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Comparison between acid mine effluents, La Zarza-Perrunal and Río Tinto (Iberian Pyritic Belt)

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Abstract. A 1.2 km long effluent from La Zarza-Perrunal mine (Iberian Pyritic Belt, IPB) was characterized and compared with Río Tinto. In La Zarza effluent microbial oxidation of ferrous iron is responsible for the drastic increase in ferric iron, from a ratio of $Fe(III)/Fe_{total}$ of 0.11 at the origin, up to 0.99 downstream. Prokaryotic and eukaryotic diversity throughout the effluent were determined. Bacteria related to the sulfur cycle as well as iron-reducing bacteria were mainly detected near the anoxic origin. Iron-oxidizing microorganisms increased along the course of the effluent following an increase in the oxygen content in the water column. Eukaryotic diversity varied drastically along the effluent. Rio Tinto (92 km length) is a natural extreme acidic environment with a rather constant acidic pH (mean pH value 2.3) and a high concentration of heavy metals. The Tinto ecosystem is under the control of iron [1]. The geomicrobiological comparisons of both habitats were performed to unravel some basic questions of biohydrometallurgical interest.

Introduction

Río Tinto is a 92-km-long river (SW Spain) which is considered a good model system for the study of extreme acidic environments and acid rock drainage (ARD). It has a low pH (mean pH 2.3) and high concentrations of heavy metals in solution (up to 20 g/l of iron, 0.7 g/l of copper and 0.6 g/l of zinc) [1, 2]. It is well established that the extreme conditions of Rio Tinto are the product of the metabolic activity of chemolithotrophic microorganisms (mainly *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) thriving in the high concentration of sulfidic minerals from the IPB. Moreover, an unexpected level of eukaryotic diversity has been described in the Tinto basin in spite of the extreme conditions found in its waters [3]. Río Tinto geomicrobiology has been studied in depth [1, 2]. Many studies have concentrated on the iron and sulfur oxidation, but little is known about the reductive part of these cycles which are of extreme importance for biohydrometalurgical processes because they reduce the efficiency of metal extraction

Although closed in the 1990s, La Zarza-Perrunal (IPB) had been mined since pre-Roman times for the recovery of Ag and Au, and in modern times, for sulfuric acid and metal retrieval (Cu, Zn and Pb). This acidic effluent has a fairly constant flow rate of ~2 l/s. [4]. After receiving fresh water, this stream merges with different creeks, until its final neutralization. Previous studies reported the geochemical evolution of this site [4], but knowledge of its correlation with microbial metabolic activities is needed to better understand the operation of the sulfur and iron cycles in these environments. In this work, the geomicrobiological characterization of La Zarza-Perrunal effluent is presented and compared with that of Rio Tinto.

Material and Methods

Study site and analytical procedures. Field work was performed in May 2007. Three different sampling stations were selected at increasing distance from the discharge point: *LZ1* (20 m) an iron reduced site, *LZ2* (900 m) a transition station and *LZ3* (1,200 m) where iron was completely oxidized. Water samples were filtrated on site through 0.45 μm membrane filters, acidified to pH<2 with HNO_3, and stored at 4°C. Sediment samples were directly taken and stored in polyethylene bottles. Physico-chemical parameters were measured *in situ* with a HANNA portable instrument. Water samples were analyzed by AAS, ICP-AES and ICP-MS. Sulfate was gravimetrically measured as $BaSO_4$. Iron speciation was determined by colorimetric digital titration (HACH). Solid samples were analyzed using ED-XRF and ICP-MS after digestion with HNO_3 and H_2O_2. Certified reference materials were used to check the accuracy of the analytical data. Solid samples were characterized by powder XRD using a PHILIPS PW 1710 diffractometer with CuKα radiation (40 kV, 40 mA).

Procedures for microbiological characterization. Identification of algae and heterotrophic protists was carried out by direct microscopy observation using different phenotypic features based on previous studies [5] and 18S rRNA gene sequence comparison. The microscope used for this work was a phase contrast Zeiss Axioscope 2. Prokaryotic microorganisms were identified by sequence analysis of their 16S rRNA genes. Samples for DNA extraction were collected with sterile pipettes and stored at -20ºC until further processing. DNA extraction, PCR amplification, sequencing and phylogenetic analysis were performed as described [6].

Results

Physico-chemical parameters. The physico-chemical characteristics of La Zarza sampling stations are summarized in Table 1. The water from the mine portal was highly anoxic, acidic (3.1), with a temperature of around 31°C and a high concentration of reduced iron. 20 m downstream, at *LZ1*, similar conditions were detected with dissolved oxygen increased up to 39% of saturation, total iron concentration at around 2.6 g/l (89% Fe(II)/Fe_{total}, pH was identical to *LZ1* with a temperature of 26ºC. At *LZ2* the situation was completely different, dissolved oxygen rose to 51% of saturation, total iron concentration was lower, of which only 36% was reduced. The pH decreased to 2.3 and temperature was 28ºC. Finally at *LZ3* iron oxidation was completed. Dissolved oxygen was 97% of saturation, total iron 2.1 g/l with only 1% as ferrous iron. The pH had the lowest value, 1.9. Redox potential clearly increased downstream. Some metals increased their concentration along the course of the effluent (Mg, Al, Mn, Cu, Zn, Ni, between others) while other metals decreased (As, Mo, Pb, Sb). Cd was rather constant during the 1200 meters analyzed.

Microbial diversity

Prokaryotic evolution. Samples from sediments, water column and photosynthetic biofilms from each station were analyzed. A total of 96 sequences of archaeal and bacterial 16S rRNA clones were obtained for each sample and compared by BLAST with the NCBI data base. In general, no significant differences were found among sediments, water and biofilms. Biofilms showed the highest diversity in every case and the water column contained the lowest prokaryotic diversity. A higher level of anaerobic microorganisms was detected in biofilms from *LZ1*. A summary of these results are shown in Table 2.

Eukaryotic evolution. In *LZ1* the stream bed of the effluent was coated with a continuous photosynthetic green biofilm. In this biofilm a species related to the *Chlorella* genus dominated the phytobenthonic community. When oxidized iron increased (between *LZ1* and *LZ2*) this continuous biofilm disappeared and was replaced by sediments. At site *LZ2* some sporadic biofilms were found. Their macroscopic appearance was completely different from those found in *LZ1*. They were small, discontinuous and composed of long green filaments. Although eukaryotic biomass decreased at this site, sampling biodiversity was higher than in *LZ1*. Filamentous algae, represented

Sample	**pH**	**ORF (mV)**	**EC (mS/cm)**	**DO (mg/l)**	**Fe^{2+} (mg/l)**	**Fe^{3+} (mg/l)**	**Fe^{2+}/Fe_t (%)**	**SO_4^{2-} (g/l)**
LZ0	3.11	269	7.26	0.8	2675	0	100	8.84
LZ1 20m	3.12	279	7.99	3.13	2325	300	89	9.06
LZ2 900m	2.23	446	7.12	4.1	845	1482	36	10.70
LZ3 1,200m	1.93	598	12.99	6.6	16	2125	1	18.60
RTorigin 20 m	2,0	464	30.50	3.7	2450	6050	40	15.60
RTBerrocal 26 km	2.3	530	6.32	6.4	50	800	6	4.84
RTLaPalmadelC 54 km	2.5	519	2.53	6.7	bdl	245	-	2.67

Table 1. Physico-chemical parameters measured along La Zarza-Perrunal effluent and compared with those of Río Tinto [1, 2].

		LZ1	***LZ2***	***LZ3***
Proteobacteria	*Alfaproteobacteria*	*Acidiphilium* spp.	*Acidiphilium* spp.	*Acidiphilium* spp.
	Betaproteobacteria	*Thiomonas* spp. Uncultured (*Sutterella*) *Ferritrophicum radicicola*	*Thiomonas* spp.	*Thiomonas* spp.
	Gammaproteobacteria	*At. ferrooxidans* Uncultured (*Thioploca*) Uncultured (*Francisella*) WJ2	WJ2 *Ps. azotoformans*	*At. ferrooxidans* Uncultured (*Thioploca*)
	Deltaproteobacteria	*Synthrophomonas* spp.		
	Planctomyces	Uncultured		
	Acidobacteria	*A. capsulate*	*A. capsulata*	
	Nitrospira	*L. ferrooxidans* *L. ferrodiazotrophum*	*L. ferrooxidans* *L. ferrodiazotrophum*	*L. ferrooxidans* *L. ferrodiazotrophum*
Firmicutes	*Bacilli*	*Allyciclobacillus* spp.	*Allyciclobacillus* spp.	*Allyciclobacillus* spp.
	Clostridiaceae	*Clostridium* spp.		
	Peptococcaceae	*Desulfosporosinus* spp.		
	Actinobacteria	*Acidimicrobiaceae*	*Acidimicrobiaceae*	*Acidimicrobiaceae*
Archaea	*Euryarchaeota*	*Thermoplasmatales*	*Thermoplasmatales*	*Thermoplasmatales*

Table 2. Prokaryotic diversity at the each of La Zarza-Perrunal effluent sampling points.

by the genera *Zygnemopsis* and *Chlamydomonas* were most abundant. *Chlorella* spp. and diatoms were also detected in low cell numbers. Finally, in *LZ3* station, where iron was completed oxidized, biofilms were smaller and intermittent. They were made up of filamentous *Zygnemopsis* and *Chlamydomonas*. Diatoms were also well represented while *Chlorella* were absent.

Discussion

The most important difference between La Zarza-Perrunal effluent and Río Tinto is the anoxic condition detected at the origin of the former, probably related to the reduced form of the iron measured at this sampling station. It is not surprising that reducing microbial activities such as those of *Desulfosporosinus* sp., *Clostridium* sp., *At. ferrooxidans* and *Acidiphilium* spp. were detected in these conditions, although the last two bacteria can also grow in the presence of oxygen [1]. The comparison with Río Tinto is rather difficult because these conditions are not found at its sampling stations, which have been selected and studied for the last fifteen years. However, the basic activities, corresponding to 80% of the diversity of the water column detected at the origin of Río Tinto (*At. ferrooxidans*, *L. ferrooxidans* and *Acidiphilium* spp.) [1, 2] have also been observed in La Zarza. After one km the physico-chemical conditions of La Zarza effluent changed drastically. Some microorganisms associated with strict anaerobic conditions were not detected (i.e., *Desulfosporosinus* sp., *Clostridium* sp.), and most of the retrieved sequences are related to iron oxidizing activities, which are routinely found all along the Tinto basin [1]. At the last La Zarza sampling station all the iron was completely oxidized, and the microbial diversity was similar to that detected in the previous sampling station and also in Río Tinto [1, 2]. The detected archaea belong to the *Thermoplasmatales* order and were found all along the effluent, similar to what has been described in Río Tinto [1, 2]. The changes detected in the eukaryotic populations of the photosynthetic biofilms are rather dramatic, and strongly suggest that some microorganisms are sensitive to changes in pH and/or redox potential. An important difference between La Zarza effluent and Río Tinto is the lack of buffer capacity exerted by ferric iron in La Zarza [1]. This is understandable in samples *LZ0* and *LZ1* in which the iron is completely reduced, but it is not clear in sample *LZ3* in which most of the iron is oxidized. One possibility is a lack of sufficient ferric hydroxide precipitates to be dissolved by the increasing proton concentration, thus impairing the buffer capacity of ferric iron.

It is clear from these results that oxygen is the limiting factor for the presence of a high concentration of ferric iron, the oxidizing agent required for efficient bioleaching of mineral sulfides. In addition, the generation of anoxic conditions favours the activity of iron reducing microorganisms, which can further reduce the effective concentration of ferric iron and, as a result, the efficiency of bioleaching processes. The anoxic conditions also favour the activity of sulfate reducing microorganisms (SRB) in the sediments, which generate H_2S that might sequester soluble metals by precipitating metal sulfides. Additionally, the generation of reduced iron could favour an increase in the pH, leading to the formation of jarosites capable of sequestering bioleached metals. All these considerations are extremely important for the design of efficient heap-leaching processes.

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