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# Effectiveness of anaerobic iron bio-reduction of jarosite and the influence of humic substances

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ABSTRACT

Nowadays there is a growing interest in developing clean mining processes free of toxic chemicals. Biotechnology could be a cost-effective and environmentally friendly alternative to traditional leaching methods. Jarosite is an important sulfate mineral that occurs naturally and also can be produced as a waste in industrial processes. In this work, bioreduction of jarosite using *Shewanella putrefaciens* and a natural consortium under anaerobic conditions is presented as an acceptable bioleaching method. Moreover, the influence of humic substances that appear in natural environments acting as electron shuttles between microorganisms and insoluble oxides has been investigated. One key factor for the industrial implementation of anaerobic bioleaching is the metal recovery from minerals. The precipitates formed as products of dissimilatory iron reduction, the bacterial ability to obtain ferrous ions in solution and the stimulation of insoluble Fe(III) ores reduction by electron shuttles have been examined in this research.



## 1. Introduction

Solid wastes from mining and metallurgy, energy production and recycling industries may contain a relatively high level of metals and can be considered as potentially valuable sources of metals (Vestola et al., 2010).

Metal solubilization in chemical leaching processes with strong acids is favorable when there are high levels of metals in wastes. The development of alternative methods when there are relatively low levels of metals has focused the interest. 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).

It is well known that the dissimilatory iron reducing bacteria play an important role in the biochemical cycling of iron in soils and sediments, where Fe(III) minerals participate as terminal electron acceptors. Anaerobic iron bioleaching is ubiquitous in subsurface environments, including mining-impacted areas. In addition, oxygen is not required and acid generation and $CO_2$ emissions are avoided in anaerobic bioleaching (Li et al., 2005). In consequence, anaerobic bioleaching may be an attractive alternative for iron extraction from recalcitrant ores and re-processing waste materials from mining operations with remarkable environmental benefits.

Humic substances are ubiquitous in soils and sediments and are formed from the degradation of plants, animals and microorganisms. The major functional groups in humic substances are carboxylic acids, phenolic and alcoholic hydroxyl, ketones and quinones. Humic substances influence the toxicity and transport of heavy metals. They can form complexes with metal ions and also alter the speciation of the metal through oxidation–reduction reactions. Humic acids play a key role as electron acceptors in microbial respiration. Dissimilatory iron-reducing bacteria oxidize organic compounds by transferring electrons to humic substances. The reduced humics can then abiotically transfer electrons to Fe(III), regenerating the oxidized form of the humics. This electron shuttling alleviates the need for bacteria to establish direct contact with the Fe(III) oxides to reduce them (Liu et al., 2001; Luu et al., 2003). Therefore, there are environments, especially those that are low in natural organic matter, with insignificant quantities of electron-shuttling compounds. In such cases, the addition of humic acids or other quinines can stimulate Fe(III) reduction.

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 iron minerals instead of soluble sources of ferric iron. Neutrophilic Fe-reducing bacteria have also been shown to cause the reductive dissolution of some ferric iron minerals. One important difference between ferric iron and other electron acceptors is that it can form different oxides and hydroxides with different crystalline structures and oxidation states of the metal. This fact has 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. Bioreduction

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of amorphous Fe(III) minerals has been extensively studied due to their poor crystalline structure in contrast with the few studies on crystalline compounds (Lovley et al., 1998).

Jarosites, $MFe_3(SO_4)_2(OH)_6$, are important iron sulfate minerals that occur in acid mine drainage environments. Moreover, jarosite has applications in base metal recovery industries. Jarosite precipitation is used in Zn industry to extract iron impurities from Zn-sulfide ores and these compounds are potential hosts for metals such as lead and silver.

However, despite the relevance of jarosite minerals, few studies have evaluated the biotic dissolution of jarosites (Bridge and Johnson, 2000; Coggon et al., 2012; Jones et al., 2006). Jarosite can be susceptible of microbial reduction. Natrojarosite reduction chemistry has been studied (Ergas et al., 2006). Assuming that the Gibbs free energy of formation of K-jarosite is −6 kJ/mol, jarosite reduction could provide the energy required for iron reducing bacteria using lactate as electron donor following the equation:

$$4MFe_3(SO_4)_2(OH)_6 + 3CH_3CHOHCOO^- \rightarrow 9H_2O + 12Fe^{2+} + 3HCO_3^- + 8SO_4^{2-} + 9OH^- + 4M^+ \quad (1)$$

Previous works have mostly used pure bacterial cultures and synthetic jarosite. Furthermore, the effect of quinones as electron acceptors for microorganisms were studied with poorly crystalline Fe(III) oxides in most of the cases (Kappler et al., 2004). The present work is focused on the anaerobic bioleaching of jarosite, a crystalline and abundant mineral, using two different cultures: a pure strain of *Shewanella putrefaciens* and a natural consortium. The kinetics of dissimilatory Fe(III) reduction was studied in the presence of the humic substances using the humic analog antraquinone-2,6-disulfonate (AQDS) to catalyze the process and keep ferrous iron in solution in an attempt to improve the potential application of bioleaching.

## 2. Materials and methods

### 2.1. Bacterial cultures and media

The natural consortium for the microbial study was collected from the edge of an open-pit lake surrounding an abandoned mine site named "Brunita" (formerly a source of Pb–Zn ores) near La Unión (Murcia, Spain). The culture of *S. putrefaciens* came from the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ).

All the cultures were grown in modified Postgate C medium supplemented with jarosites provided as electron acceptor (60 mM $Fe^{3+}$). The modified Postgate C medium contained the following salts (g/l): $KH_2PO_4$, 0.5; $NH_4Cl$, 1.0; $Na_2SO_4$, 4.5; $CaCl_2 \cdot 6H_2O$, 0.06; $MgSO_4 \cdot 7H_2O$, 0.06; sodium lactate, 6.0; yeast extract, 1.0; $FeSO_4$, 0.004; and sodium citrate heptahydrate, 0.3. pH was adjusted to 7.0 ± 0.2. Growth bacterial studies in the presence of AQDS were carried out in the same basal medium at a final concentration of 100 μM.

Standard anaerobic techniques were used in this research. Aerobic sterilized media (autoclave conditions: 121 °C, 30 min) was dispensed into the individual glass flasks and the mineral was added. 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. A 10% inoculum of active Fe(III)-reducing enrichment cultures was used. The cultures were incubated unstirred at 30 °C in the dark.

### 2.2. Synthesis of jarosite

The crystalline ammonium jarosite was biosynthesized by means of a pure culture of *Sulfolobus metallicus* grown in Norris medium. The basal medium composition was the following (salts expressed in g $l^{-1}$): $MgSO_4.7H_2O$, 0.5; $(NH_4)_2SO_4$, 0.4; $K_2HPO_4$, 0.2 and 50 mM of ferrous iron. The pH was adjusted to pH 1.8. Cultures were grown on Erlenmeyer flasks under shaking conditions (250 rpm) at 70 ± 3 °C. Once formed, jarosites were recovered, washed with acid water and dried at room temperature. XRD analysis confirmed the composition of the solid formed as ammonium and potassium jarosites.

### 2.3. Kinetic tests

The kinetic of ferric reduction was tested in vials containing 54 ml of Postgate C medium supplemented with jarosite (60 mM in $Fe^{3+}$). An inoculum of 6 ml, obtained previously after more than ten successive transfers in the same conditions, was added at the start of the experiment. Inoculated tests were maintained in anaerobic and static conditions, in the dark at 30 ± 2 °C. In addition, chemical controls prepared with cell-free culture medium were performed in order to evaluate the extent of aqueous chemical reactions that might also occur. All reported data of kinetic tests were averages of two parallel bottles. In order to avoid any discrepancy caused by physiological changes of the bacterial culture, all experiments were inoculated from the same bottle of inoculum as described by Li et al. (2006).

### 2.4. Monitoring cultures: sampling and analysis

The kinetic study of dissimilatory Fe(III)-reducing cultures was monitored by taking samples at different sampling-times. The parameters selected for checking growth were the concentration of $Fe^{2+}$ and the concentration of total iron. $Fe^{2+}$and $Fe_{total}$ were measured in the same acid extraction solution.

For sampling, 0.5 ml of the sterile gas mixture ($N_2$:$CO_2$, 80/20 v/v) was injected into the anaerobic vial and 0.5 ml of each sample was withdrawn. Sampling was performed 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 oxygen that eventually entered into the system. The sterilization of the flux was obtained by passing the mixture of gases through a sterile filter of 0.22 μm. Samples, once taken, were immediately transferred to the extraction solution in order to prevent the oxidation of iron.

Vials were sampled in a different way: two replicates were taken from the clarified supernatant solution at settlement vials and another two replicates from shaking vials. This sampling method let us to determine the speciation of iron in both the solid and liquid phases. Samples taken at decanted vials served for determining the concentration of dissolved species: $Fe^{2+}$(aq) and $Fe_{total}$ (aq). Samples taken at agitated vials served to determine the concentration of HCl-extractable Fe(II) and HCl-extractable Fe(total) (that is the sum of Fe(II) + Fe(III)).

The duplicate samples of 0.5 ml were removed from each culture at selected sampling times and placed in tubes containing the extraction solution (0.5 ml HCl solution 50% v/v) and heated to get the dissolution of solid phase. Then 4.0 ml of deionized water was added. HCl-extractable $Fe^{2+}$ was determined by the ferrozine method (Stookey, 1970). Ferrous concentration was spectrophotometrically determined by measuring the absorbance of the ferrozine-Fe(II) complex at 562 nm after 5 min of color development. 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 diluted solution by atomic absorption spectroscopy in a Perkin-Elmer HGA 700.

### 2.5. X-ray diffraction analysis

Precipitates in culture vials were recovered and preserved for identification. Oxidation was prevented by keeping samples in anaerobic conditions before analysis by bubbling a gas mixture of $CO_2$:$N_2$ (80/20 v/v).