Arsenic biomineralization: The role of the sulfur cycle in preventing arsenic groundwater contamination

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1. Introduction

Arsenic (As) is a toxic compound that is present in various concentrations in the groundwater all over the world [1]. It is known for its carcinogenic effects, which have led the Environmental Protection Agency (EPA) to set a maximum contaminant level (MCL) of 10 µg/L of As in drinking water in 2006 [2]. Several efforts are been carried out to understand the factors that contribute to As (im)mobilization in the environment. There are geological factors that influence the presence of As in groundwater, such as highly reducing conditions, young sediments, geothermal environments and high As-content parent rocks, which tend to accumulate As in the groundwater [3]. In 2000, Welch analyzed As concentration data from 300,000 different wells across the United States, and the higher As concentrations are mostly found in the Southwestern states, confirming a regional occurrence of As [4].

Arizona’s groundwater often contains high background levels of As; in fact, 30% of the 809 wells analyzed in Arizona in 2000 exceeded the MCL of As [5]. Furthermore, almost 10% of these wells with As concentration higher than 10 ppb are used for drinking purposes As-containing sulfide minerals are known to contribute to high background As groundwater concentrations in certain regions and mining districts in Arizona (Fig.1). For example, Verde Valley in central Arizona is a region where As groundwater concentrations range from 20 to 210 ppb and the geochemistry of the parent material is dominated by sulfide minerals [6, 7]. While dissolution of sulfide minerals may contribute to As release, the interaction of the As and sulfur (S) biogeochemical cycles can also be harnessed to promote the immobilization of As. [8]. In order to evaluate the environmental sources of As, it is necessary to understand the biogeochemical cycle of As and its interactions with S minerals. Realgar (AsS), arsenopyrite (FeAsS) and orpiment (As2S3) are natural As-sulfide minerals, and their biogenesis constitutes a clear example of the relationship between As and S in the environment [4].

The link between the As and S cycles is As sulfide minerals (ASM), such as orpiment (As2S3), as it can be appreciated in Fig. 2. Microorganisms play a very important role in the biogeochemical cycles of As [9] and S [10], and consequently, in the mobilization and immobilization processes of As in the environment. The two main species of As in water are arsenite (AsIII) predominant in reducing environments, and arsenate (AsV) which prevails in oxidizing environments [3]. S is mainly present as sulfide (S2-) and sulfate (SO42-) the most reduced and most oxidized species, respectively. Microorganisms will oxidize or reduce As and S depending on the redox conditions present in the aquifer. In reducing environments, AsV is reduced to AsIII by dissimilatory arsenate-reducing bacteria (DARB) and SO42- to S2- by SO42--reducing bacteria (SRB). If a concurrent microbial reduction of AsV and SO42- takes place, the biomineralization of As will occur and As2S3 as well as related minerals will be formed. Some microorganisms have the ability to act simultaneously as DARB and SRB, and to catalyze the formation of As2S3 [11]. The importance of this process in Arizona is unquestionable, where intense mining activities causes the oxidation of S2- minerals and the mobilization of the As associated with those minerals. The objective of this research is to study the interaction between these two cycles, in order to prevent As groundwater contamination, and as a possible process to bioremediate As-contaminated groundwater by stimulating the activity of the microorganisms responsible of the As biomineralization. This objective can be divided in two specific aims: (i) to study the stability of ASM in different environmental conditions; and, (ii) to study the biological mechanisms of the biomineralization of ASM.
2. Materials and Methods

2.1. Source of microorganisms

2.1.1. Aerobic microorganisms

Aerobic biomass was a Return Activated Sludge (RAS) obtained from a local wastewater treatment plant (Ina Road, Tucson, Arizona).

2.1.2. Anaerobic microorganisms

Three different anaerobic granular sludges were obtained from full scale upflow anaerobic sludge bioreactor (UASB) at different wastewater treatment plants: (i) Eerbeek (Eerbeek, The Netherlands), from recycled paper wastewater (0.135 g VSS/g wet wt); (ii) Nedalco (Bergen op Zoom, The Netherlands), from a sugar beet distillery effluent (0.0654 g VSS/g wet wt); (iii) and Mahou (Guadalajara, Spain) from beer brewery wastewater (0.0792 g VSS/g wet wt).

A S\textsuperscript{2-}-oxidizing bacteria enrichment culture was obtained from a 250 mL UASB initially inoculated with Eerbeck sludge. The reactor was fed with basal mineral medium supplemented with thiosulfate (as Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}.5 H\textsubscript{2}O) and nitrate (as KNO\textsubscript{3}) at the concentrations of 20 mM and 37.5 mM, respectively. After a stabilization period of 65 d., all the thiosulfate was successfully oxidized to SO\textsubscript{4}\textsuperscript{2-}. After more than 170 days of operation, the reactor was stopped and the enriched biomass was conserved anaerobically in the same basal mineral medium at 4°C.

2.2. Medium composition

2.2.1. Stability of ASM experiments

The stability of two different ASM was evaluated, amorphous orpiment (As\textsubscript{2}S\textsubscript{3}) and arsenopyrite (FeAsS). Both were maintained in anaerobic conditions to avoid any oxidation during storage. 104.2 g/L of orpiment or 75 g/L of arsenopyrite was added to the medium depending on the experimental conditions.

The standard basal mineral medium (pH 7.1 - 7.2) was prepared using ultra pure water (Milli-Q system; Millipore) and contained (mg/L): K\textsubscript{2}HPO\textsubscript{4} (637.5); KH\textsubscript{2}PO\textsubscript{4} (1700); NH\textsubscript{4}Cl (595); MgCl\textsubscript{2}.6H\textsubscript{2}O (165.8); CaCl\textsubscript{2}.2H\textsubscript{2}O (21.25); NaHCO\textsubscript{3} (4250), and 2 mL/L of a trace element solution containing (mg/L): FeCl\textsubscript{3}.4H\textsubscript{2}O (2,000); CoCl\textsubscript{2}.6 H\textsubscript{2}O (2,000); MnCl\textsubscript{2}.4 H\textsubscript{2}O (500); AlCl\textsubscript{3}.6 H\textsubscript{2}O (90); CuCl\textsubscript{2}.2H\textsubscript{2}O (30); ZnCl\textsubscript{2} (50); H\textsubscript{3}BO\textsubscript{3} (50); (NH\textsubscript{4})\textsubscript{6}Mo\textsubscript{7}O\textsubscript{24}.4 H\textsubscript{2}O (50); Na\textsubscript{2}SeO\textsubscript{3}.5 H\textsubscript{2}O (100); NiCl\textsubscript{2}.6 H\textsubscript{2}O (50); EDTA (1,000); resazurin (200); HCl 36% (1 mL). The system bicarbonate:CO\textsubscript{2} was used as buffer system.

The experiments were performed in aerobic and anaerobic conditions. In the aerobic experiments, O\textsubscript{2} was provided in a concentration of 2.68 mmol O\textsubscript{2}/L-liq, which was added by flushing the treatments with O\textsubscript{2}/He/CO\textsubscript{2} (20:60:20). In the anaerobic experiments, nitrate was used as the terminal electron acceptor and it was amended as KNO\textsubscript{3} in a concentration of 1.4 mM, and the treatments were flushed with He/CO\textsubscript{2} to maintain anaerobic conditions. 0.5 g VSS/L of biomass was amended in the biological treatments.

2.2.2. Biomineralization of ASM experiments

The basal medium was prepared using ultra pure water (Milli-Q system; Millipore) and contained (mg l\textsuperscript{-1}): K\textsubscript{2}HPO\textsubscript{4} (600); NaH\textsubscript{2}PO\textsubscript{4}.2H\textsubscript{2}O (899); NH\textsubscript{4}Cl (280); MgCl\textsubscript{2}.6H\textsubscript{2}O (83); CaCl\textsubscript{2}.2H\textsubscript{2}O (10); yeast extract (20), and 1 ml l\textsuperscript{-1} of the same trace element solution described
previously. \( \text{SO}_4^{2-} \) was added as Na\(_2\)SO\(_4\) (0.75 mM) and As\(^{V}\) as Na\(_2\)HAsO\(_4\).7H\(_2\)O (0.5 mM). The electron donor used was ethanol (4.9 mM). 1.5 g VSS/L of biomass was added to the treatments. The experiments were flushed with \( \text{N}_2/\text{CO}_2 \) (80:20) to ensure anaerobic conditions. NaHCO\(_3\) was used to control the pH of the solution which ranges from 6.2 (0.5 g/L \( \text{HCO}_3^- \)) to 7.0 (7 g/L \( \text{HCO}_3^- \)).

2.3. Experimental incubations

2.3.1. Stability of ASM experiments

The stability of ASM was tested through a series of batch experiments performed in 160 mL serum bottles sealed with rubber septa, maintained at 30°C in a constant agitation of 115 rpm. The assays were run in duplicates. Abiotic treatments were run with no addition of any biomass but with the presence of the mineral and the electron acceptor. Biological treatments were performed by adding any of the sludges described before to the medium with an electron acceptor and the mineral. Controls with no electron acceptor or with no mineral were run in parallel.

2.3.2. Biomineralization of ASM experiments

The biomineralization of ASM was evaluated initially in batch experiment to study the effect of different factors in the process, such as kind of inocula, pH, and electron acceptor concentrations. These experiments were performed in 160 mL serum bottles closed with rubber septa and incubated in the same conditions than for the stability experiments. The treatments were run in triplicates since one of the bottles was dedicated to pH measurements and solid phase analysis. Proper controls were set up in parallel to ensure the fidelity of the results. These controls were: (i) abiotic just amended with As\(^{V}\) and ethanol, \( \text{SO}_4^{2-} \) and ethanol, or both, As\(^{V}\) and \( \text{SO}_4^{2-} \), and ethanol; (ii) inocula with just one of the electron acceptors and the ethanol; (iii) inocula with no electron acceptor; and, (iv) inocula with one electron acceptor but no ethanol.

2.4. Analytical Methods

Liquid samples were taken from sealed anaerobic serum flasks by piercing the stoppers using syringes with 16-gauge needles. All samples were centrifuged (10 min, 14,000 g) immediately after sampling and stored in polypropylene vials. As\(^{V}\) and \( \text{SO}_4^{2-} \) were analyzed by suppressed conductivity ion chromatography using a Dionex IC-3000 system (Sunnyvale, CA, USA) fitted with a Dionex IonPac AS11 analytical column (4 x 250 mm) and AG16 guard column (4 mm x 40 mm). During each injection the eluent (KOH) used was 30 mM for 10 min. Total As was measured by using an inductively coupled plasma-optical emission spectrometry (ICP-OES) system model Optima 2100 DV from Perkin–Elmer TM (Shelton, CT, USA). Headspace samples were taken with a pressure lock gas tight syringe (1710RN, 100 \( \mu \)l (22s/2”/2), Hamilton Company). Ethanol and acetate were analyzed using a Hewlett Packard 5890 Series II gas chromatograph fitted with a Restek Stabilwax®-DA Column (30 m x 0.35 mm, ID 0.25 um) with flame ionization detector, using helium (He) as a carried gas. S\(^2-\) was determined using the methylene blue method described by Truper and Schelegel in 1964 [12]. Solid phase characterization was performed using a Scanning Electron Microscopy (SEM) Hitachi S-3400N Type II combined with a ThermoNORAN microanalyzer for energy dispersive spectroscopy (EDS).
3. Results and Discussion

3.1. Stability of ASM

The mobilization of As from ASM has been studied using orpiment and arsenopyrite as ASM examples. This process has been evaluated in aerobic and anaerobic conditions, using nitrate as electron acceptor, and in abiotic and biological treatments.

3.1.1. Aerobic conditions

The dissolution of As from orpiment in aerobic conditions is shown in Fig. 3A. The dissolved concentration of O$_2$ (DO) is 2.68 mmol/L$_{liq}$ (8.5 ppm) and the pH was 7.2 over the experiment. After 65 d. of incubation, the total As concentration was 0.044 mM for the treatment inoculated with RAS and 0.060 mM for the abiotic treatment. O$_2$ was respike this same day and the experiment was maintained for other 46 d., which was consider the end point of the experiment. The final concentration of As was 0.086 mM for the inoculated treatment and 0.116 mM for the abiotic treatment. It was also observed a release of As from the control lacking O$_2$, in the amount of 0.064 mM, suggesting that the release of As was not related with the presence of the O$_2$. The fact of a greater As concentration in the abiotic treatments could mean that part of the As was being retained by the sludge. Just 11.6% and 8.6% of the total As was mobilized in the 111 d. of experiment in the abiotic treatment and in the biological treatment, respectively. The reaction that describe the dissolution process of amorphous orpiment in aerobic conditions was defined by Lengke & Tempel in 2001 [13] as the following:

$$\text{As}_2\text{S}_3(\text{am}) + 6\text{O}_2 + 6\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{AsO}_3(\text{aq}) + 3\text{SO}_4^{2-} + 6\text{H}^+$$  \hspace{1cm} (1)

In their experiments, they calculated the rate of dissolution of As from orpiment in the neutral to alkaline range of pH in aerobic conditions without any inoculum addition, using a continuous reactor which run for 1800 min. At a pH of 7.3, 25$^\circ$C and a DO concentration of 7.01 ppm, the As concentration at the steady state was 0.029 mM, which corresponds to a mobilization of 0.15% of the total As in 1800 min. The rate of dissolution of orpiment in their conditions was 8.33x10$^{-5}$ %/min, which is very close to the rate of dissolution of orpiment in the conditions described in this report, 7.25x10$^{-5}$ %/min. In 2002, Lengke & Tempel [14] analyzed the dissolution of As from natural orpiment, in order to evaluate the effect of the orpiment solid state in the release of As. They obtained that the dissolution rate is lower by a factor of 0.002 to 0.560 in natural orpiment than in its amorphous form. This evidence, combined with the results obtained in this research, suggests that the orpiment is relatively stable in aerobic conditions over time in a circumneutral pH.

The dissolution of As from arsenopyrite in aerobic conditions was studied using the same experimental conditions. As it can be observed in Fig. 3B, after 65 d. of incubation, the total As concentration was 0.007 mM for for the abiotic treatment, whereas no As released was appreciated in the inoculated treatment. After the respike with O$_2$ and incubation for other 46 d., the final concentration of As was 0.039 mM for the abiotic treatment, and still no As was measured in dissolution in the biological treatment neither in the controls. Just 7.78% of the total As was mobilized in the abiotic treatment, while the inoculated treatment seemed to inhibit the mobilization of As into the solution. For arsenopyrite, the reaction of dissolution can be described as:

$$4\text{FeAsS} + 14\text{O}_2 + 16\text{H}_2\text{O} \rightarrow 4\text{Fe(OH)}_3 + 4\text{HAsO}_4^{2-} + 4\text{SO}_4^{2-} + 16\text{H}^+$$  \hspace{1cm} (2)
Walker et al. [15] studied the dissolution rate of arsenopyrite in aerobic conditions and circumneutral pH. At a pH of 6.3, 25°C and a DO concentration of 5.5 ppm, the As concentration after 24 h. of operation in a mixed flow reactor was of 5.30x10⁻⁴, corresponding with a dissolution of 2.78x10⁻³ % of the total As that was added as arsenopyrite. The dissolution rate in the conditions described in his article was 1.93 x 10⁻⁶ %/min, an order the magnitude lower than the rate obtained in our conditions (4.86x10⁻⁵ %/min). Walker demonstrated that the dissolution of arsenopyrite does not depend on the amount of DO, therefore a higher dissolution rate could be due to the difference in pH, from 6.3 in Walker’s experiment to 7.2 in the described experiment. Nevertheless, arsenopyrite seems to be relatively stable in aerobic conditions over time.

### 3.1.2. Anaerobic conditions

The dissolution rates of orpiment and arsenopyrite in anaerobic conditions are summarized in Table 1. Total As concentrations of 0.33 mM for orpiment and 0.15 mM for arsenopyrite were obtained in the non-inoculated treatments. Abiotic treatments showed the maximum dissolution rate for orpiment and for arsenopyrite (1.30x10⁻⁴ and 1.18x10⁻⁴ %/min, respectively) which is an order of magnitude greater than the abiotic treatments in aerobic conditions. There is no evidence in the literature of other studies done in anoxic conditions using nitrate as electron acceptor, however Lengke in his review of ASM dissolution rates [16] suggested that an increase in dissolution rate with other oxidants than O₂ could be due to complexation reactions at the surface of the mineral that could promote the release of As from the solid.

**Table 1.** Final As concentration and dissolution rates for orpiment and arsenopyrite in anaerobic conditions

<table>
<thead>
<tr>
<th></th>
<th>Orpiment</th>
<th>Arsenopyrite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final [As] (mM)</td>
<td>Exp. (days)</td>
</tr>
<tr>
<td>Abiotic</td>
<td>0.33</td>
<td>176</td>
</tr>
<tr>
<td>Nedalco</td>
<td>0.02</td>
<td>65</td>
</tr>
<tr>
<td>Eerbeck</td>
<td>0.18</td>
<td>65</td>
</tr>
<tr>
<td>Enrichment</td>
<td>0.19</td>
<td>176</td>
</tr>
</tbody>
</table>

In the inoculated treatments, the three inocula behaved different depending on the tested mineral. Orpiment was dissolved faster using Eerbeck as inocula, obtained a final As concentration of 0.18 mM, corresponding with a dissolution rate of 1.92x10⁻⁴ %/min, which was almost equivalent to the abiotic experiments. However, the dissolution rate for arsenopyrite was 9.47x10⁻⁵ %/min for Eerbeck, more than 10 times lower than for orpiment. The S²⁻-oxidizing enrichment sludge did not enhance the dissolution of the ASM, more over it seemed to promote the immobilization of As from this minerals. It is known that some microorganisms could enhance the mobilization of As from ASM (eg. *Acidithiobacillus ferrooxidans* [17, 18]), however there are also evidences that some microorganism can decrease the dissolution rate of some ASM (eg. *Thiobacillus caldus* [19]). Therefore, the effect that a specific inoculum will have in the mobilization of As from a mineral will depend on the population of microorganisms present in the sludge and the environmental conditions.

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3.2. Biomineralization of ASM

3.2.1. Biomass selection

The ability to form ASM by three different in ocula (Eerbeck, Nedalco and Mahou) has been studied. The optimum inoculum will be that that first reduces As and then \( \text{SO}_4^{2-} \) \([11, 20]\), avoiding an accumulation of \( S^{2-} \) in the liquid medium which can cause the formation of thioarsenites, dissolving the forming mineral \([21]\). Mahou was able to reduce \( \text{As}^V \) from 0.5 mM to 0.1 mM in less than 3 d., whereas no reduction was observed by Eerbeck or Nedalco. \( \text{As}^V \) reduction by Mahou was endogenous, since almost the same rate is observed in the control with no ethanol (as electron donor) addition. Therefore, Mahou was selected as the inocula and the first biomineralization experiment was set-up.

3.2.2. pH = 7

The first batch experiment was performed at a pH of 7.0. Total As, \( \text{As}^V \), \( \text{SO}_4^{2-} \), \( S^{2-} \), ethanol, acetate and the pH are monitored over time. In Fig.4, the concentrations of \( \text{As}^V \) (A) and \( \text{SO}_4^{2-} \) (B) are presented versus time. In the first 2 days, almost all the \( \text{As}^V \) was reduced at the same rate whereas \( \text{SO}_4^{2-} \) was present or not in the treatment, demonstrating that the reduction of \( \text{As}^V \) is not affected by the presence of \( \text{SO}_4^{2-} \). \( \text{SO}_4^{2-} \) reduction did not start until the \( \text{As}^V \) was reduced in the complete treatment, while the reduction of \( \text{SO}_4^{2-} \) occurred since the beginning of the experiment in the control with no \( \text{As}^V \). These results demonstrated that, indeed, Mahou had the optimum characteristics in order to precipitate an ASM. At the day 6 of the experiment, a yellowish color started to be appreciated in the complete treatment, indicating that orpiment precipitation was taking place \([11]\).

The total As concentration decreased from 0.42 mM at the beginning of the experiment to 0.22 mM after 11 d. (Fig. 5A), just in the treatment with \( \text{SO}_4^{2-} \), meaning that 47% of the As was eliminated from the solution. In the treatments with no \( \text{SO}_4^{2-} \) but inoculated there was a reduction of about a 5% in As concentration, probably due to some adsorption onto the biomass. \( \text{SO}_4^{2-} \) concentration decreased from 1.33 to 0.87 mM in the complete treatments, and from 1.33 to 0.53 mM in the treatment with no \( \text{As}^V \). Just 0.14 mM of total \( S^{2-} \) was measured in the complete treatment, whereas, when \( \text{As}^V \) was not added, the total \( S^{2-} \) concentration was 1.09 mM (Fig. 5B). Therefore, after just 11 days 0.2 mM of \( \text{As}^V \) and 0.38 mM of S were removed from the liquid phase, corresponding to a stoichiometric relation of S/As of 1.9. The theoretical stoichiometric relationship between S and As is 1.5 according with the reaction of precipitation of orpiment:

\[
2\text{As}^{III}(aq) + 3S^{2-}(aq) \rightarrow \text{As}_2S_3(s) \tag{3}
\]

The solid phase was analyzed by SEM-EDS. Different shapes of bacteria were observed under the microscope, and some of them showed a precipitated over their surface (Fig. 6). EDS characterization proved that the precipitated contained As and S, in orders of magnitude higher than the background. The precipitated under the microscope looked very similar to the one reported by Newman in 1997 \([11]\). The solid phase characterization, together with the disappearance of As and S from the solution in a stoichiometric ratio of 1.9, and the yellow color of the medium, suggest that orpiment is being form under the described conditions.

This experiment was maintained under incubation for 54 d. After 54 d., only 0.09 mM of total As were still in dissolution in the complete treatment (Fig.7). The initial reduction of total As in the inoculated controls was reversible, and the total As concentration was the same that at the
beginning of the experiment (~0.42 mM). 78.57 % of the total As was precipitated as orpiment, which was completely visible at this time (Fig. 8).

Keimovitz et al., in 2007 [20], run for over 74 d. a pilot scale bioreactor, using sediments from a landfill as inocula. Over that period of time, 85% of the arsenic in solution was eliminated, but in this case iron was present and played a very important role in the remediation. Newman demonstrated that a single microorganism can precipitate orpiment in the proper conditions [11] without the presence of iron, removing 40 to 50% of the As in 12 d. The results presented in this report demonstrate that orpiment can be formed by an inoculum from a wastewater treatment plant, confirming that the formation of ASM can be a sink for As in the environment and can potentially be used as a bioremediation strategy for groundwater systems.

3.2.3. Ongoing and future research

The effect of the pH in the formation of orpiment is being studied. An experiment at pH 6.8 has been set up and it has been running for 8 d. at the time this report was written. In Fig. 9A, the total As concentration at the beginning and at the end of the experiment is shown. In just 8 d., 0.302 mM of As was removed from the solution just when SO$_4^{2-}$ was added to the experiment, which is equivalent to a 52% of As removal, demonstrating that the pH will strongly affect the efficiency of the process, probably due to the formation of thioarsenite species at higher pH. Approximately, Just 0.14 mM of As was also removed from the inoculated treatment, as it also happened at pH 7. SO$_4^{2-}$ and S$^2-$ concentration were also measured, as shown in Fig. 9B. SO$_4^{2-}$ decreased 1.12 mM in the treatment with no As$^V$, and 0.85 mM when As$^V$ was present. 1.11 mM of total S$^2-$ was recovered from the treatment with no As$^V$, but just 0.403 mM of total S$^2-$ was recovered from the treatment with As$^V$. The stoichiometric relationship between S and As is 1.44, which is closer to the theoretical than the calculated for pH 7 experiment. The formation of a yellow precipitated has been observed. These results suggest that orpiment is being precipitated, and that lowering the pH of the solution will enhance the precipitation rate of the mineral.

A pH of 6.2 will be performed to evaluate the effect of a lower pH. With the results of the experiments at the three different pHs, a continuous, lab-scale, bioreactor will be set up and the bioremediation of As will be quantified over time.

4. Conclusions

Results have shown that ASM are relatively stable over time in aerobic and anaerobic conditions. The presence of an inoculum tends to prevent the mobilization of As from the minerals, which suggest that the dissolution rate will be lower in real natural systems. The precipitation of ASM can be promoted using sludges from wastewater treatment plants which have to own the ability of reducing As$^V$ and SO$_4^{2-}$. At pH 7, more than 75% was removed in 54 d. These evidences suggest that the precipitation of ASM could be use to remediate As from groundwater over longer period of time, leading to a more stable, long-term, AS-free system.

5. Acknowledgments

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6. References

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Appendix:

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Fig 1: Relationship between arsenic groundwater concentration (A) and mining activities in Arizona (B). (Arizona Geological Survey, 2000; State Department of Mines and Mineral Resources, 2002)
Fig. 2: The interaction between the As and S cycles can contribute to the immobilization of As and, consequently, to the prevention of As groundwater contamination.
Fig. 3: Total As concentration dissolved in aerobic conditions from orpiment (panel A) and from arsenopyrite (panel B). Legends: Biological treatment, O$_2$ and mineral (■); abiotic treatment (dashed lines O$_2$ and mineral (◊); biological treatment with no O$_2$ (○); biological treatment with no mineral EC1 (Δ).
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Fig. 4: As\textsuperscript{V} and SO\textsubscript{4}\textsuperscript{2-} reduction by Mahou with ethanol as electron donor. Legends: Complete treatment with Mahou, As\textsuperscript{V}, SO\textsubscript{4}\textsuperscript{2-}, and ethanol (■), with Mahou, SO\textsubscript{4}\textsuperscript{2-}, and ethanol (*) and with Mahou, As\textsuperscript{V} and ethanol (○); non inoculated controls (dashed lines) supplied with SO\textsubscript{4}\textsuperscript{2-} and ethanol but no As\textsuperscript{V} (◊), supplied with As\textsuperscript{V} and ethanol but no SO\textsubscript{4}\textsuperscript{2-} (x), and supplied with As\textsuperscript{V}, SO\textsubscript{4}\textsuperscript{2-}, and ethanol (Δ); inoculated controls (dotted lines) Mahou just with ethanol with no electron acceptor (●), Mahou with SO\textsubscript{4}\textsuperscript{2-} and no ethanol (-) and Mahou with As\textsuperscript{V} and no ethanol (+).
Fig. 5: Change in total As and S concentrations at pH 7. Panel A: Total As variation. Legend: Total As concentration at the beginning of the experiment (black column) and total As concentration at the day 8 of experiment (grey column). Panel B: Total S variation. Legend: $\text{SO}_4^{2-}$ change in concentration over the experiment (black column) and $\text{S}_2^-$ production over the experiment (grey column).
Fig. 6: On the top left, there is a SEM image of a bacterium with a white precipitated on top. The EDS spectra on the right correspond with the different colored areas of the SEM image on the bottom left. The first EDS spectrum corresponds with the precipitated on the surface of the bacterium (blue); the second EDS spectrum is from the bacteria background (green); and lastly, the third EDS spectrum is from the filter background (orange).
Fig. 7: Change in total As concentrations since the beginning until the day 54 of incubation at pH 7. Legend: Total As concentration at the beginning of the experiment (black column) and total As concentration at the day 8 of experiment (grey column).
Fig. 8: Visual orpiment formation as a yellow precipitate, comparison between the complete treatment with Mahou, $\text{SO}_4^{2-}$, $\text{As}^V$ and ethanol (bottle on the left) and the inoculated treatment with $\text{SO}_4^{2-}$ and ethanol but no $\text{As}^V$ (bottle on the right). Panel A shows the treatment after 6 d. of incubation and panel B after 54 d. of incubation.
Fig. 9: Change in total As and S concentrations at pH 6.8. Panel A: Total As variation. Legend: Total As concentration at the beginning of the experiment (black column) and total As concentration at the day 8 of experiment (grey column). Panel B: Total S variation. Legend: $\text{SO}_4^{2-}$ change in concentration over the experiment (black column) and $\text{S}^{2-}$ production over the experiment (grey column).