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## Bio-precipitation of arsenic and antimony in a sulfate-reducing bioreactor treating real acid mine drainage water

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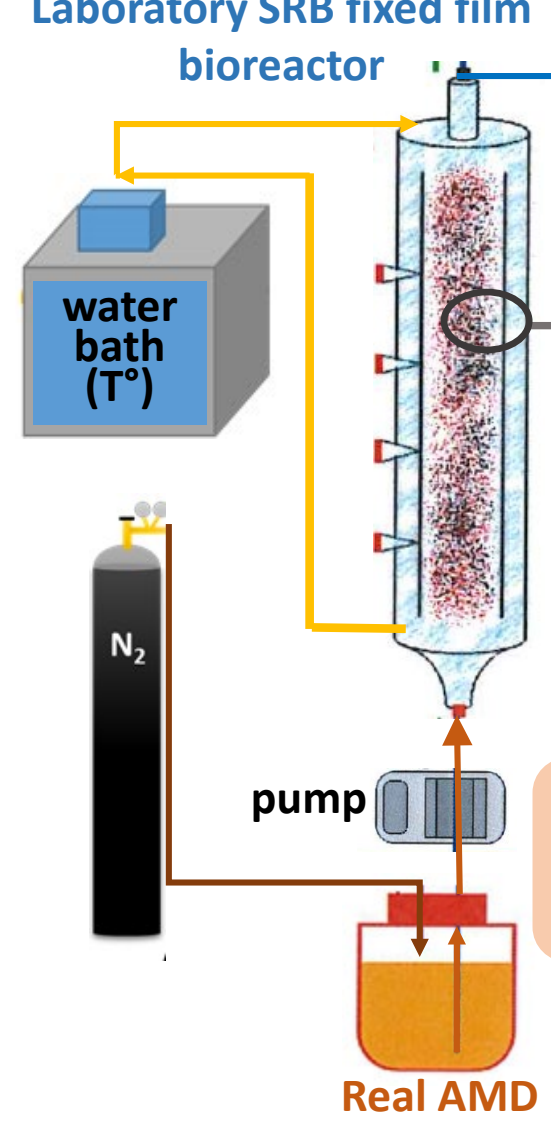
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**Bio-precipitation of antimony in a sulfate-reducing  
bioreactor treating a real As-rich acid mine drainage water**

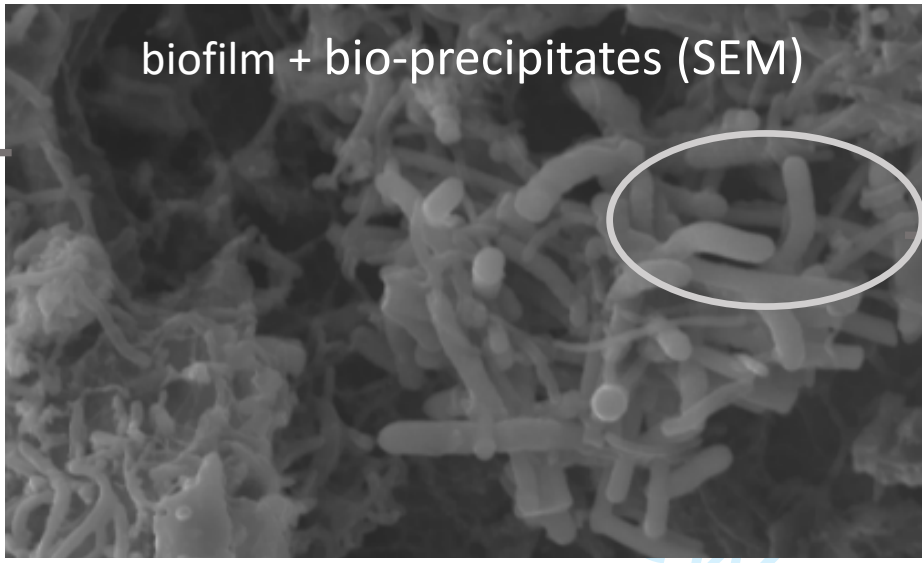
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Keywords:	antimony, arsenic, bioremediation, acid mine drainage, sulfate-reducing bacteria, Desulfosporosinus

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### Laboratory SRB fixed film bioreactor



> 96 % As removed  
> 97% Sb removed



biofilm + bio-precipitates (SEM)

16S rRNA gene metabarcoding



*Desulfosporosinus,*  
*Cellulomonas,*  
*Microbacter...*

As 1mM  
Sb from 0.01 mM to 1 mM

Real AMD

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3 **1 Bio-precipitation of antimony in a sulfate-reducing bioreactor treating a**  
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6 **2 real As-rich acid mine drainage water**  
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14 4 Elia Laroche<sup>1,2</sup>, Catherine Jouliau<sup>1</sup>, Cédric Duee<sup>1</sup>, Corinne Casiot<sup>2</sup>, Marina Héry<sup>2</sup>, Fabienne  
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36 **12 Abstract:**  
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39 13 Arsenic (As) and antimony (Sb) can be disseminated from mining sites to aquatic ecosystems  
40  
41 14 by acid mine drainage (AMD). Here, the possibility to remove As and Sb concomitantly from  
42  
43 15 acidic waters by precipitation of sulphides induced by sulfate-reducing bacteria (SRB) was  
44  
45 16 investigated in a fixed-bed column bioreactor. The real AMD water used to feed the  
46  
47 17 bioreactor contained nearly 1 mM As and increasing Sb concentrations ( $0.008 \pm 0.006$  to  $1.01$   
48  
49 18  $\pm 0.07$  mM) in order to reach a molar ratio  $Sb/As = 1$ . Results showed that the addition of Sb  
50  
51 19 did not affect the efficiency of As bio-precipitation. Sb was removed efficiently (up to 97.9%  
52  
53 20 removal) between the inlet and outlet of the bioreactor, together with As (up to 99.3%  
54  
55 21 removal) in all conditions. Sb was generally removed as it entered the bioreactor. Appreciable  
56  
57 22 sulfate reduction occurred in the bioreactor and could be linked to the stable presence of a  
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3 23 major SRB OTU affiliated to the *Desulfosporinus* genus. The bacterial community included  
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5 24 polymer degraders, fermenters, and acetate degraders. Results suggested that sulfate-reduction  
6  
7 25 could be a suitable bioremediation process for the simultaneous removal of Sb and As from  
8  
9 26 AMD.

10  
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12  
13 27 **Keywords:** antimony, arsenic, bioremediation, acid mine drainage, sulfate-reducing bacteria,  
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15 28 *Desulfosporosinus*  
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19 29

## 30 **Introduction**

31 Elevated concentrations of arsenic (As) and antimony (Sb) have been measured in diverse  
32 natural environments due to anthropogenic emissions (Smedley and Kinniburgh, 2002; Herath  
33 et al., 2017). These toxic metalloids are considered by the European Union and United States  
34 Environmental Protection Agency as pollutants of priority interest (Council of the European  
35 Communities, 1976; US EPA, 1982). Mining activities are a major source of As and Sb  
36 dissemination in the environment (Smedley and Kinniburgh, 2002; He et al., 2019). Wastes  
37 generated by mineral extraction can contain up to 10,000 ppm As and 1,000 ppm Sb  
38 associated with sulfide minerals (Smedley and Kinniburgh, 2002; Wu et al., 2011; Cidu et al.,  
39 2014). As result of inappropriate waste storage, oxidation of the sulfide minerals can generate  
40 mine drainage characterised by high concentrations of these elements, which can enter aquatic  
41 ecosystems (Manaka et al., 2007; Resongles et al., 2013; Paikaray, 2015; Sun et al., 2016).  
42 However, solubilised As and Sb can be immobilised by co-precipitation with insoluble metal  
43 (hydro)oxides (Cullen and Reimer, 1989; Filella et al., 2002). These processes have been  
44 exploited in water treatment technology, and the combined removal of these two elements  
45 following iron (Fe) addition was considered. However, variable efficiencies of As and Sb  
46 adsorption onto iron compounds have been reported due to synergic or competitive behaviour

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3 47 according to the speciation of these elements (Lan et al., 2016; Qi and Pichler, 2017; Inam et  
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5 48 al., 2018; Wu et al., 2018) and pH (Inam et al., 2018). Moreover, the disadvantages of Fe-  
6  
7 49 based treatments are the need of a constant maintenance and elevated costs. The development  
8  
9 50 of passive treatment systems exploiting biological processes is a promising low-cost strategy  
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11 51 for the remediation of metals and metalloids present in acid mine drainages (Skousen et al.,  
12  
13 52 2000). Biological processes including direct bio-reduction or bio-oxidation can be exploited  
14  
15 53 in bioremediation solutions. Therefore, Kujala et al. (2022) studied the passive treatment of  
16  
17 54 mining-affected neutral water containing both Sb and As (around 2 and 0.5  $\mu\text{M}$  respectively)  
18  
19 55 in peatlands, involving bio-oxidation and bio-reduction processes. Among the anaerobic  
20  
21 56 biological reactions, microbial sulfate-reduction has proven itself as an efficient way to  
22  
23 57 remove metals from mine waters at large scale (Mattes et al., 2011). In anaerobic bioreactors  
24  
25 58 or wetlands, the biological sulfide precipitation exclusively precipitates elements that are  
26  
27 59 already in the mine water. No external element (calcium or iron) is added, thus the final mass  
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29 60 of produced waste is lower than in competitor acid mine drainage technologies (Battaglia-  
30  
31 61 Brunet et al., 2021). Sulfate reduction has been shown to efficiently treat As and Sb  
32  
33 62 individually (Wang et al., 2013; Zhang et al., 2016; Alam and McPhedran, 2019; Xi et al.,  
34  
35 63 2020, Chen et al., 2020). Liu et al. (2018) also reported the concomitant removal of 5 mg/L  
36  
37 64 As and Sb from artificial waste water at neutral pH by bacterial sulfate reduction. In this batch  
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39 65 experiment, the removal of As depended on the presence of Fe(II) in the synthetic medium,  
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41 66 whereas Sb was removed even in absence of Fe. Although microbial sulfate-reduction has  
42  
43 67 been described for a long time as a bioprocess inhibited in acidic conditions, progress has  
44  
45 68 been made recently in the field of bio-precipitation of sulfides at low pH (Ñancucheo and  
46  
47 69 Johnson, 2012 and 2014). More specifically, bio-precipitation of arsenic sulfide at low pH  
48  
49 70 was reported (Battaglia-Brunet et al., 2012; Le Pape et al., 2017). From a chemical point of  
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51 71 view, precipitation of As sulfide should be favored at low pH, while soluble thio-As  
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3 72 complexes form at neutral and alkaline pH. The same geochemical behaviour was observed  
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5 73 with Sb in geothermal systems (Planer-Friedrich and Scheinost, 2011). The pH is likely to  
6  
7 74 affect the biogeochemical behaviour of Sb in sulfate-reducing bioreactors, as it was the case  
8  
9 75 for As. Finally, the combined removal by bacterial sulfate-reduction of both As and Sb, when  
10  
11 76 they co-occur in an AMD has not yet been studied. In this context, the aim of the present  
12  
13 77 study was to evaluate the efficiency of an upflow anaerobic fixed-bed column reactor to treat  
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15 78 concomitantly As and Sb present in an AMD by biological sulfate-reduction in acidic  
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17 79 conditions. The removal of these pollutants was investigated during ten months along the  
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19 80 bioreactor in which Sb concentration was increased progressively. Physico-chemical  
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21 81 parameters were monitored, together with mineral characterisation of the bioprecipitates. The  
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23 82 spatial and temporal evolution of the bacterial community colonizing the reactor was  
24  
25 83 investigated by 16S rRNA gene metabarcoding. Both results will document the understanding  
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27 84 of the biogeochemical behaviour of As and Sb, in the context of sulfate reduction under acidic  
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29 85 conditions.  
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## 40 **Material and methods**

### 41 **Bioreactor design and operating conditions**

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45 89 The lab-scale experiment was performed in a fixed-bed reactor, consisting of a 360 mm glass  
46  
47 90 column with an internal diameter of 36 mm (Fig. 1). A water jacket regulated the column  
48  
49 91 temperature at 25°C during the experiment and rubber-stopped sampling ports allowed liquid  
50  
51 92 sampling from the middle, bottom and upper levels. The column was sterilised by autoclaving  
52  
53 93 (120°C, 30 min) before filling with sterile materials composed of a mixture of pozzolana and  
54  
55 94 nutritive agar in order to enhance both bacterial attachment and growth, as previously  
56  
57 95 described (Battaglia-Brunet et al., 2021). The filling mixture was prepared with a total volume  
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3 96 of 400 mL pozzolana, 400 mL agar at 25 g/L, 20 mL nutritive solution at pH 4.5 (20 g/L yeast  
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5 97 extract and 7.8 g/L  $\text{KH}_2\text{PO}_4$ ) and 0.4 mL trace element solution (3 g/L EDTA; 1.1 g/L  $\text{FeSO}_4$ ,  
6  
7 98  $7\text{H}_2\text{O}$ ; 65 mg/L  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ; 89 mg/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; 28 mg/L  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ; 18 mg/L  
9  
10 99  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ; 0.3 g/L  $\text{H}_3\text{BO}_3$ ; 2 mg/L  $\text{CuCl}_2$ ; 130 mg/L  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ ).

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12  
13 100 The filled column was de-oxygenated by flushing with sterile  $\text{N}_2$  during three days, then  
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15 101 inoculated with an acid tolerant microbial consortium containing sulfate-reducing bacteria  
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17 102 (SRB) originating from the AMD of Carnoulès, France (Le Pape et al., 2017). Ten mL of this  
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19 103 inoculum were centrifuged and the pellet stored at  $-80^\circ\text{C}$  prior molecular analyses. A first step  
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21 104 promoting bacterial growth inside the column was performed in batch mode during 23 days.  
22  
23 105 Then, the column was fed in continuous mode with AMD water sampled in January 2016 at  
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25 106 the Carnoulès mine (Gard, France) and stored at  $4^\circ\text{C}$  under nitrogen until use. The main  
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27 107 characteristics of this AMD are a pH close to 3, around 100 mg/L total dissolved As, 1000  
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29 108 mg/L total dissolved Fe, 20 mg/L total dissolved Zn (Le Pape et al., 2017), and less than 1  
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31 109 mg/L total dissolved Sb (Resongles et al., 2013). For the experiment, its pH was increased  
32  
33 110 between pH 4.0 and 4.5 by adding NaOH. The water was filtered ( $0.22 \mu\text{m}$ ) and  
34  
35 111 complemented with 0.5 g/L glycerol as electron donor for the SRB. The feeding water was  
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37 112 maintained in anaerobic condition under  $\text{N}_2$  atmosphere.

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43 113 The variable parameter was the Sb concentration in the feed water: it was increased by adding  
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45 114 Sb in the real AMD water, as potassium Sb(III) tartrate, in three steps (corresponding to phase  
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47 115 1, 2 and 3) up to the molar equivalence between Sb and the As naturally present in the AMD.  
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49 116 The experimental parameters of each phase, flow rate, pH, As and Sb concentration in the  
50  
51 117 feed solution prepared with the real AMD, are summarised in Table 1. Analyses of  
52  
53 118 exploitable data started after the physico-chemical parameters (outlet pH, As, and Fe  
54  
55 119 concentration in the outlet) had stabilised for each Sb concentration in the feed in order to  
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57 120 compare the acquired results during each experimental condition.



**121 Monitoring**

122 Inlet and outlet waters were collected under anaerobic conditions and monitored over a 217  
123 days period after the first 59 days dedicated to stabilisation of physico-chemical parameters.  
124 The real flow-rate was calculated by weighing the cumulated outlet water collected in a bottle  
125 (every day except Saturdays and Sundays). Inlet and outlet water pH were measured, and 10  
126 mL were filtered at 0.45 µm, acidified with one drop of concentrated nitric acid and stored at  
127 4°C until chemical analyses. The outlet water was sampled every day whereas the inlet water  
128 sampling was done at each renewal of feed water.

129 Vertical profiles of physico-chemical and microbial parameters were determined at the end of  
130 each phase from liquid samples taken along the column. Five mL of liquid samples were  
131 collected in triplicate inside the column through the bottom, middle and upper septa with a  
132 sterile syringe. These samples were mainly constituted of solution, with bacteria in  
133 suspension, but could also contain precipitates (with or without attached bacteria). Triplicate  
134 samples were also collected from inlet and outlet water bottles. Samples were filtered with a  
135 sterile 0.22 µm cellulose acetate filter which was stored at -80°C for molecular analyses. The  
136 filtrate was acidified and kept at 4°C for chemical analysis.

137 At the end of the experiment (day 217), the column was opened in a glovebox under N<sub>2</sub>  
138 atmosphere to collect bioprecipitates deposited on the solid filling materials. Triplicate  
139 samples of solids were taken from the bottom, middle and upper parts of the column and  
140 stored at -80°C until molecular analysis. Solid samples were also collected along the column  
141 and dried under N<sub>2</sub> flux in anaerobic Hungate glass tubes for mineralogical characterisation.

**142 Chemical analyses**

143 Total As and Fe concentrations were monitored on outlet samples to check that stabilisation  
144 was reached before changing the operating conditions, by Atomic Absorption

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3 145 Spectrophotometry (AAS, Varian SpectrAA 220 FS). After stabilisation, a more complete  
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5 146 chemical characterisation was performed on selected water samples from outlet and inlet  
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8 147 (three for each feed phase, except the Sb concentration from the inlet water in phase 1 in  
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10 148 duplicate, Table 1) and from the vertical profiles collected at the end of each phase. In these  
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12 149 samples, total dissolved concentrations of As, Sb, Fe, S and other elements (Zn, Tl, Pb) were  
13  
14 150 analysed using ICP-MS on the AETE-ISO platform (University of Montpellier).

### 17 151 **Characterisation of the bioprecipitates**

20 152 The bioprecipitates collected at the end of the experiment were characterised by scanning  
21  
22  
23 153 electron microscopy (SEM). Prior to SEM, samples were submitted to plasma cleaner  
24  
25 154 (Gambetti, Colibri, 300 s under Ar plasma) in order to remove organic phases. Samples were  
26  
27 155 then covered with a 20 nm carbon coating (Cressington, Carbon Coater 208C) to make them  
28  
29 156 conductive. The SEM images were acquired on a field emission gun scanning electron  
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31 157 microscope MIRA 3 XMU (TESCAN, Brno, Czech Republic), under high vacuum conditions  
32  
33 158 with a 15 kV beam. EDS spectra and element mappings were recorded using an EDAX  
34  
35 159 Pegasus EDS system.

### 39 160 **Molecular analyses**

42 161 Genomic DNA was extracted from the pellet of the inoculum, frozen filters (5 mL aliquot)  
43  
44 162 and bioprecipitates (between 0.5 and 0.8 g), using the FastDNA Spin Kit for soil according to  
45  
46 163 the manufacturer's recommendations (MP Biomedicals, Illkirch, France), with the FastPrep®-  
47  
48 164 24 instrument at a speed of 5 ms<sup>-1</sup> for 30s. DNA extracts were quantified with the Quantifluor  
49  
50 165 dsDNA sample kit and the Quantus fluorometer, according to the manufacturer's instructions  
51  
52 166 (Promega, Madison, WI, USA), and stored at -20°C.

56  
57 167 Bacterial diversity was determined by 16S rRNA gene metabarcoding. Libraries of amplicons  
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59 168 and sequencing were performed by MetaHealth metagenomic-based services (CIRAD, PHIM,  
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3 169 Eco&Sols, Montpellier, France). The 16S rRNA V3-V4 gene region was first amplified with  
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5 170 primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-  
6  
7 171 GACTACHVGGGTATCTAATCC-3'), in duplicate in a Mastercycler ep 384 (Eppendorf)  
8  
9 172 thermocycler with 20 µL PCR reaction (0.5 g of DNA, 10 µL of Phusion Flash High-Fidelity  
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11 173 2X Master Mix (Thermo Fisher), 300 ng T4 gene 32 (MP Biomedicals), 0.25 µM for forward  
12  
13 174 and reverse primers). The following conditions were applied: initial denaturation at 95°C for 2  
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15 175 min, 25 cycles of 95°C for 30 s, 55°C for 40 s, 72°C for 30 s and a final elongation at 72°C  
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17 176 for 10 min. A second PCR was run using primers 341F and 785R modified with Illumina  
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19 177 specific overhang sequences to implement dual barcodes. PCR reactions (15 µL) contained:  
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21 178 7.5 µL Biolabs Taq 2X mastermix, 2 µL of 10 µM for forward and reverse primers, and 2 µL  
22  
23 179 of PCR product. The following conditions were applied: initial denaturation at 95°C for 2  
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25 180 min, 8 cycles of 95°C for 30 s, 55°C for 40 s, 72°C for 30 s and a final elongation at 72°C for  
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27 181 10 min. Final amplicons concentrations were quantified on a D5000 ScreenTape bioanalyzer  
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29 182 (Agilent) and validated by qPCR on a LC480 real-time thermocycler (Roche), using qPCR  
30  
31 183 primers recommended in Illumina's qPCR protocol and Illumina's PhiX control library as  
32  
33 184 standard (Illumina). All amplicon products and control library (5% PhiX library) were finally  
34  
35 185 sequenced using paired-end Illumina MiSeq sequencing (2×300 bp). The raw datasets are  
36  
37 186 available on the European Nucleotide Archive system under project accession number  
38  
39 187 PRJEB50048.

### 188 **Bioinformatic analyses**

189 Illumina reads were processed with the bioinformatics pipeline FROGS v 3.2 (Find Rapidly  
190 OTUs with Galaxy Solution) (Escudié et al., 2018) available on the GenoToul Galaxy  
191 platform (Afgan et al., 2018). Paired-end reads were firstly merged with a maximum of 10%  
192 mismatch in the overlapped region with VSERACH. Only sequences with expected length  
193 (between 340 and 450 nucleotides), no ambiguous bases (N) were clustered in two steps using

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2  
3 194 the SWARM algorithm to obtain Operational Taxonomic Units (OTUs) with minimal  
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5 195 distance ( $d_g = 1$ ), then with an aggregation distance of 3 (Mahé et al., 2014). The removal of  
6  
7 196 chimeric sequences was performed with VSEARCH with de novo UCHIME method (Edgar  
8  
9 197 et al., 2011; Rognes et al., 2016), combined with a cross-sample validation. A filtering tool  
10  
11 198 was used to select OTU according to their abundance (threshold =  $5 \cdot 10^{-5}$ , Bokulich et al.,  
12  
13 199 2013), leading to a total of 1,595,159 high quality sequences. Subsampling to the lowest  
14  
15 200 dataset (7,910 reads) was performed by random selection in order to efficiently compare the  
16  
17 201 datasets and avoid biased community comparisons. Each OTU was affiliated against the  
18  
19 202 SILVA reference database (v138). Statistics tools included in FROGS pipeline were used to  
20  
21 203 generate OTU table and to calculate the alpha and beta diversity (diversity indices and bray-  
22  
23 204 Curtis distances).

### 29 205 **Statistical analysis**

30  
31  
32 206 Statistical analyses were performed with R version 3.4.3 (R Core Team, 2018) according to  
33  
34 207 Laroche et al. (2018). The “ade4” R package was used to perform a co-inertia analysis (CIA)  
35  
36 208 and two Principal Component Analyses (PCA) (Dray and Dufour, 2007). These analyses  
37  
38 209 highlighted the common structure between the physico-chemical parameters (pH and  
39  
40 210 dissolved iron, sulfur, arsenic and antimony concentrations) and the dominant bacterial taxa in  
41  
42 211 the liquid samples at each position in the column. These dominant taxa represented more than  
43  
44 212 90% of the relative abundance of sequences in every sample during the monitoring period.  
45  
46 213 Before the CIA, the physico-chemical and biological variables were respectively standardised  
47  
48 214 and Hellinger transformed (Legendre and Gallagher, 2001). The significance of the analysis  
49  
50 215 was checked with a Monte-Carlo test (999 random permutations).  
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## 218 **Results**

### 219 **Evolution of pH**

220 The pH was always increased between the inlet and the outlet water of the bioreactor (Fig.  
221 2A). The average pH increased from  $4.29 \pm 0.03$  to  $5.10 \pm 0.25$  during the phase 1, from  $4.03$   
222  $\pm 0.06$  to  $5.05 \pm 0.19$  during the phase 2 and from  $4.35 \pm 0.20$  to  $4.91 \pm 0.29$  during phase 3  
223 (Fig. 2B). This pH increase was gradual in phase 1. In phases 2 and 3, there was a strong pH  
224 increase (around 1 unit) between the inlet and the bottom of the column, followed by little pH  
225 variation ( $< 0.3$  unit) between the bottom and the upper section and finally a pH decreases  
226 between the upper section and the outlet.

### 227 **Removal of As, Sb, Fe and S from the mine water**

228 The reactor removed 96 to 99% of dissolved As, during each of the three phases of  
229 experiment (Fig. 3A). Arsenic removal occurred at different levels of the column bioreactor  
230 depending on the experimental conditions. At the end of phase 1, 72% of As was removed  
231 between the bottom and the middle of the column. In phase 2, As concentration decreased  
232 gradually from 0.5 to 0.1 mM from the bottom to the top. In phase 3, As was mainly removed  
233 between the feed point and the bottom of the reactor (99.8%).

234 During phase 1, Sb concentration in the feed solution was that of the natural AMD (less than  
235 0.01 mM), thus it was too low to determine a precise removal rate. Sb removal during the two  
236 next phases, in which Sb concentrations were adjusted to about 0.7 and 1 mM, averaged 97%  
237 and 98% respectively. Most Sb was removed in the bottom zone of the reactor in each phase  
238 (Fig. 3B and C). As for arsenic, its concentration decreased gradually from 0.018 to 0.004  
239 mM along the column in phase 2. The dissolved Sb concentration in the outlet water was  
240 always higher than in the upper zone of the bioreactor. This phenomenon, also observed for S  
241 and Fe in phase 2, might be explained by oxidation reactions. Despite the constant flow of  $N_2$

1  
2  
3 242 flux in the outlet bottle, there may to have been some oxygen diffusion through the outlet  
4  
5 243 pipe. Other toxic chemical substances (Pb, Tl and Zn) present in minor concentrations in the  
6  
7 244 feed water were removed inside the bioreactor (Supplementary Table SM1).  
8  
9

10 245 The average dissolved Fe and sulphur (S) concentrations in the inlet water were respectively  
11  
12 246  $21 \pm 2$  and  $38 \pm 3$  mM (Fig. 3 D and E). Maximum Fe removal reached 20, 35 and 14% in  
13  
14 247 phase 1, 2 and 3, respectively (Fig. 3D). Sulfur removal reached 16, 17, and 11% in phases 1,  
15  
16 248 2 and 3 respectively. Inside the column, the S concentration decreased gradually from 33.8 to  
17  
18 249 30.4 mM at the end of the phase 1 (Fig. 3E). For the others phases, it stabilised around  $32.3 \pm$   
19  
20 250  $0.05$  mM (phase 2) and  $30.0 \pm 0.5$  mM (phase 3) between bottom and upper section and  
21  
22 251 increased slightly in the outlet water.  
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## 27 252 **Observation of the bioprecipitates**

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29  
30 253 SEM observation of the biofilm-colonised filling materials collected inside the bioreactor  
31  
32 254 revealed the presence of mineral-encrusted rod-shaped bacteria of different sizes, around 5  
33  
34 255  $\mu\text{m}$  long and 0.5 to 2  $\mu\text{m}$  wide (Fig. 4 A). The EDS spectra of the mineral phases associated  
35  
36 256 with this biofilm revealed major peaks of S and As. However, since the interaction volume of  
37  
38 257 the electron beam is ca. 1  $\mu\text{m}^3$ , it cannot be excluded that part of the EDS signal comes from  
39  
40 258 the surrounding phases. Other zones of the biofilm showed aggregates including rod cells-  
41  
42 259 shaped particles (Fig. 4C), enriched in Sb together with As and S as major peaks on the EDS  
43  
44 260 spectrum (Fig. 4D). The elemental map of one bioprecipitate (Fig. SM2) showed differences  
45  
46 261 between the distribution of As and Sb in the As-Sb-S bearing phase. Moreover, the areas  
47  
48 262 corresponding to high Fe concentrations were distinct to those of high As and Sb  
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50 263 concentrations in this bioprecipitate.  
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## 56 264 **Evolution of bacterial diversity**

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3 265 The 16S rRNA genes retrieved by metabarcoding from inoculum, liquid samples and  
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5 266 bioprecipitates collected inside the bioreactor were classified into 78 OTUs. Overall, the  
6  
7 267 richness (number of observed OTU) and the Shannon index (reflecting both richness and  
8  
9 268 evenness) indicated a low bacterial diversity in all the samples (Fig. SM3). The highest  
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11 269 bacterial diversity was observed in liquids collected at the end of phase 1 in the bottom and in  
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13  
14 270 the upper of the column ( $30 \pm 2$  OTU observed).

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18 271 Ten taxa identified at the genus level had relative abundance of reads  $\geq 2\%$  in at least one  
19  
20 272 sample (Fig. 5). The inoculum, able to reduce sulfate at acidic pH and enriched from the  
21  
22 273 Carnoulès AMD, was mainly composed of *Desulfosporosinus* (77.0%), *Microbacter* (11.6%)  
23  
24 274 and *Cellulomonas* (10.9%) These three genera were identified in all liquid samples collected  
25  
26 275 inside the column, whatever the level and the phase, with relative sequence abundances  
27  
28 276 varying from  $1.9 \pm 0.4\%$  to  $76.5 \pm 5.9\%$  (mean of triplicates) for *Desulfosporosinus*,  $4.4 \pm$   
29  
30 277  $1.3\%$  to  $41.3 \pm 2.4\%$  for *Cellulomonas* and  $16.0 \pm 4.6\%$  to  $66.5 \pm 1.2\%$  for *Microbacter*.

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34 278 Other genera were detected in lower proportions inside the column: *Acidithiobacillus* ( $0.7 \pm$   
35  
36 279  $0.2\%$  to  $16.9 \pm 2.0\%$ ), *Luteococcus* ( $0.3 \pm 0.04\%$  to  $9.3 \pm 1.7\%$ ), *Acidocella* (0 to  $4.9 \pm 1.1\%$ ),  
37  
38 280 *Metallibacterium* (0 to  $5.1 \pm 0.2\%$ ), *Sediminibacterium* (0 to  $2.6 \pm 2.2\%$ ), *Streptococcus* (0 to  
39  
40 281  $2.7 \pm 4.6\%$ ) and *Thiomonas* (0 to  $3.1 \pm 0.3\%$ ). There was a clear evolution in both space and  
41  
42 282 time of the taxonomic composition of bacterial communities inside the column.

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44  
45 283 *Desulfosporosinus* proportions were lower during phases 1 and 2 in all levels ( $1.9 \pm 0.4\%$  to  
46  
47 284  $35.7 \pm 8.9\%$ ), than in the inoculum. However, it became again dominant ( $76.5 \pm 5.9\%$ ) during  
48  
49 285 phase 3 at the bottom of the column. *Cellulomonas* was the dominant genus in bottom and  
50  
51 286 middle column samples in phase 1 ( $33.9 \pm 0.7$  and  $41.3 \pm 2.4\%$ ), and co-dominant ( $41.2 \pm$   
52  
53 287  $5.8\%$ ) in the bottom level in phase 2. *Microbacter* was dominant in the upper level in phase 1  
54  
55 288 and 2 ( $53.3 \pm 3.9\%$  and  $66.5 \pm 1.2\%$ ) and middle and upper levels in phase 3 ( $36.6 \pm 9.1\%$   
56  
57 289 and  $53.9 \pm 10.1$ ). *Luteococcus* was always present but at low relative abundance (less than 9.3  
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3 290  $\pm 1.7\%$ ) and particularly during the phases 1 (bottom and middle) and 2 (bottom). *Acidocella*  
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5 291 was more abundant in the bottom level during phase 1 ( $4.9 \pm 1.1\%$ ); its relative abundance  
6  
7 292 was the lowest during phase 3.

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9  
10 293 At the end of the experiment (phase 3), the main difference between communities from  
11  
12 294 bioprecipitates and liquid samples was the relative abundance of the *Desulfosporosinus* genus.  
13  
14 295 Its proportion was close for the bottom level and higher for the middle and top levels on the  
15  
16 296 solids ( $71.6 \pm 1.7\%$  bottom,  $81.0 \pm 1.3\%$  middle and  $47.6 \pm 5.6\%$  top) than in the liquid ( $76.5$   
17  
18 297  $\pm 1.7\%$  bottom,  $30.0 \pm 15.6\%$  middle and  $14.8 \pm 2.9\%$  top) samples. It was thus largely  
19  
20 298 dominant in the bioprecipitates collected from bottom and middle levels. *Microbacter*,  
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22 299 *Cellulomonas* and *Luteococcus* were also identified in the bioprecipitates.

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25  
26 300 A co-inertia analysis was performed to interpret the relationship between the physico-  
27  
28 301 chemical parameters and the dominant bacterial taxa (Fig. 6). The Monte-Carlo test revealed a  
29  
30 302 significant co-structure between the physico-chemical and biological variables (p-value =  
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32 303 0.03). Ninety seven percent of the total variance in the two datasets were explained by the  
33  
34 304 first two axes of the CIA. Samples were separated according their position in the column,  
35  
36 305 particularly at the bottom, along the second axis of the analysis (Fig. 6A). The dotted arrows  
37  
38 306 represent the difference between the ordination of the biological (origins of arrows) and  
39  
40 307 physico-chemical parameters (arrowheads). The principal component analysis (PCA) of  
41  
42 308 physico-chemical parameters shows that samples at the bottom of the column were associated  
43  
44 309 with lower pH values and higher dissolved As concentrations, at the opposite to those at the  
45  
46 310 middle and upper levels of the column (Fig. 6B). *Acidithiobacillus* were more abundant in the  
47  
48 311 upper level of the column according the PCA of the dominant bacterial taxa (Fig. 6C).  
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50 312 Iron and S were positively correlated with *Thiomonas* and *Metallibacterium*, but negatively  
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52 313 with *Desulfosporosinus* (Fig. 6B and 6C). A positive correlation was observed between pH  
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54 314 and *Microbacter*, whereas pH correlated negatively with *Cellulomonas* and



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3 315 *Luteococcus*, *Cellulomonas* and *Luteococcus* were also correlated positively with As, unlike  
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5 316 *Microbacter*.

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## 10 11 12 318 **Discussion**

### 13 14 15 319 **Removal of As and Sb along the experiment**

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18 320 The upflow anaerobic fixed-bed bioreactor efficiently removed As, Sb and some metals (Pb,  
19  
20 321 Tl and Zn) present in a real arsenic-rich AMD water amended with Sb. Antimony removal  
21  
22 322 was efficient even when Sb concentration was increased: it averaged 97% for initial  
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24 323 concentrations 0.7 mM (phase 2) and 1.01 mM (phase 3). The improvement of Sb sulfide  
25  
26 324 precipitation by lowering pH was observed by Chen et al. (2020), however their experimental  
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28 325 condition was less acidic (pH 6.5) than ours. Here, more than 96% of As was removed inside  
29  
30 326 the column reactor even with the increase of Sb concentration in the feeding water. Similarly,  
31  
32 327 Liu et al. (2018) reported the concomitant precipitation of As and Sb (up to 98.2% and 99.4%)  
33  
34 328 from synthetic neutral pH solution containing 0.07 mM of As(V) and 0.04 mM Sb(V), by  
35  
36 329 bacterial sulfate-reduction. Here, the increase in the fed Sb concentration did not affect As  
37  
38 330 bio-precipitation. Increasing the total metalloids concentration in the feed might have allowed  
39  
40 331 a more efficient and rapid consumption of dissolved sulfide, in particular near the acidic feed  
41  
42 332 supply at the bottom of the reactor, thus decreasing the toxicity-related stress of bacteria  
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44 333 induced by H<sub>2</sub>S in acidic conditions.

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46  
47 334 The SEM-EDS observations strongly suggest that As and Sb precipitated inside the reactor as  
48  
49 335 solid sulfides. Li et al. (2022) already observed by SEM-EDS precipitates of amorphous  
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51 336 precipitates identified as Sb<sub>2</sub>S<sub>3</sub> in their batch culture of SRB grown in presence of high Sb  
52  
53 337 concentrations. These As and Sb sulfide phases were detected in zones showing rod bacterial  
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55 338 shapes, possibly because they precipitated around the cell that excreted the dissolved sulfide.

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3 339 Such a phenomenon was already observed in batch conditions with As sulfide precipitating as  
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5 340 coatings around bacterial cells growing in batch (Le Pape et al., 2017) and continuous  
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7 341 (Battaglia-brunet et al., 2021) conditions with AMD from Carnoulès site. Liu et al. (2018)  
8  
9 342 suggested that As adsorption could occur on already formed sulfides. As a global way, the  
10  
11 343 sorption/co-precipitation of As with metal sulfides was suggested by other studies (Jong and  
12  
13 344 Parry, 2003; Sahinkaya et al., 2015). Among them, Fe sulfides were regularly cited to enhance  
14  
15 345 the removal of As inside sulfate-reducing bioreactor (Luo et al., 2008; Altun et al., 2014; Liu  
16  
17 346 et al., 2018). In our column, the soluble Fe and S concentrations varied in a similar way,  
18  
19 347 suggesting their concomitant precipitation. However, the elemental map and the low Fe  
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21 348 signals in EDS spectra indicated a minor presence of Fe sulfide in Sb-As-bearing phases.  
22  
23 349 Then, Fe does not appear to play a significant role in the removal of As or Sb in the present  
24  
25 350 study. This difference of behaviour with previous studies can be explained by the stronger  
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27 351 acidic condition of our experiment that favoured the precipitation of As and Sb as sulfide  
28  
29 352 minerals (Le Pape et al., 2017; Han et al., 2018).

### 353 **Bacterial diversity and key functional groups**

354 The bacterial communities colonising the bioreactor resulted from inoculation with a  
355 microbial consortium including sulfate-reducing bacteria, originating from the AMD of  
356 Carnoulès. Most of the taxa identified in the inoculum and in the bioreactor were previously  
357 observed in the Carnoulès ecosystem (Delavat et al., 2012 and 2013) and in anaerobic  
358 bioreactors treating artificial or real mine effluents by sulfate reduction (Battaglia-Brunet et  
359 al., 2012; Sánchez-Andrea et al., 2014). A single known SRB was detected inside the reactor,  
360 affiliated to the genus *Desulfosporinus*, which is in agreement with previous finding in  
361 similar systems (Battaglia-Brunet et al., 2012; 2021). This genus includes acid tolerant SRB  
362 isolated from mining environment (Kimura et al., 2006; Church et al., 2007; Alazard et al.,  
363 2010; Jameson et al., 2010; Sánchez-Andrea et al., 2015; Mardanov et al., 2016; Panova et al.,

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3 364 2021). It was the main bacterium composing the sulfate-reducing microbial consortium  
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5 365 inoculated in the bioreactor, and was maintained inside the column, being often one of the  
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7 366 main (bottom and/or middle levels in phases 1 and 2) and even the dominant (bottom level in  
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9 367 phase 3) genus. Its relative abundance represented thus a considerable portion of the microbial  
10  
11 368 community inside the reactor (up to  $76.5 \pm 5.9\%$  in liquid and  $81.0 \pm 1.3\%$  in bioprecipitates).  
12  
13 369 Bacterial communities gathering SRB, fermenters and cellulolytic bacteria have already been  
14  
15 370 observed in bioreactors treating mine water (Morales et al., 2005; Pruden et al., 2007; Pereyra  
16  
17 371 et al., 2008 and 2010; Vasquez et al., 2016). In the bioreactor, interactions between the  
18  
19 372 different identified metabolic groups are expected. Fermentative bacteria able to degrade  
20  
21 373 complex organic molecules accounted for a large proportion of the detected genera. Members  
22  
23 374 of the *Cellulomonas* genus are able to degrade cellulose and other complex polysaccharides  
24  
25 375 (Lynd et al., 2002) and were already found associated to acidophilic SRB consortia (Dev et  
26  
27 376 al., 2021). Cellulose degraders (*Cellulomonas*) and fermenters (*Microbacter* and *Luteococcus*)  
28  
29 377 may possibly be able to depolymerise and consume sugars from the agar phase, and generate  
30  
31 378 metabolites for SRB. The genus *Microbacter* has been first discovered in an acid rock  
32  
33 379 drainage of the Rio Tinto and is described as an anaerobic propionigenic bacterium able to  
34  
35 380 produce propionate, lactate and acetate from various sugars (Sánchez-Andrea et al., 2014).  
36  
37 381 *Luteococcus*, a genus belonging to the Propionibacteraceae family, which members are known  
38  
39 382 to produce mainly acetate, propionate and CO<sub>2</sub> from sugar fermentation (Stackebrandt et al.,  
40  
41 383 2006), is consistently detected inside the column, although at lower proportion. More  
42  
43 384 precisely, the OTU sequence classified within the genus *Luteococcus* matches with that of the  
44  
45 385 acidophilic Propionibacteraceae strain H7p isolated from sediment of the Carnoulès AMD  
46  
47 386 (Delavat et al., 2012). SRB might use metabolites produced by the complex polysaccharides  
48  
49 387 degraders and the fermenters as carbon and energy sources. Association of these diverse  
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51 388 metabolic groups inside an anaerobic process can promote synergic activities (Logan et al.,  
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3 389 2005; Pereyra et al., 2010; Sánchez-Andrea et al., 2014; Vasquez et al., 2016).  
4  
5 390 *Desulfosporosinus* species usually oxidise organic compounds incompletely, producing  
6  
7 391 acetate (Alazard et al., 2010; Mayeux et al., 2013; Sánchez-Andrea et al., 2015; Vandieken et  
8  
9 392 al., 2017); however, a recent study has reported complete oxidation of propionate, lactate, and  
10  
11 393 acetate to CO<sub>2</sub> by a *Desulfosporosinus* strain (Hausmann et al., 2019). On another hand, as the  
12  
13 394 bioreactor was fed with glycerol, a well-known substrate for acidotolerant *Desulfosporosinus*  
14  
15 395 strains but also a substrate for *Cellulomonas* and Propionibacteraceae, a competition for  
16  
17 396 glycerol may also occur and limit the organic carbon availability for SRB. As some anaerobic  
18  
19 397 bacteria can perform fermentation of tartrate (Schink et al., 1984), the supply of this organic  
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21 398 acid (with Sb) as additional potential electron donor, i.e. 0.7 mM in phase 2 and up to 1 mM  
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23 399 during phase 3, could also contribute to influence the evolution of bacterial community  
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25 400 composition during the experiment. *Acidocella* is a heterotrophic acidophilic genus that also  
26  
27 401 presents a metabolic interest. For instance, *Acidocella aromatica* uses a limited range of  
28  
29 402 organic substrates, such as acetate (Jones et al., 2013; Ñancucheo et al., 2016). Acetate is a  
30  
31 403 toxic compound at low pH, potentially produced by several bacteria in our system, including  
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33 404 SRB. In 2006, Kimura et al. proposed a syntrophic interaction between *Acidocella* and  
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35 405 *Desulfosporosinus*. *Acidocella* generated carbon dioxide and hydrogen from the acetate  
36  
37 406 produced by *Desulfosporosinus*, hydrogen being used as electron donor by  
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39 407 *Desulfosporosinus*. In our system, *Acidocella* was mainly present in phases 1 and 2, and  
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41 408 might have been replaced by other acetate degraders in phase 3.  
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52 410 **Relation between the bacterial communities, the physico-chemical parameters, and the**  
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54 411 **sulfate reducing activity**  
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3 412 There was a clear evolution in both space and time of the taxonomic composition of bacterial  
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5 413 communities inside the column (Fig. 6). The co-inertia analysis showed a significant  
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7 414 correlation between the evolution of biological and some physico-chemical parameters.  
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9  
10 415 As a fact, pH, S, As and Sb concentrations were among the most important factors explaining  
11  
12 416 the percentages of the main bacterial genera (Fig. 6). However, these parameters are directly  
13  
14 417 influenced by the sulfate-reducing reaction which consumes  $H^+$  and  $SO_4^{2-}$ , and leads to the  
15  
16 418 precipitation of As and Sb as sulfides. Sulfate reduction occurred inside the column, as  
17  
18 419 evidenced by the decrease of dissolved S concentration and metal sulfide precipitation, and  
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20 420 the gradual increase of pH in the bioreactor. In the liquid samples, the higher proportion of  
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22 421 *Desulfosporosinus*-related OTU at the bottom of the column during the phase 1 and 3 is in  
23  
24 422 agreement with the increase of pH between the inlet and the bottom of the column, but also  
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26 423 with the As and Sb precipitation between the bottom and the middle of the column. The  
27  
28 424 lowest precipitation efficiency was observed during phase 2 (Fig. 3), corresponding to the  
29  
30 425 lowest feed pH and lowest proportion of *Desulfosporosinus*-related OTU at the bioreactor  
31  
32 426 bottom (Fig. 6). The highest proportion of this sulfate reducing bacterium was found in the  
33  
34 427 bio-precipitates suggesting that *Desulfosporosinus* was mainly present in the biofilm  
35  
36 428 compartment. This may be linked to the development of micro-environments that protected  
37  
38 429 the cells against stressing parameters such as acidity or oxygen diffusion from the outlet (Yin  
39  
40 430 et al., 2019; Tran et al., 2021). The presence of *Acidithiobacillus*- and *Thiomonas*-related  
41  
42 431 OTU in the upper zone of the bioreactor, that appeared red coloured (SM4), may be linked to  
43  
44 432 oxygen diffusion from the top of the column. *Acidithiobacillus* and *Thiomonas* are S- and Fe-  
45  
46 433 oxidising bacteria already detected in Carnoulès site (Delavat et al., 2012, 2013). The glycerol  
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48 434 fed at the bottom of the bioreactor could explain higher proportions of *Cellulomonas*,  
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50 435 *Luteococcus* and *Desulfosporosinus* (all genera consuming this substrate) in the bottom zone  
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52 436 of the bioreactor (Fig. 6). The metabolism of organic substrates might have exerted influence  
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3 437 on the dynamics of the bacterial community. Hessler et al. (2018) observed a stratification of  
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5 438 microbial communities throughout a biological sulfate-reducing up-flow anaerobic packed  
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7 439 bed reactor that was attributed to variations of lactate and the volatile fatty acids produced by  
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9 440 microbial metabolisms. Further analyses would be required in order to determine the effect of  
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11 441 organic molecules resulting from the metabolism of glycerol and tartrate and biodegradation  
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13 442 of agar, on the diversity and structure of bacterial communities.  
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17 443 Globally, this study demonstrated for the first time the possibility to remove concomitantly As  
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19 444 and Sb as sulfides in a continuous column bioreactor fed with a real AMD. The reactor  
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21 445 removed up to 98%-99% of dissolved As and Sb, with no competition between As and Sb for  
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23 446 precipitation with sulfides when they co-occurred at concentrations up to 1 mM each in the  
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25 447 AMD. *Desulfosporosinus*, the only known SRB genus detected in the bioreactor, was present  
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27 448 as a major contributor to the bacterial community, which also included polymers degraders  
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29 449 and fermenters. The biological reduction of the sulfate contained in the AMD provided  
30  
31 450 enough dissolved sulfide to precipitate efficiently As and Sb. The co-occurrence of SRB,  
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33 451 polymers degraders and fermenters can be an advantage for future *in situ* passive treatment of  
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35 452 AMD, in the perspective to use low-cost complex organic substrates to sustain long-term  
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37 453 bacterial activity in a bioreactor. Results are promising for the perspective of passive bio-  
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39 454 treatments of AMD containing metalloids.  
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7 3 **Figure 1:** Schematic representation of the upflow anaerobic fixed-bed bioreactor (A). Picture  
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9 4 of the column with filling material and yellow biogenic precipitates (B).  
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15 6 **Figure 2:** pH variations of inlet and outlet waters represented by white and black circles  
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17 7 respectively (A). Histogram of pH vertical-profiles at the end of each phase in the column  
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19 8 bioreactor (B); the white circles represent the average pH of inlet and outlet waters along each  
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21 9 phase, and the error bars indicate standard deviations of the mean for three measurements for  
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23 10 each feed condition.  
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30 12 **Figure 3:** Vertical punctual profiles of dissolved arsenic (A), antimony (B, focus on low  
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32 13 concentration C), iron (D) and sulphur (E) concentrations in the column bioreactor at the end  
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34 14 of each phase (data missing for the Sb concentration in the inlet water in phase 1). Error bars  
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36 15 indicate analytical error (2%). The white circles represent the average concentrations of inlet  
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38 16 and outlet waters for each phase (three measures), and the error bars indicate standard  
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40 17 deviations of the mean for three measurements for each feed condition.  
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47 19 **Figure 4:** SEM observation of bottom bioreactor filling at the end of experiment in  
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49 20 backscattering electron mode showing biofilm morphology in a first zone (A) and the  
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51 21 corresponding EDS spectrum (B), and another zone showing Sb-enriched aggregate (C) and  
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53 22 the corresponding EDS spectrum (D).  
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**Figure 5:** Taxonomic composition of bacterial communities (at the genus level) in the inoculum, the liquid and the solid samples collected along the reactor at the end of each phases (analyses performed in triplicates except for the inoculum). “Others” represent the phylogenetic groups with a relative abundance < 1% calculated on the whole dataset.

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**Figure 6:** Co-inertia analysis of the physico-chemical parameters and the dominant bacterial taxa at each position in the column. (A) Plot represents the projection of the samples, grouped according their position inside the column, on the first two co-inertia axes. The dotted arrows represent the difference between the ordination of the biological (origins of arrows) and physico-chemical parameters (arrowheads); dotted arrows length are inversely proportional to the strength of the relationship between parameters. The upper-left barplot indicates the eigenvalues. (B) PCA of the physico-chemical parameters at each position in the column. Chemical parameters are represented by their chemical symbols as: Fe (dissolved iron concentration), S (dissolved sulfur concentration), As (dissolved arsenic concentration) and Sb (dissolved antimony concentration). (C) PCA of the dominant bacterial taxa at each position in the column.

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Figure 1

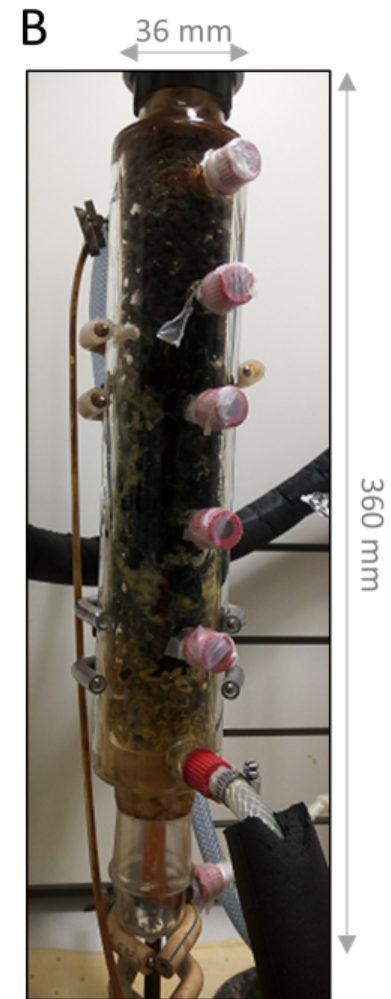
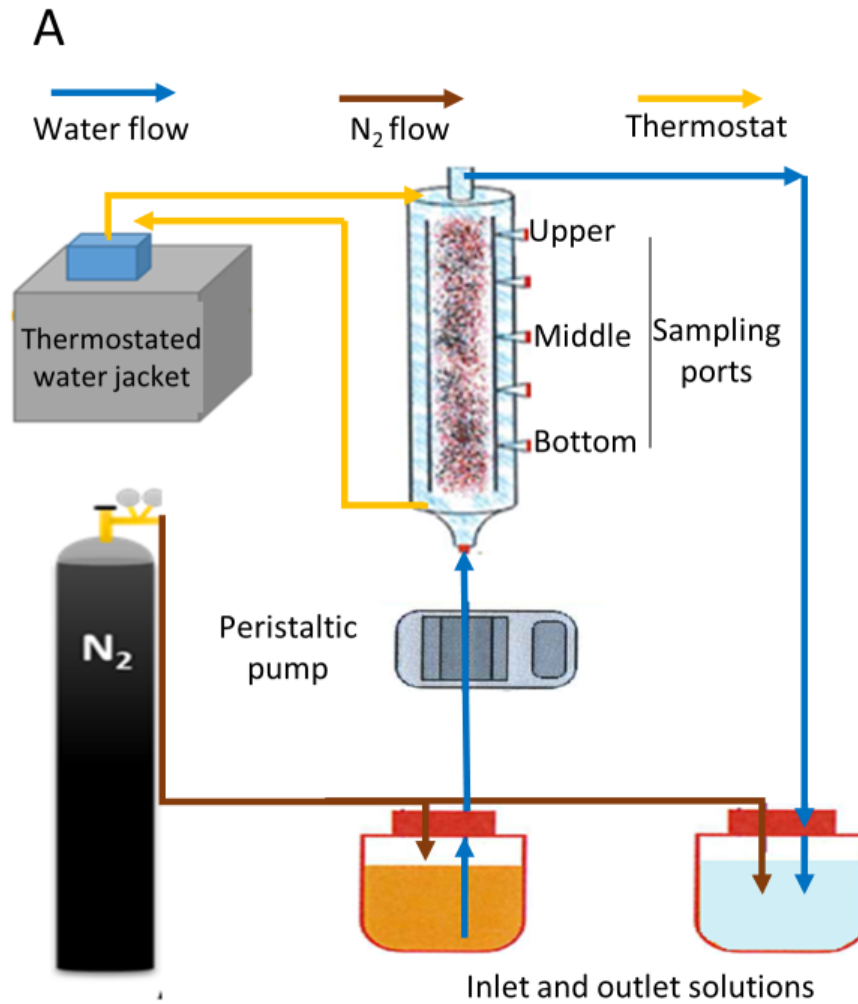


Figure 2

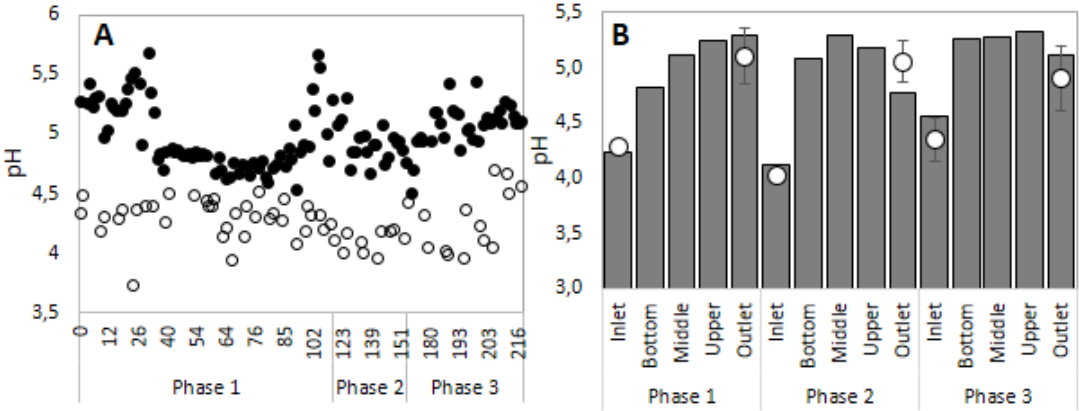


Figure 3

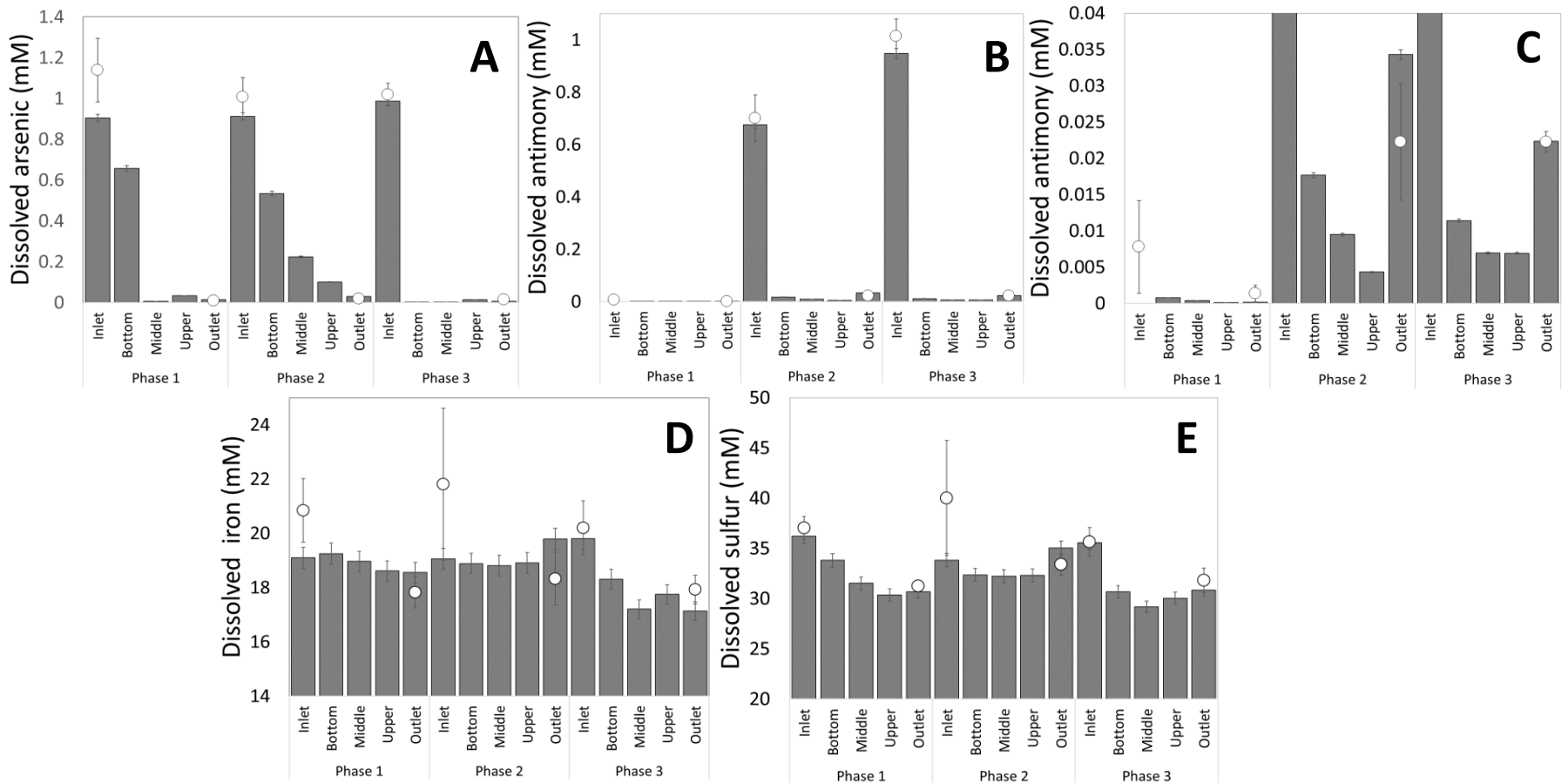




Figure 4

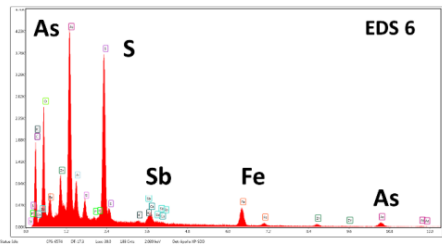
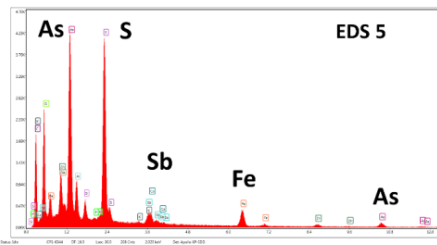
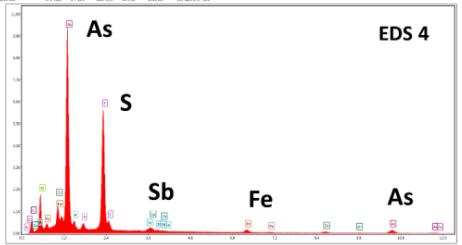
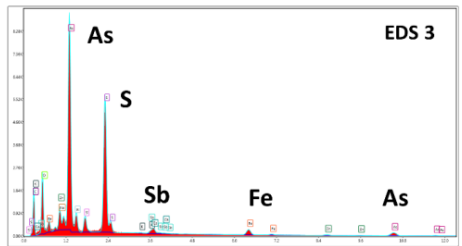
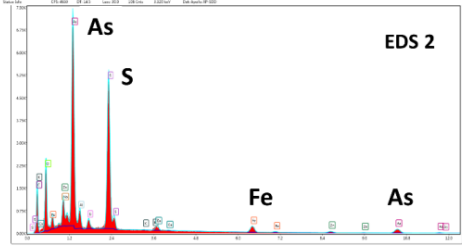
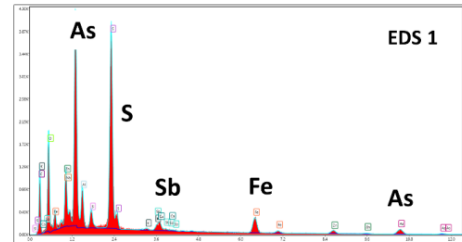
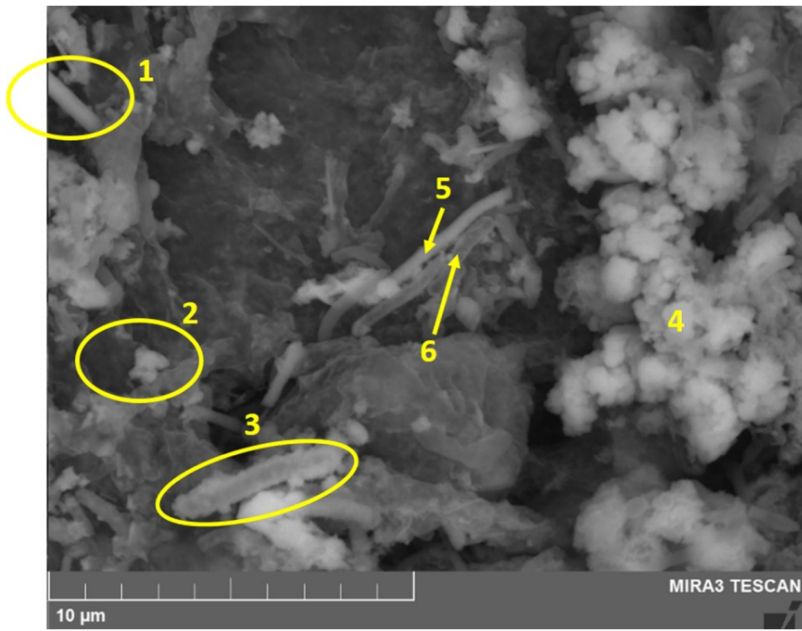
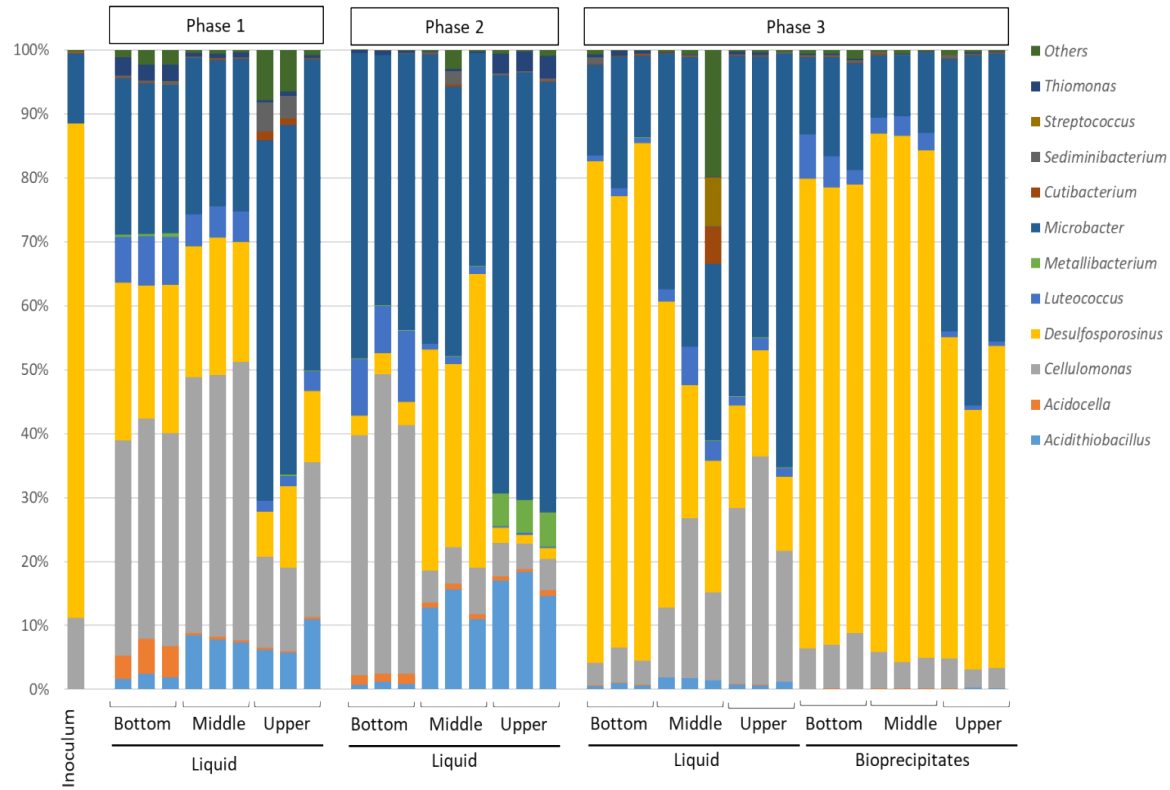
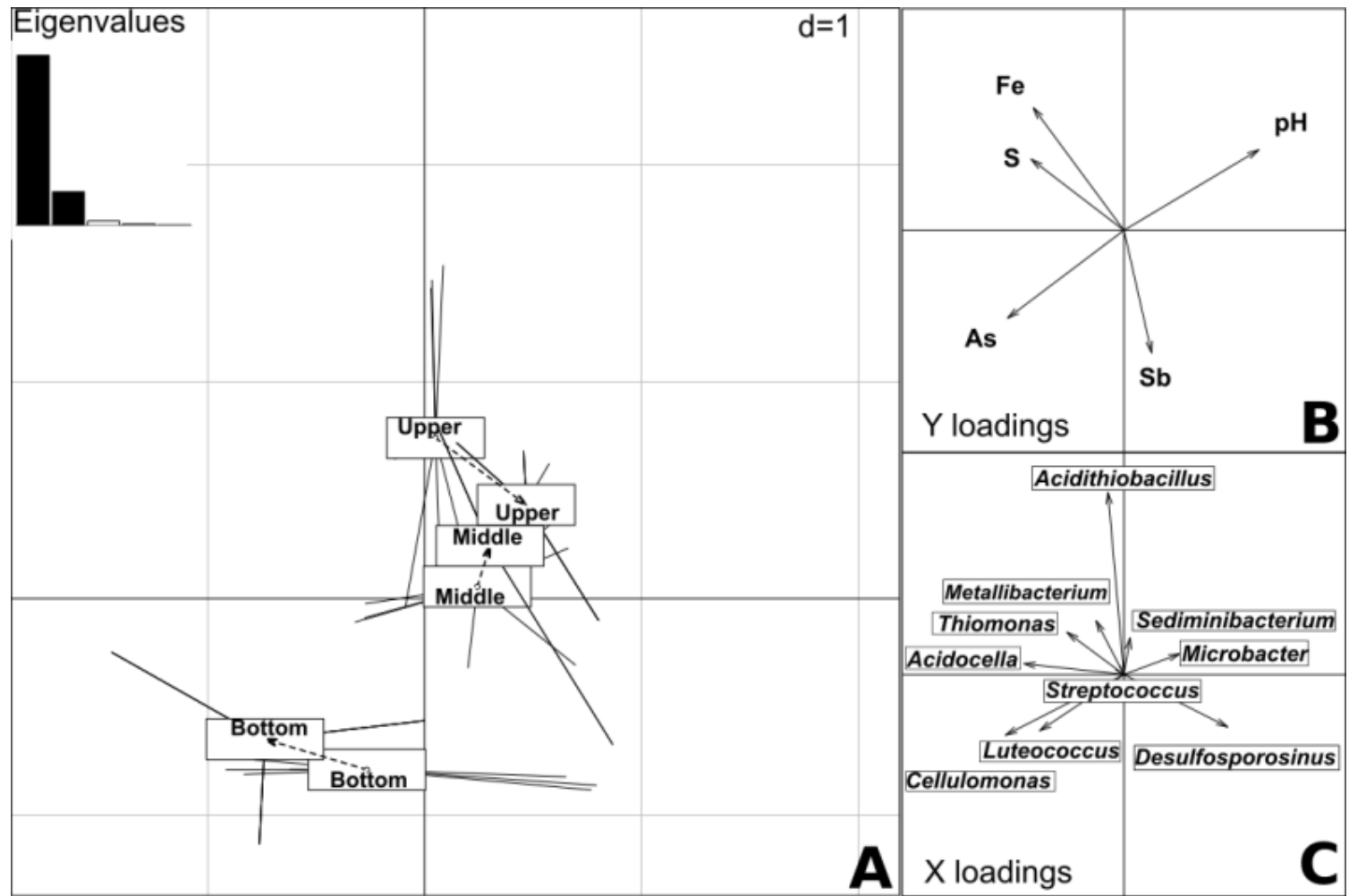


Figure 5



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4 **Figure 6**



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**Table 1:** Experimental conditions

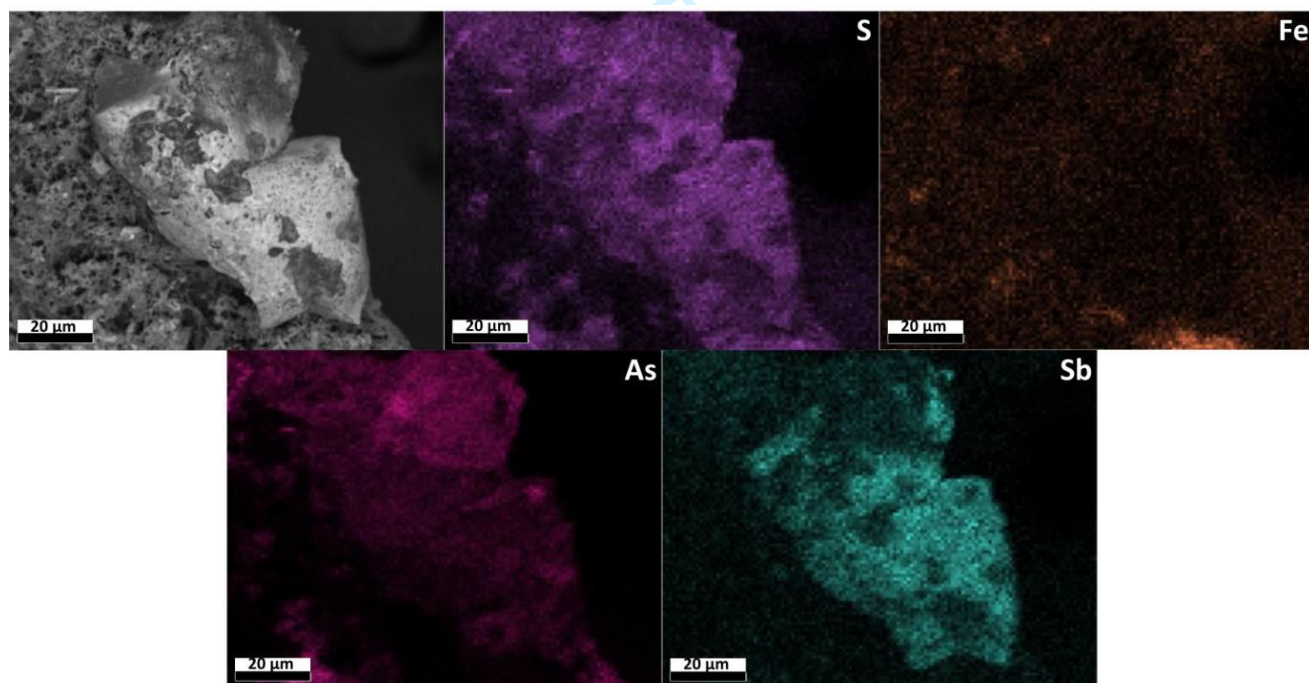
Phase	Duration		Hydraulic				
	of the phase (days)	Sampling day for ICP-MS analysis	Flow (ml/h)*	retention Time (h)*	Feed pH*	Feed [As] (mM)*	Feed [Sb] (mM)*
1	117	0, 82 and 117	1.5 ±	132 ± 10	4.29 ± 0.03	1.14 ± 0.16	0.008 ±
			0.1				0.006
2	35	123, 141 and 152	1.3 ±	163 ± 19	4.03 ± 0.06	1.01 ± 0.09	0.70 ± 0.09
			0.1				
3	65	152, 155, 204 and 217	1.4 ±	149 ± 15	4.35 ± 0.20	1.02 ±	1.01 ± 0.07
			0.1				

(\*) average values

**SM1. Average removal and concentrations of minor toxic elements from inlet and outlet waters during each phase.**

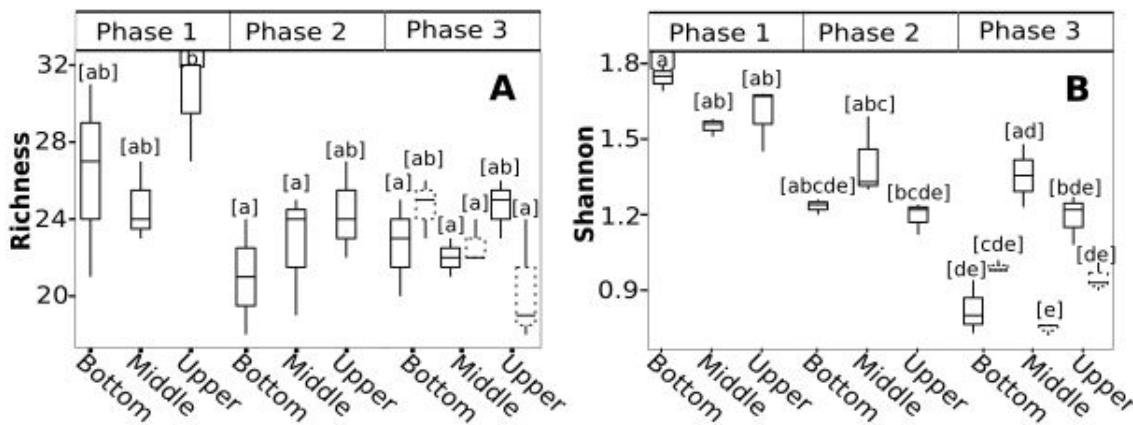
Phase		1	2	3	
<b>Pb</b>	Concentration (mM)	Inlet	$1.0 \pm 0.9$	$0.7 \pm 0.4$	$1.2 \pm 0.5$
		Outlet	$2.10^{-3} \pm 2.10^{-3}$	$< 2.10^{-5}$	$4.10^{-3} \pm 5.10^{-3}$
	Removal (%)	$99.9 \pm 0.1$	100	$99.7 \pm 0.4$	
<b>Tl</b>	Concentration (mM)	Inlet	$1.5.10^{-3} \pm 1.0.10^{-3}$	$1.5.10^{-3} \pm 3.2.10^{-2}$	$1.5.10^{-3} \pm 6.2.10^{-5}$
		Outlet	$2.1.10^{-5} \pm 1.3.10^{-5}$	$1.6.10^{-5} \pm 1.1.10^{-5}$	$2.0.10^{-5} \pm 1.5.10^{-6}$
	Removal (%)	$98.7 \pm 0.8$	$99.0 \pm 0.8$	$98.7 \pm 0.1$	
<b>Zn</b>	Concentration (mM)	Inlet	$0.36 \pm 0.02$	$0.38 \pm 0.05$	$0.35 \pm 0.05$
		Outlet	$2.9.10^{-3} \pm 1.9.10^{-3}$	$9.8.10^{-3} \pm 5.6.10^{-3}$	$7.2.10^{-3} \pm 9.0.10^{-3}$
	Removal (%)	$99.2 \pm 0.5$	$97.4 \pm 1.4$	$97.9 \pm 2.7$	

**SM2. SEM-EDS map showing the distribution of selected elements on bioprecipitate collected at the end of the experiment (middle zone of the column bioreactor).**



### SM3.Bacterial Diversity indexes

Bacterial species richness in number of OTUs observed (A) and Shannon diversity index (B) in the liquid samples (solid lines) and solid samples (dashed lines) collected inside the reactor. The different letters represent significant differences between samples ( $p < 0.05$ ).



**SM4. Pictures of the column bioreactor at the end of experiment**



Bottom of the column bioreactor  
At the end of experiment



Top of the column bioreactor  
At the end of experiment