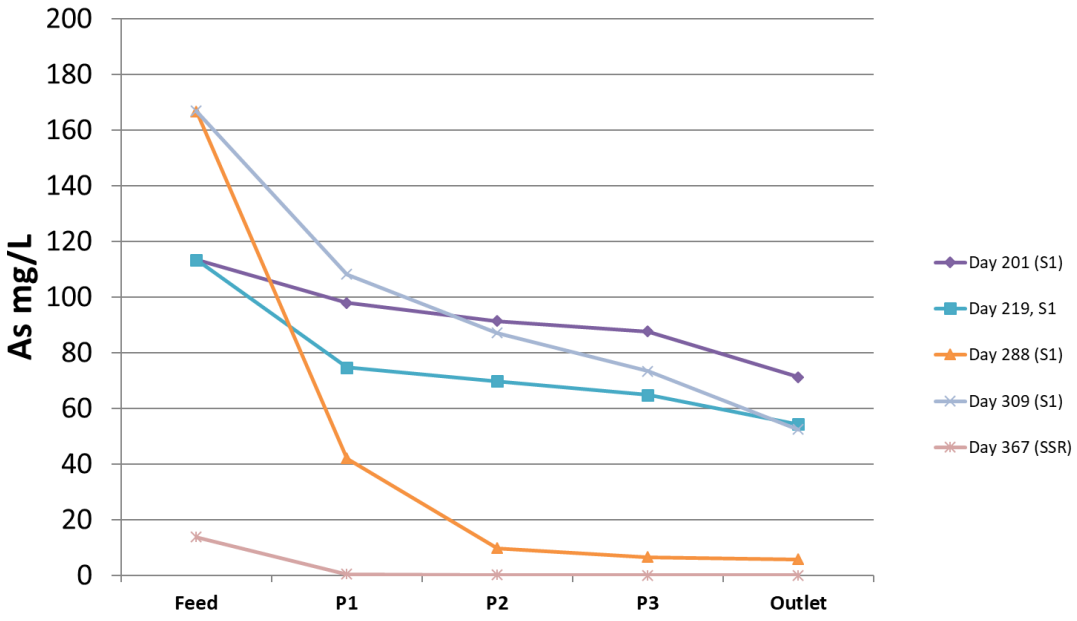
Supplementary Material SM1. Composition of the basal medium used to enrich sulfate-reducing bacteria

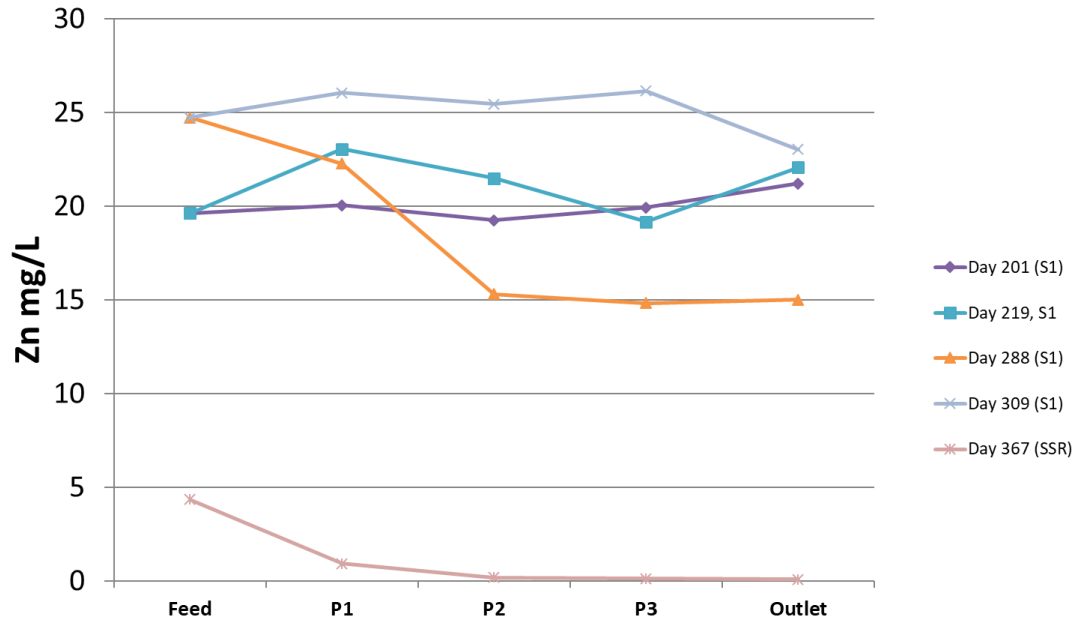
The basal enrichment medium contained the following ingredients, per litre: K_2HPO_4, 0.5 g ; NH_4Cl, 2 g ; Na_2SO_4, 1.4 g ; $MgSO_4$, 7 H_2O, 2 g ; glycerol, 0.5 g ; yeast extract, 1 g ; trace elements solution, 1 ml. The pH of this basal solution was adjusted to 4.

The trace metals solution composition was the following, per litre: EDTA, 3 g ; $FeSO_4$, 7 H_2O, 1.1 g ; $MnSO_4$, 65 mg ; $ZnSO_4$, 89 mg ; $NiCl_2$, 24 mg ; Na_2MoO_4, 2 H_2O, 18 mg ; H_3BO_3, 0.3 g ; $CuCl_2$, 2 mg and $CoSO_4$, 7 H_2O, 130 mg.

The basal medium was heated to ebullition, then distributed in 50 mL penicillin flasks equipped with rubber stoppers, flushed with N_2, and sterilized by autoclaving 30 min at 120°C.

Supplementary Material SM2. Profiles of As and Zn concentrations





Supplementary Material SM3. Acetate and total organic carbon in the anaerobic bioreactor outlet.

Table SM3. Acetate and total organic carbon in the anaerobic bioreactor outlet.

Day - condition	Acetate $mg.L^{-1}$	Theoretical acetate* $mg.L^{-1}$	Organic Carbon acetate $mg.L^{-1}$	Total organic carbon $mg.L^{-1}$
201 - S1 downflow	2.4	166	0.9	62.3
219 - S1 downflow	0.5	166	0.2	75.5
249 – S1 upflow	15.5	163	6.1	74.2
270 – S1 upflow	25.5	163	10	27.2
286 – S1 upflow	30.4	163	12	29.9
308 – S1 upflow	44.5	163	17.5	66.3
359 – SSR upflow	14.1	33	6.9	35.7
366 – SSR upflow	30.1	33	11.8	36.7

(*) Theoretical acetate through incomplete oxidation of glycerol in the feed water according to $C_3H_8O_3 + 0.75\ SO_4^{2-} + 1.5\ H^+ \Rightarrow C_2H_4O_2 + CO_2 + 0.75\ H_2S + 2\ H_2O$

Supplementary Material SM4. Characterization of the solid phases sampled in the anaerobic reactor

Sample 1: Black pouzzolana + yellow deposit
Sample 2: Gelatinous yellow colored material
Sample 3: Wolly black material

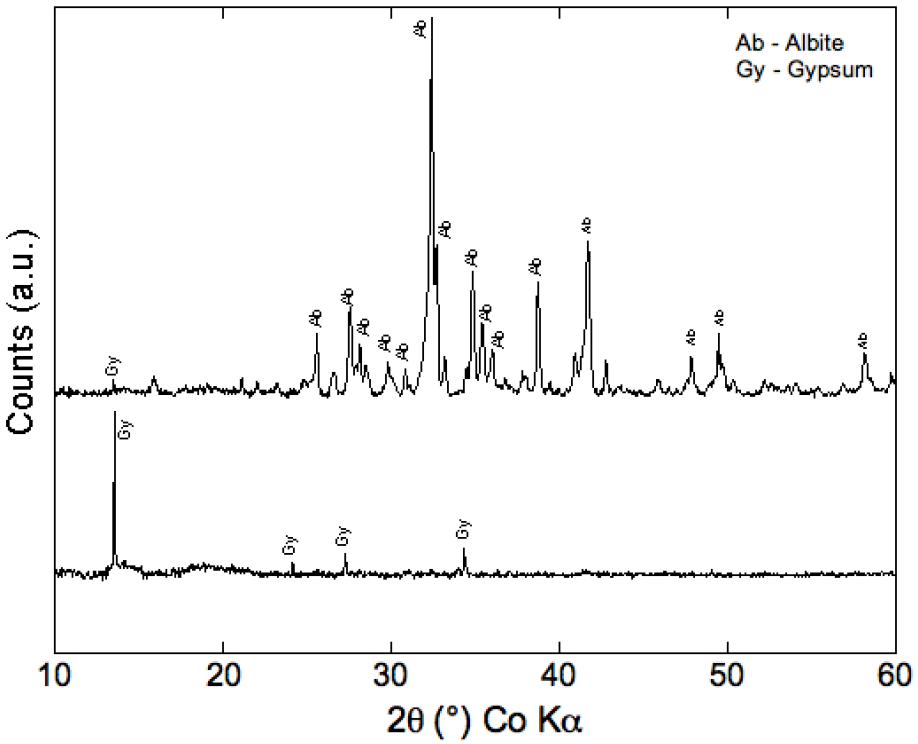


Figure SM4-A – *X-ray diffractograms of a reacted pouzzolana grain (up) and of the yellow precipitate sampled at the surface of pouzzolana grains (down). These materials were parts of sample 1.*

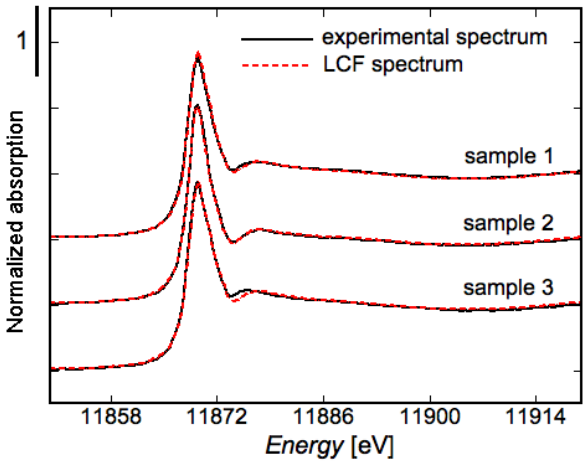


Figure SM4-B – *X-ray Absorption Near Edge Structure (XANES) experimental spectra and linear combination fits for samples 1, 2, and 3.*

	amorphous orpiment	As(III)-sorbed ferrihydrite	R-factor	Red-chi2
sample 1	100	0	0.0015	0.0004
sample 2	89	13	0.003	0.00086
sample 3	90	11	0.002	0.00055

Table SM4-C – *Relative contributions of As species in the experimental XANES spectra (Fig. 4) by performing a linear combination fitting procedure with two reference compounds, namely amorphous orpiment (As(III)-S) and arsenite-sorbed ferrihydrite (As(III)-O). R-factor and Red-chi^2 are given as indicators of the quality of fits.*

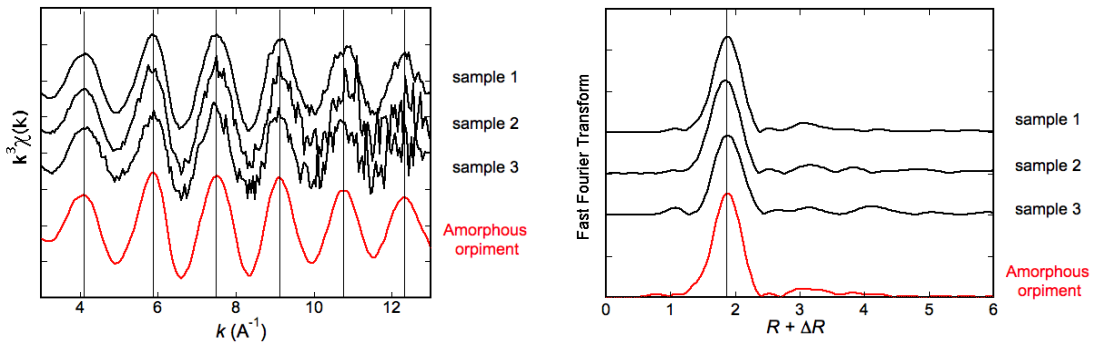


Figure SM4-D – *Extended X-Ray Absorption Fine Structure (EXAFS) presented for the experimental spectra of samples 1, 2, and 3 and for the amorphous orpiment reference mineral.*