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Authors: Erika E. Rios-Valenciana, Roberto Briones-Gallardo, Luis F. Chazaro-Ruiz, Nadia Martínez-Villegas, Lourdes B. Celis

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Role of indigenous microbiota from heavily contaminated sediments in the bioprecipitation of arsenic

Erika E. Rios-Valenciana[a], Roberto Briones-Gallardo[c], Luis F. Chazaro-Ruiz[a], Nadia Martínez-Villegas[b], Lourdes B. Celis[a]*

[a] División de Ciencias Ambientales, [b]División de Geociencias Aplicadas, Instituto Potosino de Investigación Científica y Tecnológica, Camino a la Presa San José 2055, Lomas 4a. Sección, 78216, San Luis Potosí, SLP, México.

[c] Instituto de Metalurgia, Universidad Autónoma de San Luis Potosí, Sierra Leona 550, Lomas 2a. Sección, 78216, San Luis Potosí, SLP, México.

*Corresponding author

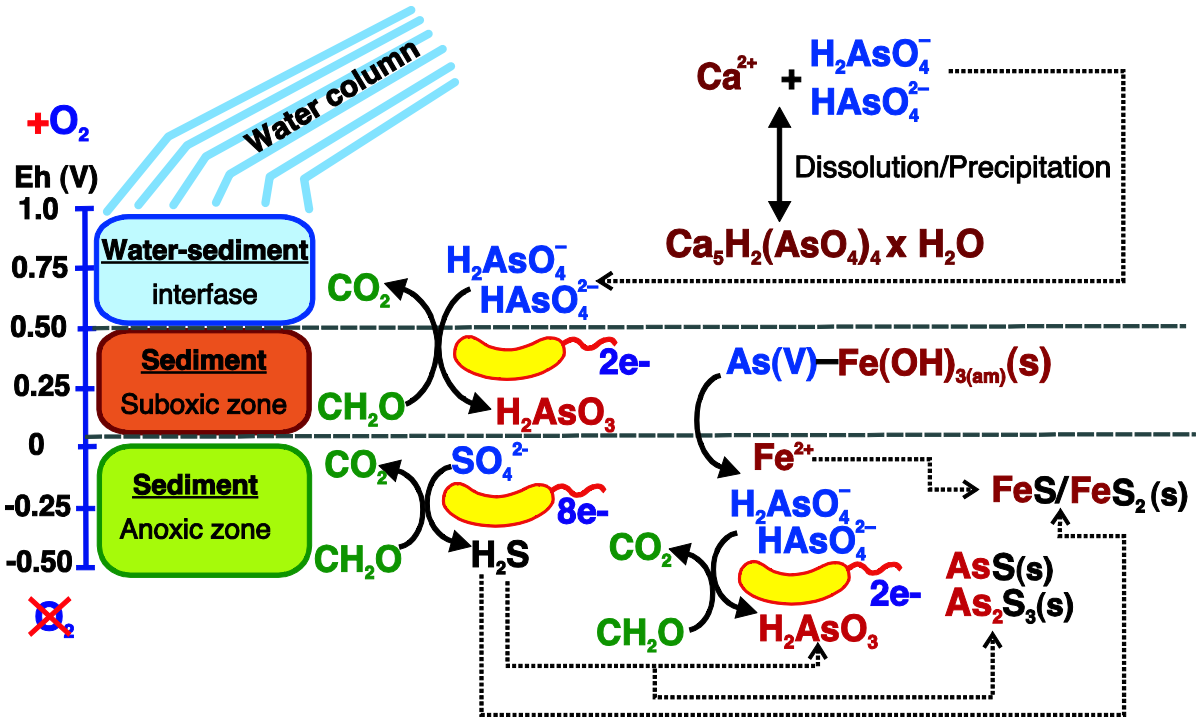
Lourdes B. Celis

email: celis@ipicyt.edu.mx

Tel.: +52-444-8342025

Fax: + 52-444-8342010

Graphical abstract



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Highlights

- Sediments (inocula) were highly contaminated with arsenic (238 or 2263.1 mg/kg)
- Precipitates were formed when arsenic and sulfate were reduced simultaneously
- As(III) was removed from the aqueous phase as arsenic sulfide
- As(III) remained in solution in the absence of sulfate-reduction

ABSTRACT

High arsenic concentrations have been detected in alluvial aquifers of arid and semi-arid zones in Mexico. This work describes the potential of microbial arsenate reduction of the indigenous

community present in sediments from an arsenic contaminated aquifer. Microcosms assays were conducted to evaluate arsenate and sulfate-reducing activities of the native microbiota. Two different sediments were used as inoculum in the assays amended with lactate (10 mM) as electron donor and with sulfate and arsenate (10 mM each) as electron acceptors. Sediments were distinguished by their concentration of total arsenic 238.3 ± 4.1 mg/kg or 2263.1 ± 167.7 mg/kg, which may be considered as highly contaminated sediments with arsenic. Microbial communities present in both sediments were able to carry out arsenate reduction, accomplished within 4 days, with the corresponding formation of arsenite; sulfate reduction took place as well. Both reducing activities occurred without previous acclimation period or enrichment, even at potential inhibitory concentrations of arsenate as high as 750 mg/L (10 mM). The formation of a yellowish colloidal precipitate was evident when both reducing processes occurred in the microcosm, which contributed to remove between 52 and 90.9% of As(III) from the liquid phase by bioprecipitation of arsenic as arsenic sulfide.

Keywords: arsenate, bioprecipitation, sediment, sulfate, reduction

1. INTRODUCTION

Arsenic-contaminated aquifers are a widespread problem and represent a latent risk for the human health [1, 2]; it is estimated that about 100 million people may be exposed to arsenic contaminated water [3]. Therefore, the study of the chemical and biological processes involved in the transformation of this metalloid is highly relevant. Multiple works have been conducted, at laboratory scale in microcosm or bioreactors, to study the biological transformations of arsenic and their impact on the speciation and distribution of arsenic in contaminated sediments. Most of the reports have focused on iron-rich environments [4–6], in which the predominance of iron oxides and oxyhydroxides controls the mobilization of arsenic. In these environments, one important mechanism that causes the desorption and release of arsenic into the aqueous phase is the reductive dissolution of iron oxyhydroxides as consequence of the microbial reduction of Fe(III) [6]. Conversely there is a lack of studies that focus on iron depleted environments, in this case it is important to take into account the interrelationship between sulfur and arsenic biogeochemical cycles because the microbial sulfate reduction plays an important role on arsenic speciation. The mobilization of arsenic and sulfur has been documented in sediments from lakes, rivers and aquifers [7–9], the extent of mobilization depends on the geochemical and mineralogical composition of the sediment, including the organic matter availability which may stimulate the indigenous microbial community and promote a series of biological processes that ultimately affect the chemical composition of the water [10].

The biological reduction of arsenate or As(V) to arsenite or As(III) is an undesirable process because it modifies the extent of arsenic toxicity in accordance to its oxidation state, from the less toxic form, As(V), to the more toxic and mobile form, As(III) [1].

To counteract the negative effect of arsenate-reduction this microbial process can be associated with the sulfate-reduction process, the availability of As(III) and sulfide can promote the bio-mineralization of arsenic by removing it from the aqueous phase by its immobilization as As-bearing sulfide minerals [11,12]; of course the process may be limited by the redox conditions and the concentration of As(III) and sulfide [5,13]. The biogenic arsenic sulfides promoted by microbial reduction of As(V) and sulfate are mainly orpiment (As_2S_3) and realgar (AsS), the stoichiometry of these minerals is presented in Reactions 1 and 2 [14].

$2\ H_3AsO_3 + 3\ HS^- + 3\ H^+ \rightarrow As_2S_{3(s)} + 6\ H_2O$ (Reaction 1)

$H_3AsO_3 + HS^- + 2\ H^+ + e^- \rightarrow AsS_{(s)} + 3\ H_2O$ (Reaction 2)

However, microbial sulfate-reduction may cause important changes in arsenic speciation such as the formation of dissolved thioarsenates or thioarsenites, which may maintain arsenic in soluble form [15].

Mexico is amongst the countries affected by high concentrations of arsenic in groundwater and several detailed studies have shown this problem, however most of those studies have only focused on the hydrogeochemical aspects [16–19]. There is a lack of studies conducted on calcareous and gypsic sediments with high concentrations of arsenic, calcite ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$), and depleted in iron, that investigate up to what extent the biological processes affect the mobilization of arsenic in such type of environments.

The aim of this work was to assess the potential of arsenic- and sulfate-reduction activities by the indigenous microbiota of sediments, from a calcareous hydraulic system highly contaminated with arsenic, without previous acclimation or enrichment. In addition, our

interest was to determine if the coupled processes of microbial arsenic- and sulfate-reduction linked to the oxidation of organic matter lead to the mineralization of arsenic.

2. MATERIALS AND METHODS

2.1 Source of inoculum

Surface sediments (5-10 cm depth) from two sampling sites were used as inoculum, sediment CB was obtained from the site identified as Cerrito Blanco (100º36’41” W and 23º40’23” N) and sediment CT was obtained from the site identified as Club de Tiro (100º38´22” W and 23º38’22” N). The sampling sites are near to the mining district of Santa Maria de La Paz (Matehuala, San Luis Potosi, Mexico); this mining district presents a high degree of contamination by arsenic. Further information about the hydrogeochemical characterization of both sites can be found in Martinez-Villegas *et al.* [18].

2.2 Characterization of the sediments

The characterization of the sediment, pore- and colum-water was accomplished through their elemental composition, total carbon, organic carbon and sulfate content, as well as by the quantification of pH, redox potential, and the total, volatile and fixed solids content. In addition the sediments were analyzed using Scanning Electron Microscopy (SEM) combined with energy dispersive spectroscopy (EDS), and by X-ray diffraction. The pore water of sediments was obtained by centrifugation at 10,500 *g* during 30 minutes; the liquid obtained was filtered (0.22 µm) and acidified (HNO_3 1 M) prior to analysis. The sediments were homogenized and sieved (1.7 mm) in an anaerobic chamber (COY 14500) before use them as inoculum.

2.3 Microcosms assays

Microbial sulfate- and arsenate-reducing activities were evaluated in serum bottles (125 mL) containing 120 mL of basal medium (supplementary material), pH adjusted to 6.8 and 10% (wet weight/volume) of sediment either from site Cerrito Blanco or Club de Tiro. The assays were prepared in the anaerobic chamber and the headspace (~5 mL) was purged with N_2/CO_2 (80:20) for 3 minutes; each assay was done in triplicate. Proper controls were set up as well: endogenous (without the addition of lactate, sulfate or arsenate); endogenous of sulfate-reduction (with lactate only); inhibition of sulfate-reduction (with 25 mM Na_2MoO_4, lactate, arsenate and sulfate); chemical (basal medium with lactate, arsenate and sulfate, but without sediment), sterile (with lactate, arsenate and sulfate but sterilized after the addition of the sediment). Lactate, sulfate and arsenate were added from stock solutions to a final concentration of 10 mM each, unless otherwise specified. The assays were incubated in the dark at 30 ºC, without agitation. Microcosms were periodically sampled, each 2 or 5 days during 20 days, to follow the concentrations of lactate, acetate, sulfate, sulfide and arsenic species (V and III). At the end of the experiments (30 days), the precipitates from selected assays were recovered and subjected to acid digestion for further elemental analysis by inductively coupled plasma-optic emission spectroscopy (ICP-OES, Varian 730-ES), the precipitates were also characterized by SEM-EDS and X-ray diffraction.

2.4 Most probable number estimates of arsenate-reducing microorganisms

The number of indigenous arsenate-reducing bacteria in the original sediments was estimated by the most probable number technique according to Kuai et al. [20]. Briefly, serial dilutions (10^{-1} to 10^{-10}) were inoculated, in triplicate, in serum vials containing 9 mL of medium amended with 10 mM acetate, 5 mM lactate and 5 mM arsenate. Vials were incubated in the dark for 30 days at 30 ºC without agitation. To determine if arsenate reduction took place, we

added 100 µL HCl (1 M) and 1.5 mL of sodium sulfide (15 mM) to each vial, the immediate formation (about 30 seconds) of a yellow precipitate was considered as positive result of arsenate-reduction. The most probable number was estimated from the indexes reported in the appropriate tables of standard methods [21].

2.5 Analytic procedures

The elemental composition of the sediments (after acid digestion), pore- and column-water, was analyzed by ICP-OES. Total arsenic was analyzed by Atomic Absorption Spectroscopy. Sulfate, lactate and acetate were determined by capillary electrophoresis [22]. Total carbon and organic carbon were determined in a Total Organic Carbon analyzer, equipped with a solid sample module. Arsenic species (V and III) were separated by anion-exchange chromatography (supplementary material). Dissolved sulfide was determined using the method of Cord-Ruwisch [23]. The pH, redox potential (Eh) and volatile suspend solids (VSS) were determined according to standard methods [21]. To recover the biogenic precipitates, the bottles were opened inside an anaerobic chamber; the solid phase was cleaned by centrifugation (12000 rpm) after washing it with O_2 free deionized water. The precipitates and original sediments were characterized using scanning electron microscopy (FEI-QUANTA 2000) combined with energy dispersive spectroscopy (EDAX - DX4). The precipitates and sediments were also characterized by X-ray diffraction (Bruker D8 Advance); the X-ray diffraction patterns were recorded from 10° to 80° 2θ with a step time of 10 s and step size of 0.02° 2θ. Phase identification was made by matching the experimental diffractogram with data from the PDF-4 of the ICDD (International Center of Diffraction Data).

3. RESULTS AND DISCUSSION

3.1 Sediment, pore water and water column characteristics

The results of the physicochemical parameters determined in the sediment samples as well as in the column water and pore water, of both sampling sites, are summarized in Table 1. The X-ray diffraction patterns and SEM-EDS analysis of both sediments are provided in Figures S1 and S2 of supplementary material. The mineralogy of sediment CB was dominated by gypsum ($CaSO_4 \cdot 2H_2O$), followed by calcite ($CaCO_3$) and quartz (SiO_2), while in the sediment sample from the site CT only calcite and quartz were identified. These results were in agreement with the morphological and elemental microanalysis using SEM-EDS that showed sulfur and calcium in sediment CB, whereas in sediment CT the main elements were silicon and calcium. The elemental analysis of the sediments (Table 1) showed that sediment CT had an iron concentration of 15.4 g/kg (6.3% dry weight) whereas sediment CB had 5.0 times less iron concentration 3.0 g/kg (1% dry weight). In general the sediments may be classified as sulfate-rich sediment (sediment CB) with 329 g of SO_4^{2-}/kg, and sulfate-poor sediment (sediment CT) with 18.8 g SO_4^{2-}/kg but enriched with iron. In sediments with high iron content, that usually contain more than 15% of iron, arsenic mobility can be limited by adsorption or co-precipitation with iron minerals; in reducing environments the lower solubility of iron sulfides limits the precipitation of arsenic sulfides because iron sulfides maintain low dissolved sulfide concentrations [24].

The arsenic content in sediment CT (2263.1 mg/kg) was almost ten times higher than in sediment CB (238.3 mg/kg), the concentration of arsenic in uncontaminated sediments is typically around 5-10 mg/kg [2], therefore the sediments had an extremely high content of arsenic. The concentration of arsenic in the pore water of sediment CB was higher than in the column water which pointed out to the release of some arsenic from the solid phase that may

accumulate in the pore water [15]. This situation is feasible because the dissolution of minerals containing arsenic, such as calcium arsenates, can occur. In addition, from a hydrogeochemical study, it was concluded that the main processes that control the arsenic concentration in the water column of the sites Cerrito Blanco and Club de Tiro, are related to the precipitation and dissolution of calcium arsenates [18]. Furthermore, sediment CB was rich in gypsum, a highly soluble mineral (2,600 mg/L) that could contribute to the high concentrations of arsenic in the pore water due to the dissolution of co-precipitates of calcium arsenates and gypsum [25]. This phenomenon could be enhanced by reducing conditions, oxidation of organic matter and microbial activity. For example, sulfate reducing bacteria (SRB) which consume sulfate, could eventually break the balance of sulfate in solution and promote gypsum dissolution; SRB could also accelerate the dissolution of gypsum by the production of extracellular polymeric substances [26] . In contrast, in sediment CT a similar concentration of arsenic (~48 mg/L) in both, the column water and pore water, was found; possibly in this site the dissolution processes did not occur due to the mineralogical composition of sediment CT which is dominated by calcite and quartz that have lower solubility than gypsum [27].

3.2 Arsenate reduction in a sulfate-rich scenario

In the experiments inoculated with sediment CB, arsenate reduction occurred in the assays that contained lactate, as electron donor, and As(V) and SO_4^{2-}, as electron acceptors. In the assay in which sulfate reduction was inhibited with molybdate, arsenate was also reduced (Fig. 1a). The complete reduction of As(V) was reached around 4 days of incubation and it was clearly associated with the increase of As(III) concentration (Fig. 1b). After 5 days of incubation

As(III) decreased gradually which was associated with the formation of a yellowish precipitate in the assays containing lactate, sulfate and arsenate. Apparently, there was no consumption of sulfate in any assay (Fig. 1c), although the formation of sulfide was evident and close to stoichiometry in those assays amended only with lactate or lactate and sulfate (Fig. 1d). Sulfide production was also detected in the endogenous control (without the addition of lactate, As(V) or SO_4^{2-}), pointing out to the presence of an intrinsic source of carbon and sulfate in sediment CB (Figs. 1c and d). Sediment CB was composed mainly by gypsum, this mineral tends to dissolve easily in water maintaining in equilibrium the concentration of sulfate in the solution. The solubility of gypsum is 2600 mg/L [26], which results in a sulfate concentration of 1450 mg/L equivalent to 15 mM, which was approximately the concentration in the experiments not amended with sulfate (Fig. 1c).

Lactate was completely consumed in the assays containing only lactate and lactate and sulfate, but it was not completely consumed in the experiments containing As(V) and SO_4^{2-} as electron acceptors (Fig. 1e), hence it is possible that there was some degree of inhibition caused by arsenic and for this reason the remnant of lactate was not invested in sulfate-reduction, even when the electron acceptor (SO_4^{2-}) was available. The inhibition of SRB by arsenic is a possibility because 10 mM of As(V) equivalent to 749.2 mg/L were added to the assays, and it has been reported that arsenic concentrations higher than 8 mM (600 mg/L) can be inhibitory to SRB [28]. On the other hand, when a high sulfate concentration is present, as it was the case of sediment CB, limited sulfate-reducing activity is desirable to avoid the formation of soluble arsenic-sulfide compounds, since it has been observed that in the presence of high sulfide concentrations the arsenic removal from the solution is poor, due to the formation of soluble complexes of thioarsenates and thioarsenites [4, 29], which are favored at S/As ratios between 1 and 4 [29].

The assay in which sulfate reduction was inhibited with molybdate (25 mM) allowed us to corroborate that no yellowish precipitate was formed during the experiment, pointing out that the reduction of both, As(V) and SO_4^{2-}, was required for the formation of such yellowish precipitate, which, *a priori,* we believed was mainly composed by arsenic sulfides. This observation was also supported by the fact that in the assays in which arsenate reduction occurred, sulfide concentration remained around 2.5 mM, even below the concentration of sulfide produced in the endogenous assays (Fig. 1d), possibly due to the precipitation of As(III) and sulfide.

Acetate accumulated in the assays that contained As(V), while it was completely consumed in the assays without arsenate (Fig. 1f). This observation can be explained by the presence of SRB which are able to metabolize acetate to CO_2, and it is supported by the sulfide production in the assays with either lactate only or with lactate and sulfate, that reached a sulfide concentration higher than 20 mM at the end of the experiment, which was more than the expected by the incomplete oxidation of 10 mM of lactate (Fig. 1d). Possibly, SRB were able to metabolize acetate whereas arsenate-reducing bacteria were not. Most of the reported arsenate-respiring bacteria can oxidize lactate incompletely [11,30]; although *Chrysiogenes arsenatis and Bacterium MPA-C3* are arsenate-respiring bacteria able to grow using acetate as electron donor/carbon source, besides other substrates [30,31].

3.3 Arsenate reduction in sediments poor in sulfate with some iron

Arsenate- and sulfate-reduction were evaluated using sediment CT as inoculum, which showed a low sulfate concentration and contained some iron. Figure 2 shows the concentration profiles of As(V), As(III), sulfate, sulfide, lactate and acetate over the incubation time of the batch experiments. The assays amended with As(V) presented arsenate-reducing activity (Figs. 2a

and b) coupled to the consumption of lactate and acetate production (Fig. 2e and f). The biological reduction of As(V) was clearly associated to the formation of As(III), and this occurred in all the assays amended with lactate, sulfate and As(V), even in the one were sulfate reduction was inhibited by molybdate (Figs. 2a and b). It is worth to note that in the absence of molybdate, from day 10 onwards, a significant decrease of As(III) concentration was observed, this fact was clearly related to the formation of a yellowish precipitate; while in the treatment with molybdate such a decrease did not happen, and there was no formation of precipitate. To properly analyze the results of this set of experiments, it is important to highlight some issues. In the assays with sediment CT the addition of sulfate was crucial to sustain the microbial sulfate reduction process because this sediment had low sulfate concentration. In the assay with lactate and sulfate, the sulfide concentration (9.1 mM), was very close to the concentration corresponding to the stoichiometric reduction of the supplemented sulfate (10 mM). In contrast, the results of the assay amended only with lactate, showed low sulfide production (0.9 mM) despite of the endogenous sulfate consumption (4.5 mM) (Figs. 2c and d). This low sulfide concentration can be related with the evident formation of a black precipitate at about 6 days after inoculation where the sulfide concentration did not increase whereupon (Fig. 2d). The black precipitate was analyzed by ICP-OES and SEM-EDS and it was found that the precipitate mainly contained calcium, iron, sulfur and arsenic (Fig. S3). This kind of precipitate was not formed in the assay prepared in the same way but inoculated with sediment CB, possibly due to the lower concentration of iron in sediment CB.

To prove if the microbiota of this sediment had the ability to use both electron acceptors at the same time, when the carbon source was not limited, a treatment with 14 mM lactate, 2.5 mM As(V) and 10 mM sulfate was assayed. The concentration of lactate was increased to 14 mM to ensure that reducing equivalents of the electron donor will allow the occurrence of both

microbial processes: arsenate- and sulfate-reduction. The complete reduction of arsenate (2.5 mM) occurred within two days and sulfate reduction occurred subsequently, reaching 6.8 mM sulfide at day 20 (Fig. 2d). This observation strongly suggested that As(V) was preferred as electron acceptor over sulfate, most probably because As(V) reduction provides higher free energy (-172 kJ/mol) when coupled to the oxidation of lactate to acetate [11]. It is worth to note that As(V)-reduction is necessary prior to sulfate reduction to achieve arsenic mineralization [12]. However in this assay, we did not observe the formation of the yellowish precipitate, sulfide accumulated in the liquid media (~7 mM), and the concentration of total dissolved arsenic remained the same as at the beginning of the experiment (2.5 mM). Under some conditions, sulfate-reduction can trigger and increase the mobility of arsenic, for instance amorphous orpiment (As_2S_3) can be dissolved when equimolar or higher concentrations of sulfide are present [13]. In addition, at high concentrations of sulfide the formation of thioarsenites species becomes important because these compounds remain in solution triggering arsenic mobility [15]. Newman *et al*. [11]examined the growth of *D. auripigmentum* with 1 mM As(V), 10 mM SO_4^{2-} and 20 mM lactate, and although they observed the formation of a yellowish precipitate, the authors found that As(V) was reduced to As(III) concurrently with sulfate reduction and the formation of As_2S_3. The authors argued that sulfate-reduction occurred at a low rate, which suggests that the formation of a biogenic arsenic sulfide precipitate is not only impacted by substrate availability but also by the rate of sulfate reduction.

Regarding the substrate, lactate was consumed totally in all the experiments except in the assay with MoO_4^{2-} that showed some lactate at the end (2 mM), which obeys to an excess of lactate, when sulfate reduction was inhibited, around 5 times the concentration necessary for the reduction of 9.2 mM of As(V) supplemented in the assay (Fig. 2e). Acetate was not consumed

completely in any of the As(V) amended assays which may point out to a detrimental effect of arsenic in the consumption of acetate. In contrast in the sulfate reduction assay (lactate + SO_4^{2-}) a tendency towards acetate consumption was observed at the end of the experiment (Fig. 2f). It is worth mentioning that in the endogenous control, microbial sulfate reduction was not observed, due to the low organic carbon content in the sediment, which was insufficient to support the sulfate-reduction process.

3.4 Arsenic removal and formation of precipitates

We observed that sediment CT and its pore water contained higher concentration of arsenic than sediment CB (Table 1). Accordingly, the rates of arsenate-reduction were higher in the assays with sediment CT than in the assays with sediment CB (Table 2), this result was expected if we consider that the indigenous microbiota present in sediment CT was adapted to high concentrations of arsenic compared to the microbiota present in sediment CB. In addition, the most probable number of arsenate-reducing bacteria in the original sediment CT was 6.2 times higher (4.7×10^8 cells/g sediment dry weight) than in sediment CB (7.6×10^7 cells/g sediment dry weight). As a global result, the microorganisms in sediment CT had the ability to perform arsenate-reduction much faster than those in sediment CB. In the assays with both electron acceptors, As(V) and SO_4^{2-}, the rates of arsenate-reduction were 0.10 and 0.26 mmol/L·h with sediment CB and CT, respectively. In our study, the maximum arsenate-reduction rate was obtained in the assay in which sulfate reduction was inhibited with molybdate (Table 2), it has been reported that molybdate favors arsenate-reduction, because molybdenum is a necessary cofactor for the As(V) reductases [7].

Concerning the precipitates, the assays amended with lactate, As(V) and SO_4^{2-} (10 mM each), either with sediment CB or CT, showed the formation of a yellowish precipitate (Fig. S4),

which *a-priori* was presumed to be composed by an arsenic sulfide. The analysis of the precipitates by ICP-OES showed that the elements present in major concentrations were S, As and Ca (Fig. 3), the high calcium content in both precipitates may be due to the mineral composition of the sediments (calcite and gypsum). The sample of the yellowish precipitate formed in the assay with sediment CB had a higher concentration of arsenic (50.9 mg/g of precipitate) than the precipitate formed in the assay inoculated with sediment CT (18.8 mg/g of precipitate). These results agree with the total arsenic removed from the aqueous phase, since in the assay inoculated with sediment CB the arsenic removal from the aqueous phase was 90.9% (693.6 mg/L), while in the assay inoculated with sediment CT the arsenic removed was 52% (332.2 mg/L) (Table 2). It is important to note that although the rates of arsenate-reduction were higher in the assays with sediment CT than with sediment CB, the removal of dissolved arsenic was lower in the assays with sediment CT. Most probably the reason is that sediment CB showed endogenous sulfate-reducing activity due to its indigenous microbiota and a considerable initial sulfide concentration (~2.5 mM) that allowed starting the precipitation of arsenic. The redox potential measured at the end of the experiments performed with sediment, lactate, As(V) and sulfate (10 mM each), showed that using sediment CB the ORP was -258.9 mV, while in the assay with sediment CT it was -67.3 mV. It has been documented before that the precipitation of sulfide minerals is highly favored by extremely reduced conditions (redox potentials close to -200 mV) [1]. In addition, the indigenous microbiota of sediment CB showed higher sulfate reduction rate (0.09 mmol SO_4^{2-}/L·h) than that of sediment CT (0.04 mmol SO_4^{2-}/L·h) supporting As(III) removal from the aqueous phase by sulfide precipitation.

In the bioprocesses studied, the sediment and its native microbiota were closely integrated. Although the chemical and mineralogical composition of the sediments may provide the

electron acceptors (i.e. sulfate and As(V)) and also the electron donor (in the case of sediment CB) these were not present in the necessary oxidation state and concentrations to form precipitates. For instance, the reduction of sulfate to sulfide, is only feasible by microbial activity or, in the absence of microbial activity, by thermal sulfate reduction at high temperature conditions (>250 °C) [32]. Similarly, the fast reduction of As(V) to As(III), in the period of time covered by the experiments (few days), would be only effective in the presence of sulfide or bacterial activity [1, 33]. In order to observe the development of the precipitate it is necessary the presence of both species, dissolved sulfide and dissolved As(III), therefore the activity of the indigenous microbiota is a prerequisite to achieve arsenic precipitation.

The microanalysis by SEM-EDS confirmed the presence of arsenic and sulfur in the precipitate, but also the presence of calcium and silicon, whereas the X-ray diffraction patterns corresponded to mineral phases of gypsum, calcite and quartz (data not shown), which were the main matrix components of the sediments. Unfortunately both analysis, XRD and SEM-EDS, were biased by the composition of the sediment interfering with the analysis of the precipitates (Fig. 4 and S5). Therefore, it was not possible to know if the relative calculated S/As molar ratio corresponded to arsenic sulfides, i.e. orpiment (As_2S_3) or realgar (AsS); another possibility is that the yellowish precipitate could be a mixture of orpiment and realgar [12]. The composition of a yellowish precipitate, without the interference of the sediment matrix, showed that it was composed by arsenic and sulfur (Fig. S6). The precipitation of As(III) as orpiment and realgar is attractive and desirable because the formation of these minerals promotes the removal of dissolved arsenic, as these minerals may contain between 60 and 70% of As(III) [4]. In an ideal scenario, under reducing conditions, the arsenic

immobilization as arsenic sulfides is convenient because of their stability and high capacity to retain highly toxic arsenic, As(III), in solid phase.

CONCLUSIONS

The physicochemical composition of sediments is a key factor for the outcome of the microbial processes carried out by their indigenous microbiota. In the case of sediments contaminated with arsenic, microbial processes are heavily impacted by the availability of organic carbon, the presence of potential electron acceptors and incidence of chemical elements that may interfere in the arsenic cycle, as it is the case of iron and sulfur. The microbial communities present in both sediments were able to perform arsenate and sulfate reduction without prior acclimation period or enrichment, even with potentially toxic arsenate concentrations (~750 mg/ L).

Although sulfate was present as electron acceptor, the process most favored in the microcosms was arsenate-reduction. However, if there is an available carbon source, in this case lactate, sulfate-reduction was also performed. The biological processes of arsenate- and sulfate-reduction carried out by the indigenous microbiota present in the sediments can potentially modify the speciation of arsenic in the original sites by driving As(V) reduction to As(III) and removing the latter from solution through bioprecipitation as mineral sulfides.

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Legends to figures

Figure 1. Time profiles of the assays performed with sediment CB. (a) As(V) reduction; (b) As(III) production; (c) sulfate reduction; (d) sulfide production; (e) lactate consumption; (f) acetate production. Lactate, As(V), SO_4^{2-} 10 mM each (■); lactate 14 mM, As(V), SO_4^{2-} 10 mM (□); lactate 10 mM (●); lactate, SO_4^{2-}10 mM each (○); lactate, As(V), SO_4^{2-} 10 mM each (+ MoO_4^{2-}) (▲), endogenous control, without lactate, As(V) and sulfate (♦). The upper line in (b) indicates the theoretical concentration of As(III) expected from the reduction of As(V).

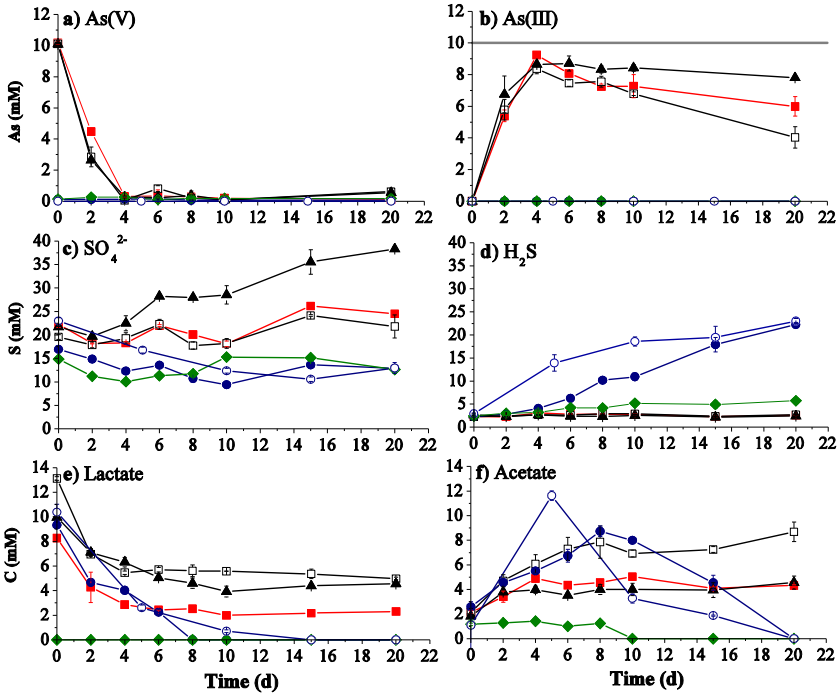

Figure 2. Time profiles of the assays performed with sediment CT. (a) As(V) reduction; (b) As(III) production; (c) sulfate reduction; (d) sulfide production; (e) lactate consumption; (f) acetate production. Lactate, As(V), SO_4^{2-} 10 mM each (■); lactate 14 mM, As(V) 2.5 mM,

sulfate 10 mM (□); lactate 10 mM (●); lactate, SO_4^{2-} 10 mM each (○); lactate, As(V), sulfate 10 mM each (+ MoO_4^{2-}) (▲); endogenous control, without lactate, As(V) and sulfate (♦). The upper line in (b) indicates the theoretical concentration of As(III) expected from the reduction of As(V).

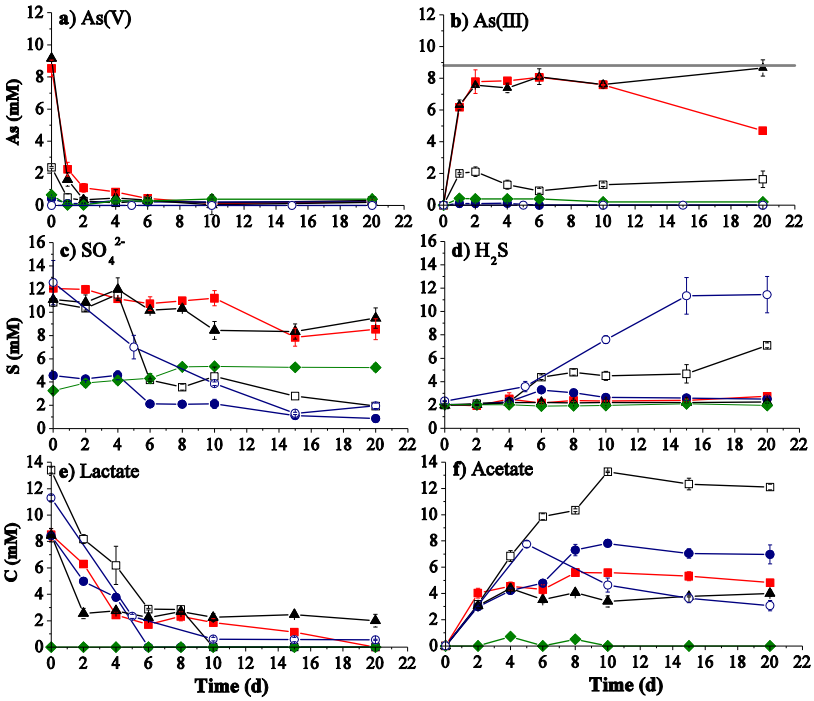

Figure 3. Elemental composition by ICP-OES of the yellow biogenic precipitates recovered from the batch assays inoculated with sediment CB or CT and amended with lactate, As(V) and sulfate 10 mM each.

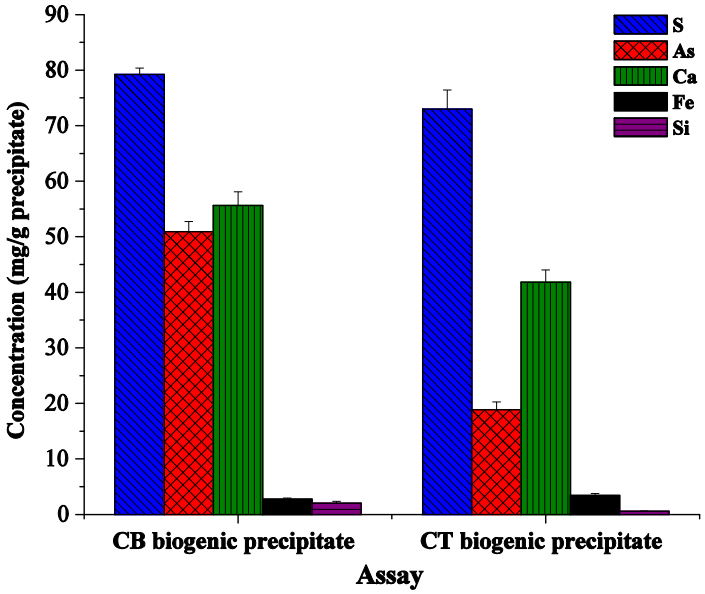

Figure 4. Analysis of the precipitate formed in the assay inoculated with sediment CB (lactate, As(V), sulfate 10 mM each) after 30 days of incubation. (a) SEM micrograph, (b) representative EDS analyses.

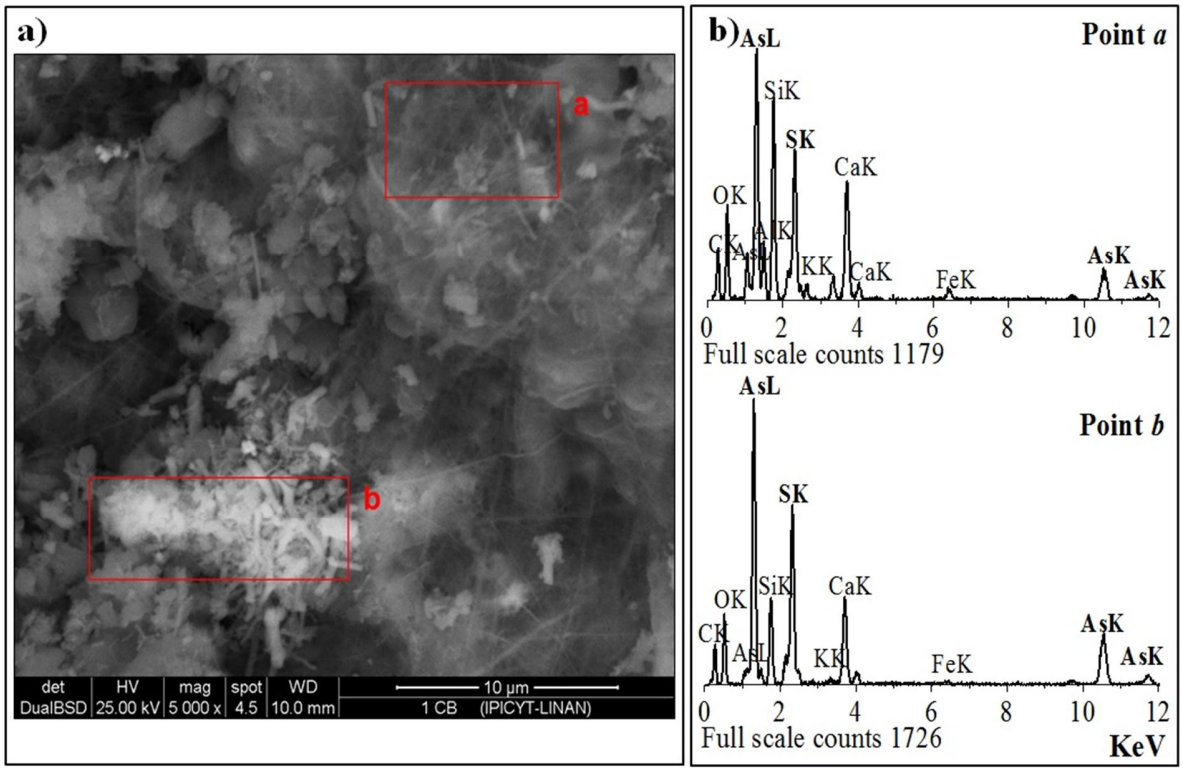

Table 1. Major physicochemical characteristics of the sediment, column water, and pore water obtained from the sampling sites Cerrito Blanco (CB) and Club de Tiro (CT).

Sampling site	Cerrito Blanco (CB)			Club de Tiro (CT)		
Parameter	Sediment	Column water	Pore water	Sediment	Column water	Pore water
pH	8.5	8.2	8.2	8.4	7.8	7.7
Eh (mV)	-97.4	-78.4	-80.3	-81.4	-57.2	-46.8
Alkalinity (mg $CaCO_3$/L)	–	180	–	–	312	–
Volatile Solids (%)	12.4 ± 0.06	–	–	6.5 ± 0.05	–	–
Total organic carbon (mg/L)	–	2.2 ± 0.33	16.7 ± 1.21	–	4.1 ± 1.62	18.6 ± 2.84
Total carbon (mg/g)	12.2 ± 0.50	–	–	31.83 ± 3.67	–	–
SO_4^{2-} (g/kg, or mg/L) [a]	329.8 ±10.96	1594.7 ± 18.37	1409 ± 76.10	18.8 ± 0.29	1718.4 ± 13.30	1215.7 ± 77.99
Major elements (g/kg or mg/L)[a]						
S	111.8 ± 6.09	473.1 ± 14.63	541.1 ± 24.58	6.0 ± 0.57	443.3 ± 12.22	406.0 ± 10.27
Ca	158.4 ± 11.57	738.7 ± 12.68	297.9 ± 13.31	149.2 ± 1.0	1031.2 ± 7.70	281.5 ± 4.94
Fe	3.0 ± 0.07	ND	0.50 ± 0.04	15.4 ± 0.19	ND	0.27 ± 0.04
Si	16.0 ± 0.20	116.4 ± 33.13	33.5 ± 8.64	36.7 ± 2.05	188.0 ± 5.81	17.72 ± 4.03
Al	3.5 ± 0.18	0.04 ± 0.00	0.19 ± 0.020	23.3 ± 2.20	0.24 ± 0.01	0.09 ± 0.05
Minor elements (mg/kg or mg/L)[b]						
As	238.3 ±4.13	5 ± 0.24	13.1 ± 2.21	2263.1 ±167.72	48.3 ± 0.45	47.8 ± 0.85
Na	131.7 ± 13.85	368.9 ± 4.55	61.72 ± 2.73	253.7 ± 7.14	1138.8 ± 12.48	136 ± 2.81
Mn	77.0 ± 1.84	ND	0.26 ± 0.01	234.5 ± 5.10	ND	1.9 ± 0.15
Mg	497.5 ± 37.71	102.5 ± 2.73	36.64 ± 2.69	2013.6 ±114.86	178.8 ± 14.36	31.8 ± 2.07
P	243 ± 19.18	ND	0.61 ± 0.03	1275.5 ± 36.74	ND	0.63 ± 0.04

[a] Concentration in g/kg for sediments and in mg/L for water samples

[b] Concentration in mg/kg for sediments and in mg/L for water samples

ND = not detected, – = not quantified.

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Table 2. Arsenate-reduction rates and removal efficiency of dissolved arsenic obtained in the assays inoculated with sediment from Cerrito Blanco (CB) and Club de Tiro (CT).

Assay	Rate of As(V) reduction (mmol/L·h)		% As removal	
	CB	CT	CB	CT
Lactate, As(V) and SO_4^{2-} (10 mM each)	0.10 ±0.002	0.26 ±0.003	90.9 ±1.24	52.0 ±1.36
Lactate (14 mM), As(V) and SO_4^{2-} (10 mM each)	0.15 ±0.02	NP	93.0 ±1.00	NP
Lactate (14 mM), As(V) (2.5 mM) and SO_4^{2-} (10 mM)	NP	0.15 ±0.02	NP	0
Lactate, As(V) and sulfate (10 mM each), and MoO_4^{2-} (25 mM)	0.15 ±0.001	0.31 ±0.01	19.5 ±0.31	4.8 ±0.20

NP: Assay not performed