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Reductive leaching of jarosites by *Aeromonas hydrophila*

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ABSTRACT

The mineral industry has suffered in recent years two significant impacts: the raise on energy cost and more strict environmental regulations. In parallel to the above restrictions, the depletion of high grade mineral resources leads the mining industry to search for alternative processes to avoid the high energy consumption and exploit low grade mineral deposits. Jarosite is a common product in mining and industrial operations. This basic sulfate mineral incorporates several metals by substitution and the dissolution of jarosite by dissimilatory iron-reducing bacteria influences the mobility of many heavy metals present in the mineral structure.

In this work, the anaerobic bioreduction of three ferric solids (a biogenic mixture of ammonium and potassium jarosites, synthetic silver jarosite and natural gossan ore from RioTinto, Spain) by an isolated strain of *Aeromonas hydrophila* has been investigated. The release of ferrous ion indicated that the microbial reduction of jarosite takes place using lactate as electron donor. This process was accompanied by the subsequent secondary mineralization, leading to the formation of various iron-bearing minerals such as siderite, vivianite or magnetite. In addition, the influence of chelating agents (citrate and EDTA) and humic substances' analogue (AQDS) as electron shuttling molecule were studied. While the chelants increased iron reduction and dissolution rate, the humic substances had no effect in the process. These results suggest that direct contact between *A. hydrophila* and minerals is required for the reduction of Fe(III).

1. Introduction

Mineral ore processing in the recovery of metals by conventional technologies involve high energy and reagents costs and environmental risks. Nowadays, there is a growing interest in the development of environmentally friendly sustainable treatments. In this context, the role of some microorganisms in mineral dissolution makes bioleaching an alternative method to obtain different metals (Eisele and Gabby, 2014; Vera et al., 2013). Biological options involve considerably less energy or reagent expenditure and consequently are more cost-effective and environmentally friendly (Hallberg et al., 2011; Papassiopi et al., 2010). Recent advances in fields such as molecular biology, chemical analysis, surface science or nanobiotechnology, have contributed to a better understanding of this bioprocess.

Traditionally, bioleaching has been related to Fe(II)-oxidizing bacteria. Nevertheless, microbes can also mobilize metals and attack mineral surfaces by redox processes. Many microorganisms able to reduce Fe(III) are heterotrophs and play a key role in the biochemical cycling of iron and organic matter degradation in soils and sediments (Lovley, 1991; Wang et al., 2009). Solubilization mechanisms provide a route for removal of metals from industrial wastes and by-products, low-grade ores and metal-bearing minerals, which is relevant to bioremediation of soil matrices and solid wastes, metal recovery and recycling. Anaerobic iron bioleaching may be an attractive alternative for iron extraction from recalcitrant ores and re-processing waste materials from mining operations. In addition, oxygen requirements, acid generation and CO_2 emissions are avoided (Li et al., 2005). However, anaerobic bioleaching could be also used to remove iron impurities from materials where iron gives undesirable properties. An example of this is the upgrading of kaolin and silica, which require a high degree of whiteness for many of their markets. This would preferably be done using low-cost reagents, using a sufficiently gentle process that the desirable mineral is not itself altered and a microbiological process could be an alternative (Eisele and Gabby, 2014).

Fe(III) has a very low solubility and is usually present as insoluble oxides at neutral pH. One of the main challenges for the industrial implementation of anaerobic bioleaching, as well as a decisive research stage, is the adaptation of microbial cultures to grow on

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iron minerals instead of soluble sources of ferric iron. Iron can form different oxides and hydroxides with different crystalline structures and oxidation states of the metal. These characteristics have an important effect on its bioavailability (Bonneville et al., 2009). In general, the more crystalline the Fe(III) oxide, the less susceptible it is to microbial reduction; therefore, most of the studies have been performed using amorphous Fe(III) minerals instead of crystalline compounds (Lovley et al., 1998).

Dissimilatory Fe(III)-reducing microorganisms transfer electrons derived from central metabolism to a site of reduction somewhere outside the membrane. Nonetheless, there are different mechanisms by which microorganisms access and reduce insoluble Fe(III)-bearing minerals (Luu and Ramsay, 2003). At first, it was considered that bacteria required a direct contact with the solids for Fe(III) reduction. However, later findings have focused on the role of outer-membrane c-type cytochromes in electron transfer to Fe(III) (Richardson et al., 2012) or on the use of "electron shuttling" molecules either produced by the cells (Stintzi et al., 2000) or present in their environment (Castro et al., 2013). The present investigations evidence that some bacterial pili are electrically conductive and these "wires" would act as conduits transporting the electrons to metal acceptors during respiration (Gorby et al., 2006; Reguera et al., 2005).

Our research interest was focused on the ability of certain microorganisms to reduce and dissolve different jarosite species, $MFe_3(SO_4)_2(OH)_6$, in order to recover the metals contained in its crystal lattice. The reductive dissolution of ferric iron minerals has been already studied and facilitate the extraction of nickel from a lateritic ore (Hallberg et al., 2011). Jarosites can be considered a valuable source to recover metals (Dutrizac and Jambor, 2000). In this study, the iron reducing behavior of a strain of *Aeromonas hydrophila* isolated from a natural microbial consortium and its effect in the bioreduction of jarosite have been examined. *A. hydrophila* persists in its aquatic environments (including polluted waters); however, the influence of *Aeromonas* spp. in the elemental cycles are relatively unknown, as well as its potential for biotechnological applications (Seshadri et al., 2006). Fe(III) reduction by *A. hydrophila* is dissimilatory in nature: growth and Fe(III) reduction occurred concomitantly, and no growth occurred in the absence of added Fe(III) (Knight and Blakemore, 1998). The kinetics of dissimilatory Fe(III) reduction from this iron-hydrosulfate mineral was studied considering both the soluble and insoluble ferrous iron obtained. The presence of silver in the mineral and its inherent toxicity to most microorganisms may affect cell growth and biological mineral transformations. Therefore, the influence of chelating agents and humic substances present in the environment was also evaluated.

2. Materials and methods

2.1. Bacterial culture

The strain A of *A. hydrophila* was isolated from a sample collected from the edge of an open-pit lake surrounding an abandoned mine site named "Brunita" (Pb-Zn ores) near La Unión (Murcia, Spain). The pure isolate was sent to the Spanish Collection of Type Cultures (CECT, University of Valencia, Valencia, Spain) for its identification. Partial 16S rRNA sequences were obtained from the CECT microbial identification service as described by Arahal et al. (2008). *A. hydrophila* strain A was deposited in the collection under the number CECT 8424. After isolation, the culture was grown in modified Postgate C medium (g/l): KH_2PO_4, 0.5; NH_4Cl, 1.0; Na_2SO_4, 4.5; $CaCl_2{\cdot}2H_2O$, 0.04; $MgSO_4{\cdot}7H_2O$, 0.06; sodium lactate, 6.0; yeast extract, 1.0; and sodium citrate, 0.22. pH was adjusted to 7.0 ± 0.2. The medium was supplemented with soluble ferric citrate or ferric minerals as electron acceptor (60 mM Fe^{3+}).

Aerobic sterilized medium (121 °C, 30 min) was dispensed into individual glass flasks. Then, the solution was vigorously bubbled with N_2:CO_2 (80/20, v/v) to strip dissolved oxygen. The flasks were capped with butyl rubber stoppers and sealed with aluminum crimps. Batch cultures were incubated unstirred at 30 °C in darkness.

2.2. Preparation of minerals

A mixture of ammonium and potassium jarosite were biosynthesized using a pure culture of *Sulfolobus metallicus* grown in Norris medium (Norris and Barr, 1985) ($MgSO_4{\cdot}7H_2O$, 0.5 g/l; $(NH_4)_2SO_4$, 0.4 g/l and K_2HPO_4, 0.2 g/l) supplemented with 50 mM Fe^{2+} at pH 1.8, 250 rpm and 70 ± 3 °C for 7 days. The jarosites were washed with deionized water and dried at room temperature.

Silver jarosite was chemically synthesized using the method proposed by May et al. (1973). According to the procedure, 3.27 g of Ag_2SO_4 were added to 430 ml of boiling Milli Q water followed by the addition of 5.7 ml of 17.6 N H_2SO_4 and 60 ml of 0.5 M Fe_2SO_4 solution. The solution was stirred and heated at 95 °C for 200 h in a flask with a condenser. The final product was washed with deionized water.

The gossan mineral was a representative sample from the Rio Tinto mine (Spain) used in a cyanidation plant in 1991 and supplied by Rio Tinto Minera S.A. The sample was crushed and a fraction <40 μm was obtained.

2.3. Bioreductions tests

All the experiments were performed in duplicate at 60 mM Fe (III) adding 60 ml of medium to the vials containing the mineral. The kinetic tests were monitored periodically. The parameters selected for checking growth were the concentration of Fe^{2+} and the concentration of total iron.

Before sampling, the stoppers were disinfected externally with ethanol. Sampling was carried out with a deoxygenated and sterile syringe. The syringes were oxygen cleaned by previously injecting a sterile mixture of N_2/CO_2 in order to displace the oxygen that eventually entered into the system. The gas flow was sterilized by passing the mixture of gases through a sterile filter of 0.22 μm. Two replicates were taken from the clarified supernatant at settled vials and another two replicates from shaking vials. This method let to determine the speciation of iron in both the solid and liquid phases. The concentration of dissolved species (Fe^{2+}(aq)) was determined in samples taken from the supernatant. While samples taken from agitated vials served to determine the concentration of $Fe(II)_{total}$. Once removed, the samples were immediately transferred to the acid solution in order to prevent the oxidation of iron. Duplicate samples of 0.5 ml removed from each culture at selected sampling times were placed in tubes containing the extraction solution (0.5 ml HCl solution 50% v/v) and heated until complete dissolution of the solid phase. Then, 4.0 ml of deionized water were added.

HCl-extractable Fe^{2+} was determined spectrophotometrically by the ferrozine method measuring the absorbance of the ferrozine-Fe(II) complex at 562 nm (Stookey, 1970). Standards of ferrous iron for the ferrozine assay were prepared with ferrous ethylene diammonium sulfate tetrahydrate dissolved in 0.5 N HCl (Lee et al., 2007). HCl-extractable total iron was determined in the same acid dilute solution by atomic absorption spectroscopy in a Perkin-Elmer HGA 700.