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Copper resistance, motility and the mineral dissolution behavior were assessed as novel factors involved in bacterial adhesion in bioleaching

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ABSTRACT

A study was carried out on the adhesion to sulfide minerals of chemolithoautotrophic acidophilic bacteria obtained from industrial copper bioleaching operation. For this purpose, a mixed culture obtained from an industrial process and two metabolically different pure strains of *Acidithiobacillus*: *A. ferrooxidans* and *A. ferridurans* were used. These microorganisms showed significant differences in adhesion with respect to pyrite and chalcocite in terms of the temporal dynamics patterns and preference. A complex dynamics that involve cycles of attachment and detachment can only be explained by considering both, the intrinsic characteristics of the microorganisms, such as hydrophobicity, resistance and motility, and the properties of each mineral like their hydrophobicity, dissolution behavior and ionic contribution.

1. Introduction

Copper sulfide bioleaching makes it possible to process low-grade ores at low cost and with low environmental impact. Bioleaching involves chemical and biological processes in which microorganisms have a fundamental role. The interaction between these microorganisms and minerals is one of the principal factors driving the efficiency of the copper recovery. This interaction has been thoroughly studied (e.g., reviews by Watling, 2006 and Schippers et al., 2013), and has been designated as an "indirect contact mechanism", a process that depends on the contact between microorganisms and the mineral substrate and in which the extracellular polymeric substances (EPS) secreted by the cells play a fundamental role (García-Meza et al., 2013; Kinzler et al., 2003; Yu et al., 2008; Zeng et al., 2011). Attached microorganisms are capable of catalyzing chemical reactions that lead to a build-up of Fe(III) in the EPS, which causes mineral oxidation and the subsequent release of metal ions (Kinzler et al., 2003; Schippers et al., 2013).

In the biofilm development, the mineral–cell interaction is a complex process that involves a cycle with three stages — attachment, growth and detachment of the cells (Davey and O'Toole, 2000) — and bacterial adhesion to a given substrate depends on selective preference (Rodríguez et al., 2003). Previous to the attachment process, there is a searching stage, in which the cells look for available substrata. This phase is thought to be governed by physical and chemical interactions between the planktonic cells and the mineral (Hermansson, 1999). Particularly, hydrophobicity (Liu et al., 2004b; Pakshirajan, 2007; Takeuchi and Suzuki, 1997; Zhu et al., 2012), ionic chemical interactions (Devasia et al., 1993; Gehrke et al., 1998; Hermansson, 1999; Sand and Gehrke, 2006) and physical factors (Africa et al., 2010; Edwards and Rutenberg, 2001; Zhu et al., 2012) are relevant in this stage. In addition, stability in the next stages of the long-term community on the solid substrate is hardly influenced by biological factors, such as the production of polymeric substances (Sand and Gehrke, 2006; Zhu et al., 2012), resistance to metals (Alvarez and Jerez, 2004; Dopson et al., 2003), microbial differential metabolic traits (Dopson et al., 2003), microbial interactions (Florian et al., 2011) and Quorum Sensing (González et al., 2012). In bioleaching, most of the factors just mentioned have been studied considering macroscopically measurable variables. However, the characteristics of the mineral–bacterium interstitial zone differ from those of the surrounding area, and it is at this microscopic level that the above mentioned factors actually determine the result. At present, there is only an incipient understanding of the interactions at ionic and molecular level as well as of bacterial communication and the importance of the dissolved-metal concentration gradient resulting from bioleaching. In addition, it is well known that each microorganism present in bioleaching has distinctive characteristics and the composition of the communities impacts the operative results (Demergasso et al., 2010; Florian et al., 2011). Current knowledge on the matter has been derived from adhesion studies with bioleaching bacteria involving pure cultures as well as mixed cultures. However, the medium-term temporal dynamics of attached populations and communities have not been completely understood up to now. These dynamics are complex and there are still no mathematical models capable of predicting with any precision temporal fluctuations in the community

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Table 1
Brief description of the microorganisms used in this investigation.

Bacterial strains	Phylogenetic affiliation	Resistance to $CuSO_4$ (mM)	Fe(II) oxidation rate (mg h^{-1})	S° oxidation rate (mg h^{-1})
Acidithiobacillus ferridurans (*Af*D2)	FOA/Group II	325	64	10.6
Acidithiobacillus ferrooxidans IESL32 (*Af*DM)	FOA/Group I	100	74	11.7
Leptospirillum ferriphilum	*Leptospirillum*/Group II	100	–	–

FOA = Fe(II)-oxidizing acidithiobacilli (Amouric et al., 2011). Resistance to CuSO4 and oxidation rates were obtained from Araya, 2012.

(Demergasso et al., 2010). It has been reported that highest copper extraction occurs in the first stages of the industrial processes (Demergasso et al., 2010), when *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferriphilum* are the most important microorganisms (Demergasso et al., 2005b; Olson et al., 2003; Rawlings et al., 2003) and early-stage adhesion phenomena are determinant (Kinzler et al., 2003). This means that, within a few hours, microorganisms must be capable of choosing a suitable mineral substrate and adhering to its surface so as to obtain the energy they need for their metabolism. This stage is critical to the process and, since industrial progress depends on scientific understanding, this topic has become extremely important in biotechnology. Therefore, the present report aims at studying adhesion phenomena at microscopic level and to follow them over some time in order to gain valuable information to better understand these bioleaching systems.

Chalcocite and pyrite are two commonly found minerals in most of the industrial bioleaching processes. Chalcocite is the one yielding the greatest amount of copper in these processes (Demergasso et al., 2010; Gentina and Acevedo, 2013). It is well known that chemical chalcocite dissolution and copper release occur in stages (Adewale-Bolorunduro, 1999; Leahy et al., 2007; Thomas et al., 1967), and require the presence of iron oxidizing microorganisms (Leahy et al., 2007; Ruan et al., 2010). However, presently, only a partial description of the dynamics of microorganism adhesion on chalcocite exists (Echeverría and Demergasso, 2009). Pyrite (FeS_2) is also very common in these processes and its relation with oxidizing microorganisms has been thoroughly studied (Norris and Kelly, 1978; Rodríguez et al., 2003; Ruan et al., 2010; Schippers et al., 2000, 2013). Both minerals are used by oxidizing bacteria as sources of energy, but they differ in their chemical composition, which has a significant effect on substrate selection by microorganisms (Ruan et al., 2010).

By means of microscopy techniques, we were able to follow the adhesion dynamics directly over the mineral surface. In this way, we saw the effect of the mineral composition and of the surrounding media in pure and mixed bacterial cultures.

2. Materials and methods

2.1. Microorganisms

Adhesion tests were performed on a mixed culture of microorganisms obtained from an industrial bioleaching process. The microorganisms were characterized by means of Real Time PCR using specific probes for *A. ferrooxidans*, *A. thiooxidans* and *Leptospirillum*. The cells present were identified as *A. ferrooxidans*, 7.2×10^6 cells mL^{-1}, *A. thiooxidans*, 1.0×10^7 cells mL^{-1}, and *L. ferriphilum*, 2. 9×10^4 cells mL^{-1}. In addition, two pure strains isolated from industrial processes were used in adhesion dynamics and inhibition assays: *Acidithiobacillus ferridurans* D2 (*Af*D2 — formerly Group II *A. ferrooxidans*) and *A. ferrooxidans* IESL32 (*Af*DM). The strains *Af*D2 and *Af*DM were chosen because they are predominant in an industrial bioleaching process, and present over 99% similarity with the type strain *A. ferridurans* ATCC 33020 and *A. ferrooxidans* ATCC 23270, respectively. The characteristics of the microorganisms used in this investigation are listed in Table 1.

2.2. Mineral substrata

The minerals used in this study are greater than 99% pure chalcocite and pyrite (from Museo Mineralógico, Universidad de Atacama, Copiapó, Chile). They were ground to 3 mm fragments and suspended in an epoxy resin solution (Epoxy Cure 2 Epoxy System, Buehler, USA), so that one side could be polished to a shiny finish (Fig. 1). The fragments were then removed from the resin, pyrite was washed with boiling 6-N hydrochloric acid to eliminate iron ions, and then both minerals were passed through an acetone wash to remove any remaining sulfur, as specified in the protocol described (Schippers et al., 1999).

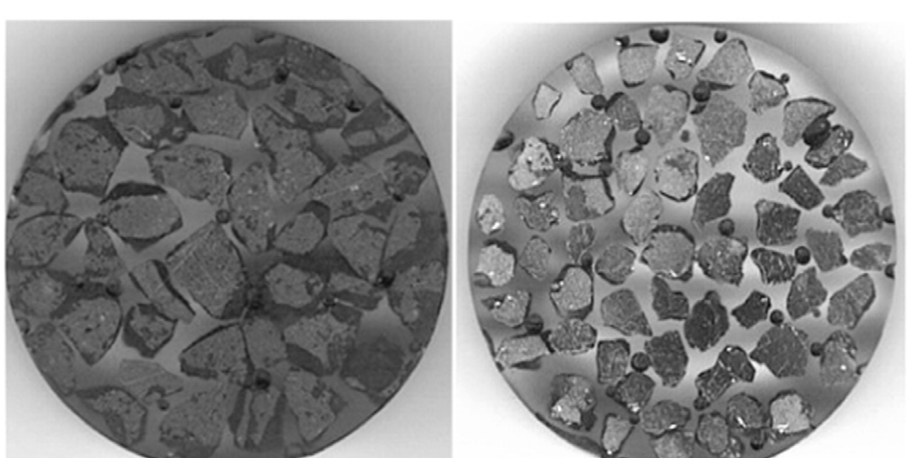

Fig. 1. Polished mineral fragments. On the left, chalcocite; on the right, pyrite. Diameter of the plates: 3 cm.