

Communication

Removal of Arsenic Using Acid/Metal-Tolerant Sulfate Reducing Bacteria: A New Approach for Bioremediation of High-Arsenic Acid Mine Waters

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Abstract: Fluvial sediments, soils, and natural waters in northern Chile are characterized by high arsenic (As) content. Mining operations in this area are potential sources of As and other metal contaminants, due to acid mine drainage (AMD) generation. Sulfate Reducing Bacteria (SRB) has been used for the treatment of AMD, as they allow for the reduction of sulfate, the generation of alkalinity, and the removal of dissolved heavy metals and metalloids by precipitation as insoluble metal sulfides. Thus, SRB could be used to remove As and other heavy metals from AMD, however the tolerance of SRB to high metal concentrations and low pH is limited. The present study aimed to quantify the impact of SRB in As removal under acidic and As-Fe-rich conditions. Our results show that SRB tolerate low pH (up to 3.5) and high concentrations of As ($\sim 3.6 \text{ mg}\cdot\text{L}^{-1}$). Batch experiments showed As removal of up to 73%, Iron (Fe) removal higher than 78% and a neutralization of pH from acidic to circum-neutral conditions (pH 6–8). In addition, XRD analysis showed the dominance of amorphous minerals, while Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM-EDX) analysis showed associations between As, Fe, and sulfur, indicating the presence of Fe-S-As compounds or interaction of As species with amorphous and/or nanocrystalline phases by sorption processes. These results indicate that the As removal was mediated by acid/metal-tolerant SRB and open the potential for the application of new strains of acid/metal-tolerant SRB for the remediation of high-As acid mine waters.

Keywords: Arsenic; sulfate reducing bacteria; acid mine drainage; acidic waters; acid-tolerant; metal-tolerant

1. Introduction

Arsenic (As) is a ubiquitous and toxic trace metalloid, which is widely distributed in natural environments [1]. Several groundwaters, sediments and minerals of Northern Chile are enriched in As [2–5]. Specifically, the Chilean Altiplano has high concentrations of As and sulfate in fluvial waters, seriously affecting the quality of water resources and limiting the use of water for consumption, agricultural, and industrial purposes [4].

In the Azufre River sub-basin, the release of contaminants from natural and anthropogenic sources negatively impact the quality of rivers and surface waters. In this area, hydrothermal waters emerge with high concentrations of dissolved As ($>3 \text{ mg}\cdot\text{L}^{-1}$), iron (Fe) ($>80 \text{ mg}\cdot\text{L}^{-1}$), and a pH lower than

3 [4,5]. The As occurrence in fluvial waters is linked to the presence of ferric Fe(III)-oxyhydroxide ores, because As is sorbed onto these minerals [6]. In addition, arid and semiarid climates strongly contribute to the generation of As-rich waters, due to high evaporation rates that concentrate surface runoff [7]. Despite this, little is known about the microbial speciation, precipitation/dissolution, or sorption processes that are involved in the fate of As in these systems and how they could be optimized in treatment systems.

The main source of As in the environment comes from its release from As-rich minerals (e.g., arsenopyrite (FeAsS)) [1], but mining operations can accelerate their release to the aqueous phase [8]. Particularly, acid mine drainage (AMD) can enhance the release of As from different minerals [9–11]. AMD is characterized by the release of acidic waters ($\text{pH} < 4$) with high dissolved metals (e.g., Fe and As, among others) and sulfate concentrations [12,13]. AMD has a negative impact on the mobilization of As and can promote the release of As directly through the oxidation of As-bearing sulfide or by dissolution of As-rich mineral phases (i.e., As-rich Fe oxyhydroxides) [10,11]. The removal of As from acid waters is a great challenge, because acidic conditions favor the dissolution of As species and prevent its removal. Nevertheless, microbial communities that are selected under acidic conditions may result in an efficient treatment for As removal.

Conventional treatment technologies for As removal, acid neutralization, and salt removal, such as co-precipitation, adsorption, chemical neutralization, or ion exchange processes have several limitations, both economic and operational [14]. Sulfate reduction processes mediated by Sulfate Reducing Bacteria (SRB) allow for the reduction of sulfate concentrations, precipitation of metals, and potentially increase the pH [15]. However, most strains of SRB grow and reduce sulfate optimally at a circum-neutral pH (6–8) [16]. Low pH severely affects the growth of bacteria and sulfate removal rates [17]. Likewise, metal and metalloid concentrations (e.g., Fe and As) have a negative effect on the sulfate removal rates [18]. Particularly, the growth of SRB can decline significantly under high As concentrations, due to its toxicity. Even so, several studies have shown that bacteria can tolerate low pH and can be an alternative for the treatment of acid mine waters [19–22]. Thus, bioremediation systems that are based on acid/metal-tolerant SRB could be used for the treatment of acidic metallic waters, but it is still necessary to study its feasibility of operation under specific conditions. The present study shows the removal of As from AMD waters by biological sulfate reduction processes under acidic and As-rich conditions. The relevance of these findings lies in that new strains of acid/metal-tolerant SRB can potentially be used to optimize As removal and AMD treatment simultaneously through its bacterial activity.

2. Materials and Methods

2.1. Study Site: Aguas Calientes Area in the Upper Section of the Azufre River Sub-Basin

The Aguas Calientes area is located in the Azufre River sub-basin near the Tacora volcano, in the Lluta River Watershed (LRW) in northern Chile ($18^{\circ}00' - 18^{\circ}30' \text{ S}$ and $70^{\circ}20' - 69^{\circ}22' \text{ W}$). The area is characterized by water scarcity, high altitude ($>4000 \text{ m a.s.l.}$), and high evaporation rates ($4.9 \pm 0.5 \text{ mm d}^{-1}$). The Azufre River originates in the upper section of the LRW in the Altiplano with high concentrations of [As] ($3.6 \pm 0.46 \text{ mg}\cdot\text{L}^{-1}$), [Fe] ($81.6 \pm 13.5 \text{ mg}\cdot\text{L}^{-1}$), [Al] ($205.3 \pm 25.7 \text{ mg}\cdot\text{L}^{-1}$), [Zn] ($7.4 \pm 1.2 \text{ mg}\cdot\text{L}^{-1}$), $[\text{SO}_4^{2-}]$ ($4678 \pm 16.9 \text{ mg}\cdot\text{L}^{-1}$), and a pH less than 2.0 [4,5] due to contributions from hydrothermal springs and acidic metal-rich runoffs.

The Aguas Calientes area is impacted by a legacy sulfur mine site on the slopes of the Tacora volcano, where there are mineral deposits of elemental sulfur and at the base of the volcano there is an accumulation of fine elemental sulfur (S^0) tailings (Figure 1). In addition, there is AMD generation that is associated with S^0 tailings, characterized by an acidic pH (1.9 to 4.0), high specific conductivity (1.0 to $5.7 \text{ mS}\cdot\text{cm}^{-1}$), and elevated concentrations of dissolved Fe ($>10 \text{ mg}\cdot\text{L}^{-1}$) and As ($>0.4 \text{ mg}\cdot\text{L}^{-1}$). The S^0 tailings are disposed near a natural wetland, where the decomposition of plant organic

matter occurs and generates anaerobic environments, enabling sulfate reduction processes to occur continuously (Figure 1).

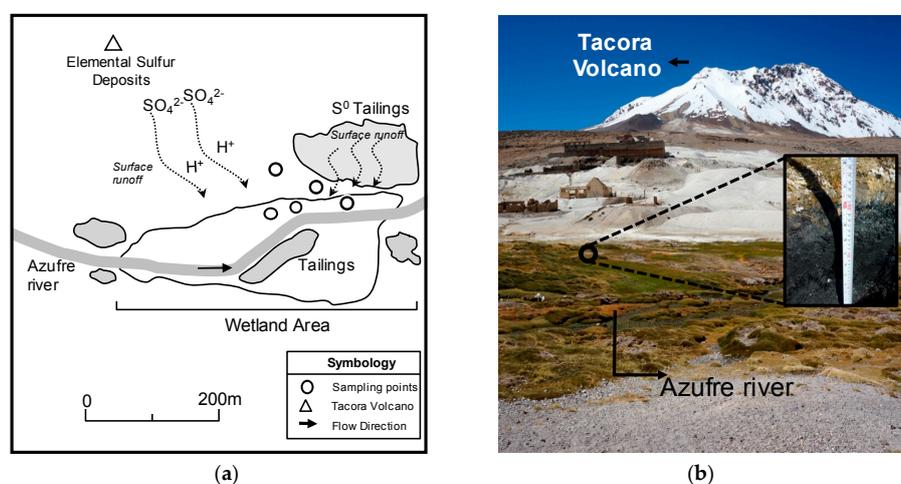


Figure 1. (a) Schematic representation of the study site in the Aguas Calientes area, Azufre River sub-basin in northern Chile; (b) Photograph of the wetland area with a representative sulfate reduction sediment profile.

2.2. Sampling

To evaluate sulfate reduction processes, sediment profiles were sampled at different points that were located at the interface of S^0 tailings with the wetland growth zone. These sediments receive an AMD runoff and have Fe and As concentrations of up to $187.2 \text{ g}\cdot\text{kg}^{-1}$ and $9.5 \text{ g}\cdot\text{kg}^{-1}$, respectively. Five sampling points were selected for hydrogeochemical analysis, elemental analysis in solid phase, and cultures of microorganisms in anaerobic reactors. Sampling points were selected based on monitoring of on-site geochemical parameters (pH, Eh, conductivity, and dissolved oxygen (DO)) in surface waters and saturated sediments in the Aguas Calientes area carried out in May 2012. The presence of a sulfate reduction process was evidenced by black spots on gray surface sediments, which becomes completely black at 5 cm of depth. Sediment profiles were collected using polyethylene cores (25 cm depth) that were inserted by hand in sediments. Immediately after collection, the cores were sliced into 5 cm deep layers. The undisturbed layers were stored in 300 mL High Density Polyethylene (HDPE) bottles and were kept in the dark at 4°C until processing and microbial culture.

Surface water samples were collected near each sediment profile for elemental analysis. Water samples were collected in 300 mL HDPE bottles and were filtered in the field using $0.22 \mu\text{m}$ nylon filters. Furthermore, on-site water analyses included temperature (PHC301, HACH), pH (PHC301, HACH), dissolved oxygen (DO) (LDO101, HACH), and electrical conductivity (CDC401, HACH) (Hq40d Multi, HACH, Loveland, CO, USA).

2.3. Batch Experiments

Batch reactors were inoculated with SRB-enriched cultures that were obtained from Winogradsky columns prepared with high concentrations of dissolved As, Fe, and sulfate ($10 \text{ mg}\cdot\text{L}^{-1}$ of As and $100 \text{ mg}\cdot\text{L}^{-1}$ of Fe and $3070 \text{ mg}\cdot\text{L}^{-1}$ of sulfate). Acid tolerance was evaluated through microbial growth in culture media with variable pH. Each anaerobic batch reactor consisted of 250 mL glass bottle inoculates (in duplicate), with actively growing planktonic SRB cell suspensions and SRB biofilms (50–100 mg wet weight) in a working volume of 250 mL (without empty space volume). The initial cell suspension was set at $\sim 5.5 \times 10^8 \text{ cells}\cdot\text{mL}^{-1}$. The planktonic SRB growth was quantified through optical density at 600 nm. The culture media that was used in the experiments was a modified-Postgate C medium composed of 0.5 g KH_2PO_4 , 4.5 g Na_2SO_4 , 1 g NH_4Cl , 0.06 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 g $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.3 g sodium citrate, 0.004 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and completed with H_2O to 1 L. Additionally, $5 \text{ g}\cdot\text{L}^{-1}$

of fresh plant organic matter (wet weight, preserved at 4 °C) that was extracted from a wetland located in the Aguas Calientes area was added to the culture medium to favor microbial growth. Decomposition of this plant organic matter occurs naturally in the proximity of sediment profiles that were sampled for this study. The pH was adjusted to 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5 (seven reactors and one control reactor [pH = 7.0]), and every three days 5 mL samples were taken and the pH evolution and chemical oxygen demand (COD) consumption were monitored. The reactors were operated without agitation (static state) at 25 °C in the dark. The reactors had teflon-lined septa at the top for periodic sampling and to bubble N₂ gas every three days to remove oxygen and generate an anaerobic environment.

Sulfate reduction experiments were conducted with the same SRB-enriched culture, under the same operating conditions mentioned above (static state, 25 °C in the dark), and with the same culture media (modified-Postgate C medium) enriched with plant organic matter (5 g·L⁻¹ wet weight), but with natural water from the Azufre River as culture medium (See Section 2.1). Four batch reactors (two replicates) were installed in the laboratory and the initial pH of the natural water was adjusted using 2M NaOH to 3.5, according to acid tolerance assays. Similarly, reactors were bubbled with N₂ gas to remove oxygen and were kept in the dark at room temperature (23 ± 1 °C). The batch reactors were kept for 30 days without sampling to allow for the adaptation and acclimatization of acid tolerance in SRBs.

2.4. Geochemical Analyses

Water samples from the reactors were taken every seven days, for a total of 80 days. The bioreactor performance was assessed by monitoring pH, electrical conductivity, sulfate, and by As and Fe removal. Measurements of pH (PHC301, HACH), DO (LDO101, HACH), and electrical conductivity (CDC401, HACH) were determined immediately after the collection of the sample. Sulfate was analyzed by ion chromatography (882 compact IC plus, Metrohm, Herisau, Switzerland), whereas total dissolved metal concentrations (As and Fe) were carried out by Inductively Coupled Plasma Optical Emission Spectrometry (IC-OES).

Solid phases that precipitated during sulfate reduction experiments (with natural water from the Azufre River) were examined by X-ray diffraction (XRD) and Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM-EDX). XRD analyses were performed on a Bruker D8 Advance (40 kV, 40 mA Cu K α radiation, $\lambda = 1.5406 \text{ \AA}$) and surface characterization of solid phases were conducted using a scanning electron microscope (JSM-IT300LV; JEOL Ltd., Tokyo, Japan), equipped with an Oxford EDX AZtec detector (Oxford Instruments, High Wycombe, UK). Prior to the observation by SEM-EDX, the samples were fixed with 2% glutaraldehyde solution in a sodium cacodylate buffer (0.2 M, pH 7.2, at 4 °C) and dehydrated in water/ethanol solutions (50, 70, 90 and 100%). The samples were then treated for 45 minutes with a critical point dryer (Autosamdri-815; Tousimis, Rockville, MD, USA), and were coated with carbon in a desktop sputterer (Desk V; Denton Vacuum, Moorestown, NJ, USA).

3. Results and Discussion

3.1. Metal-Tolerant Sulfate Reducing Bacteria

Sediments from sampling site were used to evaluate sulfate reduction processes under extreme conditions (Low pH, high concentration of heavy metals). From these sediments, metal-tolerant SRB were enriched in Winogradsky columns. We observed the precipitation of sulfide minerals inside the Winogradsky columns through the increase of black areas over time in the external face of the acrylic column and sulfate removal of up to 76%. Metal-tolerant bacterium can successfully grow under these conditions, withstanding highly toxic As concentrations. These results suggest that SRB can tolerate high concentrations of As and Fe in solid and dissolved phases, and can develop metal resistance mechanisms to protect their cellular components.

High concentrations of dissolved and precipitated metals have a negative effect on bacterial growth, and cause a reduction of sulfate removal rates in SRB [18]. Arsenic, Fe, and other metals compete with

essential cations and may inactivate enzymes and other proteins [18]. The toxicity depends on the type of metal and the type of bacteria or the exposed microbial community, ranging from a few $\text{mg}\cdot\text{L}^{-1}$ to concentrations above $10 \text{ mg}\cdot\text{L}^{-1}$ [18]. Interestingly, our results show that SRB tolerate concentrations to the order of $\text{g}\cdot\text{kg}^{-1}$ in the solid phase, and higher than $10 \text{ mg}\cdot\text{L}^{-1}$ in the aqueous phase. The mechanisms for toleration of high concentrations of metals may be associated with processes of surface adsorption, oxidation/reduction reaction, enzymatic speciation, cellular respiration, bioaccumulation, biosynthesis of metal-binding proteins or extracellular polymers, among others [23,24]. Operationally, for SRB, the metal tolerance is more relevant because it does not allow for precipitated metal sulfides to alter their metabolic activity. Even so, the increase in metal precipitates may reduce the growth kinetics of SRB and sulfate removal rates when Fe and As precipitates reach values that are higher than those reported in sediments. However, field conditions in the Aguas Calientes area suggest that SRB strains can tolerate high dissolved and solid phase metal concentrations. Therefore, the isolation and growth of these microorganisms under controlled conditions can enhance the efficiency of treatment systems for waters with extremely high concentrations of As and Fe.

3.2. Metal-Tolerant Sulfate Reducing Bacteria Are Tolerant to Low pH

SRB tolerance to low pH conditions was evaluated in batch reactors. The results showed microbial growth in pH values that were higher than 3.5 (Figure 2). This growth is evidenced by the COD consumption ($-\Delta 30\text{--}78\%$), pH increase, and by the counting of total cells in the culture (Data not shown). Under pH 3.5, no increase in cell number was observed and organic matter degradation activity (COD consumption) was not reported, this suggests that under pH 3.5, the acidity was lethal for microbial activity (associated to SRB). Conversely, in reactors with a pH of greater than pH 3.5, an increase in the pH that is associated with the COD consumption was observed, which is explained by the increase in the total alkalinity of the culture medium, mediated by sulfate reduction activity. In the sulfate reduction process, SRB reduce sulfate to sulfide and oxidize the electron donor (organic carbon source) into bicarbonate [25,26]. The bicarbonate increases the alkalinity causing pH increase [25,26].

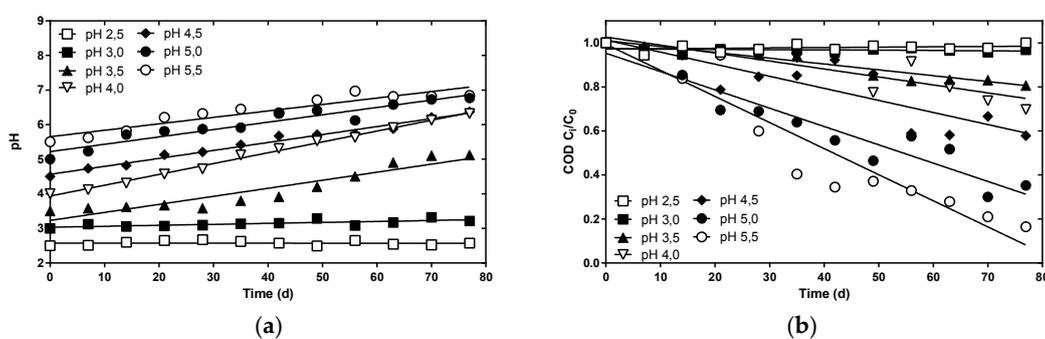


Figure 2. Sulfate Reducing Bacteria (SRB) acid tolerance. (a) Variation of pH and chemical oxygen demand (COD) consumption (b) in anaerobic batch reactors. The COD consumption is expressed as the relative concentration (C_i/C_0) related to the initial COD concentration of the culture medium (C_i = concentration at time i , C_0 = initial COD concentration). C_0 in the aqueous phase of all reactors was $1040 \pm 5 \text{ mg}\cdot\text{L}^{-1}$. Initial $\text{SO}_4^{2-} = 3067.9 \text{ mg}\cdot\text{L}^{-1}$, $\text{Fe} = 0.8 \text{ mg}\cdot\text{L}^{-1}$. A linear regression was fitted to the data in different pH conditions.

Typically, optimal growth conditions for SRB in different natural systems occur at pH values of greater than 5.0, because SRB activity is reduced under this pH [16]. Acidophilic SRB can survive in natural waters with $\text{pH} < 3.0$, but the sulfate reduction rates are significantly reduced [27,28]. This occurs because SRB must maintain a higher/circum-neutral internal cell pH, which would produce a high consumption of energy, and therefore they would have less energy to grow [19]. Nevertheless, although acidic conditions are not preferable, the Gibbs free energy calculations show that the reaction is more favorable at a lower pH, obtaining a higher energy gain [19]. Sánchez-Andrea, et al. [19]

propose that the extra energy that is produced at low pH could compensate the higher energy required to pump the protons out of the cell. Other studies support the presence of SRB at low pH based on the use of non-ionic substrates, such as glycerol, hydrogen, alcohols, or sugars [29–31]. However, it is also necessary to consider that at acidic pH, H₂S is found almost completely non-dissociated (pK_a = 7, 30 °C), and undissociated sulfide (H₂S) has an inhibitory effect on growth [28–30]. These mechanisms support the lack of growth observed in reactors under pH 3.5. However, at a pH higher than 3.5, the sulfate-reducing metabolic activity may generate total alkalinity, raising the pH and neutralizing the inhibitory effect, thus increasing the growth rates and the sulfate reduction rates when approaching circum-neutral conditions.

AMD treatment has been extensively studied in recent years [25,31–34]. Typically, AMD waters have a pH below 4.0 [12,35]. SRB grow optimally under circum-neutral conditions, therefore the AMD treatment that is mediated by SRB requires pH neutralization. However, many researchers have studied acid-tolerant and acidophilic SRB [21,36–40]. Kimura, et al. [41] have reported a reduction of sulfate at pH 3.8, and Bijmans, et al. [42] reported a high rate of sulfate reduction at pH 4.0 and 4.5 using H₂ and formate as electron donors in laboratory experiments. Despite this, few studies have linked SRB acid tolerance with tolerance to high concentrations of heavy metals and their potential impact on As removal from acid mine drainage waters [22,43]. We found SRB to be capable of tolerating pH up to 3.5 and concentrations of As higher than 10 mg·L⁻¹, and the interaction between Fe and As may be relevant for the removal process. The native acid/metal-tolerant SRB may be adequate for removal of As concentrations from contaminated waters.

3.3. Arsenic and Iron Are Removed in Sulfate Reduction Reactors

Batch tests were conducted to probe the removal of As and Fe under acidic conditions. The pH neutralization was observed over time in two reactors. The pH increased rapidly from 3.5 to between 6.0 and 7.5 (Figure 3). Additionally, an increase in the total alkalinity was observed (from 5 mg·L⁻¹ to 500 mg·L⁻¹ CaCO₃), suggesting that the processes of acid neutralization may be due to biological sulfate reduction. Interestingly, both of the reactors showed a constant pH above 6.5 after 35 days of operation, suggesting that microbial consortia are able to overcome the acclimatization stage and maintain circum-neutral conditions. The pH values reported during the batch experiments suggest that SRB are active during the operation and tolerate the initial acidity. Additionally, in the two reactors SRB activity was confirmed by sulfate removal of more than 60% (data not shown).

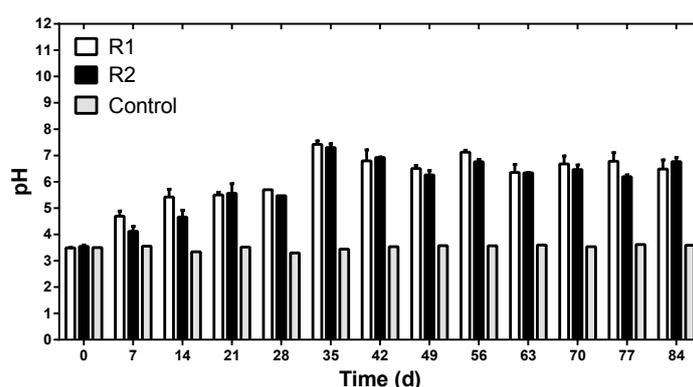


Figure 3. pH evolution in batch reactors inoculated with SRBs enriched from anaerobic sediments. Initial SO₄²⁻ = 3067.9 mg·L⁻¹, Fe = 0.8 mg·L⁻¹. The data are presented as the mean ± SEM (error bars).

To reveal if pH neutralization and the sulfate reduction process promote As removal, we evaluated the change in Fe and As concentrations in natural water from the Azufre River that was used as culture medium in sulfate reduction batch reactors. The results showed around 90% and 80% removal of Fe and As, respectively. Figure 4 shows that the highest removal of As and Fe occurred in correlation

with the increase of pH (Figure 3), suggesting an initial pH-dependent mechanism that is associated with the sulfate reduction.

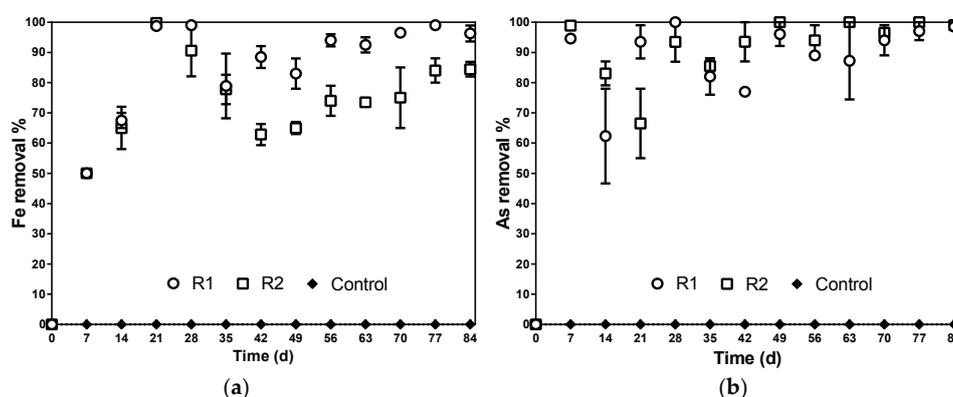


Figure 4. Total dissolved Fe (a) and As (b) removal during batch reactor operation. A marked removal of As and Fe was observed after 14 days of operation. The metal removal data are the mean of two reactors (average of two replicates). Initial concentrations in batch reactors were As = 3.6 mg·L⁻¹, Fe = 81 mg·L⁻¹ and SO₄²⁻ = 4678 mg·L⁻¹. The data are presented as the mean ± SEM (error bars).

X-ray diffraction that was performed on the precipitated solids (lyophilized) recovered at the end of batch sulfate reduction experiments shows a strongly bulging amorphous baseline (Figure 5). Peaks of crystalline minerals for As sulfides were not observed, but weak peaks were detected for sulfate phases, such as Gypsum (CaSO₄·2H₂O) and Fe such as Jacobsonite (MnFe₂O₄) (Figure 5). Poor crystallization of minerals could be a consequence of early stages of sulfide precipitation. Electron microscopy images confirmed the presence of minerals that are associated with microorganisms (Figure 6a) and EDX microanalysis revealed Fe, As, and S signals for different surface particles indicating the presence of Fe-S-As, Fe-As or Fe-S compounds in the precipitated solids (Figure 6b). Thus, solid phase analysis indicates that the removal of As and Fe is associated with compounds precipitated in sulfate reduction reactors.

Removal of As may be associated with the formation of Fe sulfides, which in anoxic environments are capable of sorbing dissolved As [44]. In anoxic environments, the formation of sulfide mineral phases control the mobility and speciation of As [44]. Arsenic can coprecipitate with sulfide minerals (realgar, orpiment) [45] or sorb onto sulfide minerals (mackinawite or troilite) [46]. Changes in species distribution of As and chemical associations with Fe and S minerals are determined by variations in pH and redox potential [46–48]. Recent studies have shown that As can be removed as orpiment (As₂S₃) or realgar (AsS) under anaerobic conditions from acidic waters [43,49–52]. In turn, Le Pape et al., [22] showed the complete removal of As from AMD waters by an indigenous SRB consortium. Using Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy, they showed that As precipitated as thiol-bound As^{III}, amorphous orpiment (am-As^{III}₂S₃) and realgar (As^{III}S). This supports the idea that As removal could be explained by the formation of amorphous Fe-bearing sulfides and amorphous As-bearing sulfides and can occur by precipitation, and/or coprecipitation processes. However, As speciation analysis at the molecular level is necessary to confirm that this mechanism is relevant for the As removal, as mediated by acid/metal-tolerant SRBs.

Also, it is not possible to rule out As removal by an indirect mechanism of adsorption of As species onto Fe minerals (amorphous and/or nanocrystalline phases). The As sorption has been extensively studied for Fe oxides as ferrihydrite or goethite and Fe sulfides as mackinawite or pyrite. [53–55]. For these processes to occur, tolerance of SRB to high concentrations of dissolved metals (As and Fe) must be a prerequisite. Our results indicate that acid/metal-tolerant SRB could be decisive for As removal from polluted waters, and the integration of both resistance processes (acid and metal tolerance) would allow for the treatment of acidic waters enriched with As and other heavy metals.

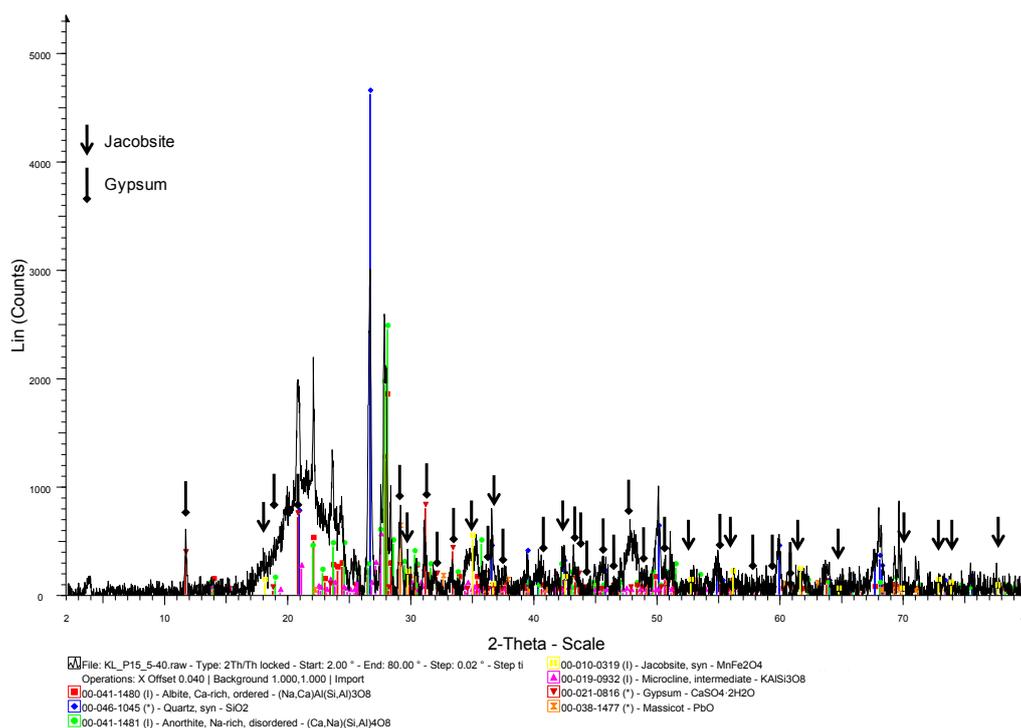


Figure 5. Representative X-ray diffraction (XRD) pattern for biologically produced solid precipitates collected at the end of sulfate reduction experiments (80 days). Peaks marked by arrows have been identified as Jacobsite and Gypsum. The formation of amorphous precipitates is shown as a bulking of the baseline (Black line). The vertical bar shows the scale of relative counts. Initial concentrations in batch reactors were As = 3.6 mg·L⁻¹, Fe = 81 mg·L⁻¹ and SO₄²⁻ = 4678 mg·L⁻¹.

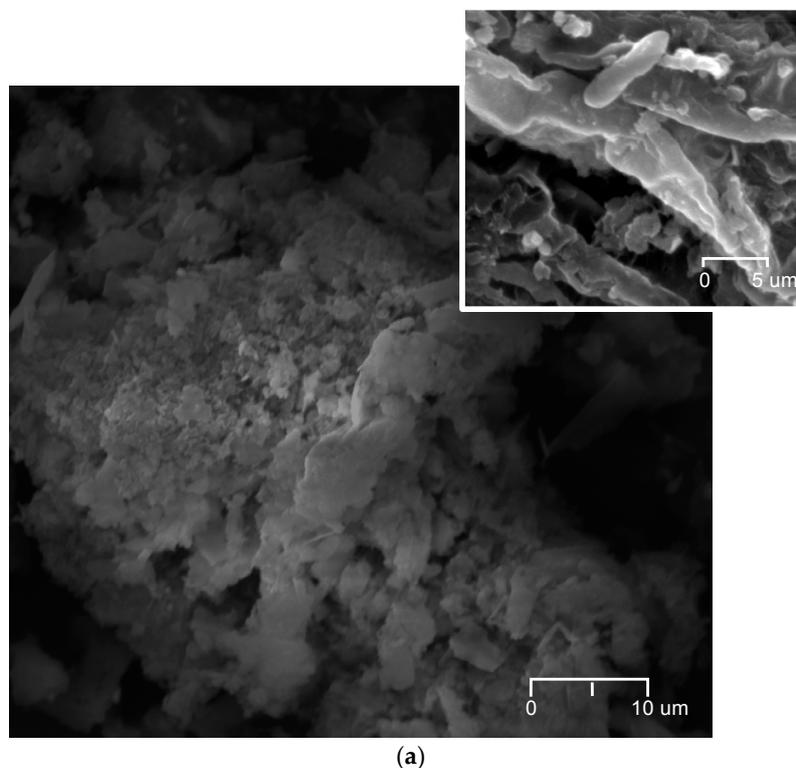
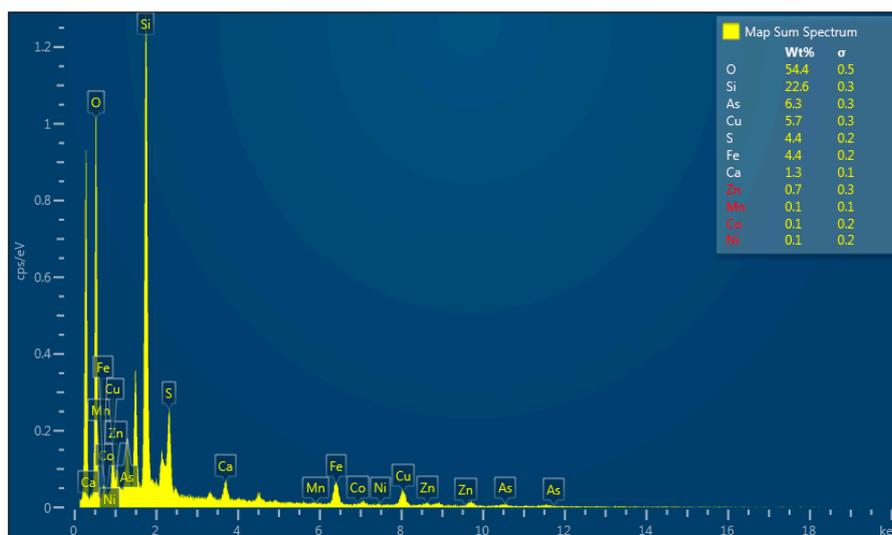


Figure 6. Cont.



(b)

Figure 6. (a) Scanning electron micrographs of SRBs and solid phases precipitates inside the sulfate reduction reactors, carbon was used as a surface coating; (b) Energy dispersive X-ray spectrum of precipitates solids. Intensity of As, Fe, and S emission lines are shown in the Energy Dispersive X-ray Spectroscopy (EDX) spectrum. Other elements such as Mn, Zn, Ni, Co, Pb, and Al have also been identified. The natural water of the Azufre river has concentrations of these elements, which can also precipitate during sulfate reduction processes. Initial concentrations in batch reactors were $\text{As} = 3.6 \text{ mg}\cdot\text{L}^{-1}$, $\text{Fe} = 81 \text{ mg}\cdot\text{L}^{-1}$ and $\text{SO}_4^{2-} = 4678 \text{ mg}\cdot\text{L}^{-1}$ (The natural water of Azufre river has others heavy metals identified in the EDX spectrum).

4. Conclusions

Our results show that SRB tolerate low pH and can grow under high concentrations of As and Fe. pH neutralization that is mediated by microbial activity derived from acid/metal-tolerant SRB was observed in batch reactors. pH values remain in circum-neutral ranges due to the increase in alkalinity during the sulfate reduction process and this promotes the precipitation of metal carbonates and hydroxides in oxic microenvironments. Fe and As removal is influenced by the pH increase that is mediated by acid/metal-tolerant SRB. The increase in pH and sulfide concentration produces Fe removal associated with the precipitation of Fe-ores. Conversely, As removal may be associated with the precipitation of amorphous Fe-bearing sulfides and/or amorphous As-bearing sulfides (i.e., amorphous orpiment; $\text{am-As}^{\text{III}}_2\text{S}_3$) or by an indirect mechanism of adsorption onto Fe minerals (Fe-sulfides). These results are a step forward in understanding sulfate reduction systems under acidic and high metal conditions, and suggest the potential future applications for the bioremediation of acidic waters enriched in As.

These findings contribute to the understanding of biological sulfate reduction processes under extreme conditions of low pH and high concentrations of As and Fe. Simultaneous tolerance to low pH and high concentrations of metals can be transformed into an effective alternative for As removal and could be expanded towards the treatment of other heavy metals derived from natural sources or mining operations. In the same way, the study and application of these processes could improve traditional SRB-mediated AMD treatment systems, which have the constraints of the initial operating conditions (i.e., low pH, dissolved heavy metals). Additional efforts are needed to describe the mechanisms that are involved in the tolerance to As, Fe and low pH, and the tolerance to other metals. Future research should include the study of these processes and the interaction of acid/metal-tolerant SRB with other acidophilic or acid-tolerant microorganisms living in AMD waters.

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Conflicts of Interest: The authors declare no conflict of interest.

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