BIOELECTROCHEMICAL SYSTEMS AS ENRICHMENT STRATEGY FOR LITHOAUTOTROPHIC BACTERIA IN AN ARSENIC CONTAMINATED ANDEAN WATERSHED

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ABSTRACT

The study of microorganisms involved in the arsenic (As) biogeochemistry is important as they can regulate As speciation and mobility in the environment. In this work, we studied bacterial communities from a natural As rich environment with the aim to prospect for microorganisms related to the cycle of this element. We used biolectrochemical systems (BESs) as a method for microbial enrichment since they have been recently proposed as a promising strategy to grow lithoautotrophs, and as a sustainable alternative for bioremediation. The objective of this study was to prospect for potential EAM in As-rich sediments from a hydrothermal source in northern Chile.

Our study site was located at the upper part of the Lluta river watershed (Arica and Parinacota Region, northern Chile). In this location, sediments to construct BESs were collected within an area where natural attenuation of As concentration from a hydrothermal source is thought to occur due to an initial microbial oxidation of As(III) to As(V), followed by the adsorption of As(V) onto Fe(III) oxy(hydr)oxides. BESs were constructed in the laboratory as duplicated Winogradsky columns with electrodes (anodes and cathodes) inserted and operated for 15 months.

Microbial growth on BES cathodes was confirmed by scanning electron microscopy. BESs registered power densities of about 10 μWcm^{-2}. Electrochemical test conducted on selected cathodes from duplicated columns (BC1 and BC2) showed peaks previously associated with biological catalysis of electron transfer from electrodes to the medium. Pyrosequencing evidenced differenced in bacterial composition between sediments from the field and cathode biofilms. In the former, communities were dominated by Chlorobi, Chloroflexi, and Nitrospirae, while the latter were greatly dominated by Proteobacteria. The Xanthomonadaceae family was the most dominant Proteobacteria in BC1 (~46%) while Acetobacteraceae dominated in BC2 (92%). Interestingly, BES operation also enriched members of the genus *Acidocella* (BC1~16% and BC2~27%), which are known to favor reductive dissolution of Fe(III) oxy(hydr)oxides. Bacteria isolated from cathodes, using a lithoautotrophic As oxidizers culture medium, included *Methylobacterium* spp*., Herbaspirillum* sp. and *Pseudomonas fluorescens,* all of which have been previously reported in cathodic biofilms. This work represents the first step in the exploration of the electrochemical capabilities of microbial communities from As-rich environments in the northern Andean region of Chile.

Keywords: Arsenic, biological arsenic oxidation, bioelectrochemical systems, biocathodes.

1. Introduction

Andean basins in northern Chile exhibit high As concentration in rivers and soils, compromising health and sustainability of communities that depend on these critical resources for urban and rural development. In water As exists predominantly as arsenite (As(III)) and arsenate (As(V)). The latter is generally removed from the systems due to its high sorption affinity to Fe(III) oxy(hydr)oxides within a wide range of pH (4-9) conditions. The understanding of this biogeochemical cycle and its

implications in water and soil quality is crucial to develop strategies and smart technologies that control this environment risk.

Bioelectrochemical systems (BESs) represent a promising strategy to enrich and cultivate lithoautotrophic microorganisms that can replace their natural electron donors by electrodes (Carbajosa *et al*., 2010). These electrochemically active microorganisms (EAM) can be found in metal-rich contaminated environments such as soil and sediments. However, they are difficult to culture with traditional methods. If used in BESs, EAMs can modify biogeochemical processes that control the mobilization and fate of emerging contaminant.

Recent studies on EAM have revealed the metabolic versatility of these microorganisms. They have been used to treat contaminants including chromium (VI) (Huang *et al*., 2010), and uranium (VI) (Gregory and Lovley, 2005), among others. However, their potential use in BESs for As bioremediation is poorly understood. The objective of this study was to prospect for potential EAM in As-rich sediments from a hydrothermal source in northern Chile. The knowledge gained from this research could allow improving and designing novel bioremediation strategies to be used in mine-impacted environments.

2. Materials and Methods

2.1. Site description and sample collection

Sediments with high As contents (6.4 ± 1.7 mg kg^{-1}) were obtained in two campaigns from a hydrothermal source (As 0.8 ± 0.2 mg L^{-1}; pH 5.9 ± 0.1) located at the upper part of the Lluta River basin in northern Chile (Fig. 1a) (Leiva *et al*., 2014). In the first campaign (May, 2012), sediment were collected in 1L bottles and transported on ice to the laboratory where they were keept at 4°C during seven months until BESs contruction. In the second campaign (March, 2013), sediments were aseptically collected in 50 mL polypropylene tubes and mainatined at 4°C for DNA extraction within a week of sample collection.

2.2. BES setup, operation and electrochemical analysis

Setup and operation. The BESs were operated for 15 months at ~25°C; they consisted of duplicate 250 mL graduated cylinders, each filled with 230 mL sediments; 40 mL of water from the hydrothermal source; and 40 mL of deionized-filtered water. BESs were configured with eight carbon-felt cathodes (geometric surface of 9.1 cm^2) submerged in the water zone at the same depth. Each of the cathodes was connected to an anode through a 1 kΩ resistor. Anodes were made of two different materials, four of graphite (geometric surface of 6.4 cm^2), and four of titanium (geometric surface of 0.7 cm^2). An anode of each material was buried in the sediment at different depths (distance between cathode and anode: 7.5 cm, 12 cm, 15.5 cm, and 21 cm) (Fig. 1a). Closed-circuit voltages were measured using a data acquisition system (Multimeter 2700, Keithly Instruments Inc.)

Electrochemical analysis. Cathodic Linear Sweep Voltammetry (LSV) was performed at the end of the BESs operation. LSVs were conducted using three-electrode cells with cathodes as working electrodes, a counter electrode of platinum (CHI115, CH Instruments Inc.), and a reference electrode of silver/silver chloride (CHI111, CH Instruments Inc.). The electrolyte consisted of water from the BESs (pH = 4.2 ± 0.1; DO = 8.6 ± 0.1 mg L^{-1}; EC = 3.8 ± 0.4 mS cm^{-1}). LSVs were performed from 0.1 to -1 V (vs. Ag/AgCl) at 1 mV s^{-1} using a potentiostat Reference 600 (Gamry Instrument Inc.).

2.3. Scanning electron microscopy (SEM) and bacterial isolation from BES cathodes

Microbial growth on silver-coated cathode surfaces was confirmed by SEM using a LEO 1420VP equipment coupled to an Oxford 7424 solid-state detector.

Isolation of potential As(III) oxidizing bacteria was done using cathode electrodes as inoculum. A basal growth medium was prepared using (per L of water): 30 mg Na_2SO_4; 100 mg KCl, 80 mg $MgCl_2$; 100 mg $CaCl_2 \cdot 2H_2O$; 200 mg $(NH_4)_2SO_4$; 6.8 mg KH_2PO_4; 0.018 mg $AlCl_3 \cdot 6H_2O$; 0.03 mg $Na_2WO_4 \cdot 2H_2O$; 0.2 mg Na_2EDTA and trace elements added according to Bahar (2012). This medium

was autoclaved at 121°C for 20 min and supplemented with filtered (PTFE filters, pore size 0.22, Clarinert) stock solutions of $NaHCO_3$ (8400 mg L^{-1}, 10X), $NaAsO_2$ (1299 mg L^{-1}, 10X), HCl (3.7%, 143X), and vitamins (Santini *et al*., 2000). Each of the cathode portions were placed in 5 mL of the final culture media, and maintained in a orbital shaker (at 200 rpm and 30°C) until turbidity was observed (~ 4 weeks after incubation). After incubation, 100 µL of liquid cultures were spread on the same media described above but solidified using bacto agar (1.5%).

DNA was extracted from isolates using a Genomic DNA Kits PureLink® and subjected to 16S rRNA PCR amplification using primers 8F/1392R. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea). Sequences were compared to the GenBank database using Blastn for taxonomic assignments.

2.4. Microbial community characterization

Community DNA was extracted from two sediment subsamples obtained from the field (2nd campaign) and from representative cathodes from both BESs, at the end of the incubation period, using the Power Soil® DNA isolation kit (MoBio, CA). All DNA concentrations were measured using a NanoDrop 2000c spectrophotometer (Thermo Scientific). Community DNAs were subjected to bar-coded amplicon library preparation by PCR to amplify 16S rRNA genes using the primers 28F/519F. Targeted sequences were then pyrosequenced using the 454 FLX Titanium system at the Research and Testing laboratory (Texas, USA). Pyrosequencing data was processed and analyzed with QIIME v1.9 (Caporaso *et al*., 2010).

3. Results

3.1. Operation and electrochemical analysis of BESs

BESs were operated for 15 months producing about 10 $\mu W cm^{-2,}$, which is comparable to similar systems using marine sediments and soil (Dumas *et al*., 2008). LSVs revealed a shift in the cathodic potential from about -0.2 V vs Ag/AgCl observed in abiotic controls to 0 V vs Ag/AgCl in biocathodes from columns 1 (BC1) and column 2 (BC2) (Fig 1b). Cathodes associated with the deeper graphite anodes inserted in each column were selected to compare two different electrochemical performance observed among replicates. Interestingly, BC1 showed a cathodic current peak of 30 $\mu A\ cm^{-2}$ at -0.4 V vs Ag/AgCl (0 V vs SHE; at pH=4.2). This peak has been previously linked to *A. ferrooxidans* catalyzed oxygen reduction (Carbajosa *et al*., 2010). In contrast, BC2 showed a clear cathodic current plateau of 37 $\mu A\ cm^{-2}$ at -0.5 V vs Ag/AgCl (-0.1 V vs SHE; at pH=4.2), revealing a different catalytic effect. Differences observed on cathodic potential could be associated with different EAB that catalyze electron transfer from electrodes to the medium.

3.2. Bacterial community characterization

The sediment bacterial communities were dominated mainly by the phyla Chlorobi, Chloroflexi, and Nitrospirae, all of which have been reported as dominant groups in As contaminated environments (Yamamura and Amachi, 2014). In both cathode biofilms Proteobacteria was the most abundant phylum (BC 1 ~90% and BC 2 ~96%). The Xanthomonadaceae family was the most dominant Proteobacteria in BC 1 (~46%) while Acetobacteraceae was in BC2 (92%). Recently, the Xanthomonadaceae family was identified as important member (6%) of an electroactive denitrifying biofilms in biocathodes of BESs (Gregoire *et al*., 2014). Additionally, members of this taxa have shown iron oxidation capability (Lu *et al*., 2010). Acetobacteraceae was dominated mainly by *Acidocella* species in both biocathodes (BC1 ~16% and BC2 ~27%). *Acidocella* species are known dissimilatory Fe(III) reducers which play a role in Fe(III) oxy(hydr)oxides dissolution (Coupland, 2008). As this microorganisms can indirectly facilitate changes in As mobilization in the environment, their abundance in cathodic biofilms demand further investigation.

3.3. Identification and characterization of isolates from BESs cathodes

Biocathode microbial colonization was confirmed by SEM (Fig. 1c and 1d). Similar morphologies have been observed for electroactive bacteria responsible for current production via direct electron transfer mechanism (Gorby *et al*., 2006).

Bacteria isolated from cathodes, using a lithoautotrophic arsenic oxidizers culture medium, included *Methylobacterium* spp*., Herbaspirillum* spp. and *Pseudomonas fluorescens,* all of which have been previously reported in cathodic biofilms (Reimers *et al*., 2006; Erable *et al*., 2010; Lefebvre *et al*., 2012). However, these potential EABs have not been isolated from BESs.

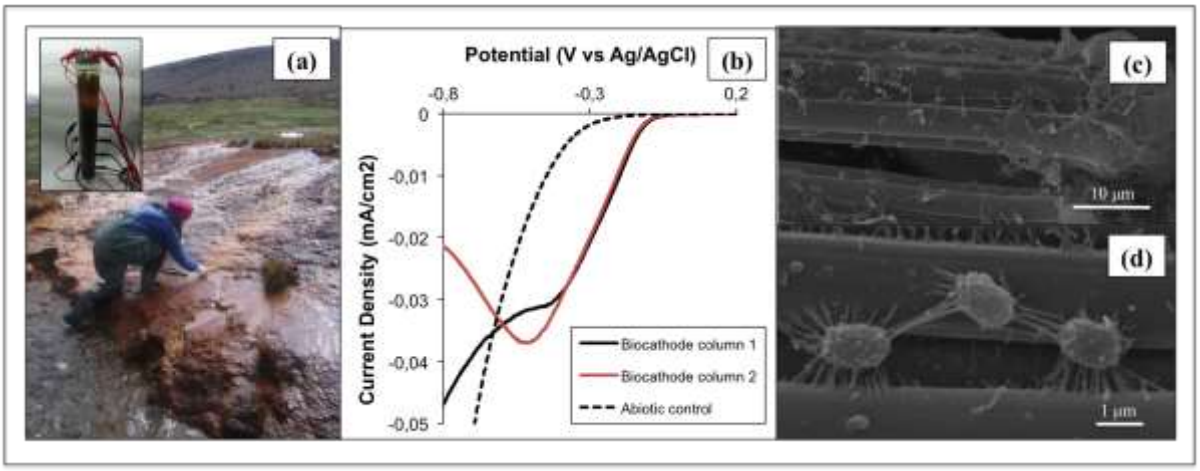

Figure 1: (a) Study site and BESs configuration; (b) LSV of biocathodes from 15-months duplicated columns (BC1 and BC2); (c) SEM showing biofilm on graphite cathode electrodes; (d) close-up of previous SEM image showing cells attached to the electrodes and connected among them.

4. Conclusions

This work represents the first step in the exploration of the electrochemical capabilities of microbial communities in As rich environments in northern Chile. Our results evidenced the presence of Fe(II) reducers in cathode biofilms that can potentially regulate Fe(III) oxy(hydr)oxide solubility, and as a result, As mobility. In spite of the presence of putative lithotrophic organisms present in cathode biofilms, further research is needed to identify potential candidates that can improve BES performance and regulate As cycle. In this line, further exploration of cathode isolates grown in a culture medium with As as the only electron donor is under way.

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