



Improving zinc recovery by thermoacidophilic archaeon *Acidianus copahuensis* using tetrathionate

Camila CASTRO, Edgardo R. DONATI

CINDEFI (CCT La Plata—CONICET, UNLP), Facultad de Ciencias Exactas,
Universidad Nacional de La Plata (UNLP), Calle 47 y 115, (1900) La Plata, Argentina

Received 5 November 2015; accepted 8 March 2016

Abstract: The attachment and bioleaching experiments were conducted to evaluate the zinc recovery from Hualilan ore by the thermoacidophilic archaeon *Acidianus copahuensis*. Cells of this species pregrown on tetrathionate showed higher capability of attachment to the ore than cells pregrown on other energy sources and such attachment seemed to be mediated by the product of extracellular polymeric substances. *A. copahuensis* achieved a successful bioleaching of the ore reaching 100% of zinc recovery when tetrathionate was added. Simultaneous addition of yeast extract and tetrathionate maintained the zinc extraction at higher rate. Zinc dissolution kinetics was controlled by chemical reaction in cultures with the external addition of tetrathionate but by the diffusion through a product layer of jarosite in the other cultures.

Key words: bioleaching; zinc; tetrathionate; microbial attachment; *Acidianus copahuensis*; thermophiles

1 Introduction

Zinc is mainly produced through the extraction of zinc from sphalerite by roast-leach-electrowinning (RLE) and pressure hydrometallurgy. These processes consume a lot of energy for roasting and smelting, and often are associated with the emission of gases such as sulfur dioxide and fumes into the environment [1,2].

In the last decades there has been much interest in the development of biohydrometallurgical methods for metal extraction from sulfide minerals. Biohydrometallurgy has some advantages over pyrometallurgical and hydrometallurgical techniques [3,4]. These advantages comprise low operation costs, low investment in infrastructure, reduced emissions to air, simplicity of operation, and applicability to refractory ores and low-value ores or mineral resources that cannot be treated by conventional mining techniques [5,6]. In primary copper production, bioleaching share is about 20% or more. Also, there are several biooxidation projects in gold mining. Gold is not really bioleached but the microbial activity facilitates the liberation of gold from a sulfidic matrix. In comparison to copper and gold, the application of bioleaching for recovering other metals is still an exception. It has been proved that bioleaching

may be applied as an alternative to increase zinc production, especially from low-grade ores and in the treatment of zinc concentrates which are difficult to process using conventional technologies. Mesophilic microorganisms such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, and *Acidithiobacillus thiooxidans* have been most extensively investigated in sphalerite bioleaching processes [7–10]. In order to make it economically feasible in primary zinc production, new microbial species capable of rapid and more efficient metal extraction are being tested. Increasing attention has been focused on moderate and extreme thermophiles that facilitate high zinc extraction rates due to the high temperatures but also due to the metabolic characteristics of these microorganisms [2,10–14].

Recently, we have isolated a new species of thermophilic archaea belonging to *Acidianus* genera from the geothermal Cavihue-Copahue system, located in the north-west of Neuquén Province, Argentina [15,16]. *Acidianus copahuensis* has shown a broad metabolic versatility. It grows on sulfur, tetrathionate, ferrous iron, and glucose under aerobic conditions; but it can also function under anaerobic conditions. This metabolic versatility can potentially be exploited for the extraction of metals from other substrates.

Hualilan area is considered one of the most important sources of gold in San Juan Province (Argentina) in the Southern Andes. This refractory ore required a pretreatment like biooxidation to liberate the gold. In addition, this ore has considerable zinc content (about 8%) mainly as sphalerite which is one of the most important sources for zinc production in the world. It seems to be possible to recover zinc as a subproduct through the biooxidation pretreatment of Hualilan ore [9].

The main objective of this research was to investigate the zinc extraction using the process of bioleaching (biooxidation) for the Hualilan ore using the recently isolated thermophilic archaeon *A. copahuensis*. The effect of culture history on the attachment to the mineral and the influence of external addition of other energy sources (ferrous iron, tetrathionate, elemental sulfur, and yeast extract) on the zinc extraction from the mineral were also investigated. Finally, the kinetics for the bioleaching of zinc was analyzed by applying the shrinking core model.

2 Experimental

2.1 Mineral

Mineral samples from Hualilan mining area (San Juan Province, Argentina) were used throughout this study. The main mineralogical species detected into the mineral were pyrite, sphalerite, pyrrhotite, galena, and chalcopyrite. The main chemical composition (mass fraction) of the mineral was: 11.53% Fe, 8.12% Zn, 1.3% Mn, 0.140% Cu, 0.070% Pb, 0.048% Cd, 0.019% Ag, and 0.003% Au. Mineral samples were reduced in size through consecutive steps of crushing and grinding, until particles diameters were less than 74 μm . The specific surface area of the mineral was 6.13 m^2/g (BET surface area).

2.2 Strain and culture conditions

Acidianus copahuensis strain ALE1 DSM 29038 [15] was cultured in flasks containing Medium 88 basal salt solution (M88) which is a selective medium for thermophilic, acidophilic archaea recommended by the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), adjusted to pH 2.0 with 5 mol/L sulfuric acid. The medium was sterilized by autoclaving at 2.026×10^5 Pa for 20 min. The basal medium was supplemented with potassium tetrathionate (3.0 g/L) from a stock solution sterilized by filtration with a 0.22 μm pore-size membrane. Yeast extract (1.0 g/L) was separately sterilized and then added to the medium. Cultures were incubated in Erlenmeyer flasks containing 50 mL of fresh medium at 65 °C with agitation at 150 r/min. After growth, cultures were

filtered and cells were harvested by filtration and centrifugation at 8000 r/min for 10 min. Cell pellets were washed twice with M88 solution in order to remove any trapped ions and then resuspended in fresh M88 solution.

For attachment experiments, *A. copahuensis* was cultured using Mackintosh basal salt solution (MAC) [17] at pH 2 supplemented with 10 g/L elemental sulfur powder, 3.0 g/L potassium tetrathionate, 6.0 g/L ferrous iron as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0% (w/v) Hualilan ore, 1.0 g/L glucose, and/or 1.0 g/L yeast extract as energy source.

2.3 Attachment experiments

Attachment experiments were performed in 100 mL flasks, each containing 50 mL MAC solution (pH 2) with 5.0 g of Hualilan ore, and an initial cell number of 5×10^8 cell/mL. As inocula, cultures of *A. copahuensis* grown on different energy sources (elemental sulfur, potassium tetrathionate, ferrous iron, or Hualilan ore in mixotrophic conditions with yeast extract additions; or in heterotrophic condition using just glucose) were used. Flasks were incubated at 65 °C with shaking at 120 r/min. Aliquots of 1 mL were taken periodically up to 420 min. Cell number was determined in the liquid phase with a counting chamber by using a phase-contrast microscope. The amount of cells attached to the mineral surface was calculated as the difference between the number of initial cells inoculated and the number of planktonic cells. In order to test the nonspecific attachment to the glass wall, some flasks were inoculated without mineral. Nonspecific attachment values were subtracted from the total attachment values to obtain the specific attachment to the mineral under study.

2.4 Bioleaching experiments

Bioleaching experiments were carried out in sterile 250 mL narrow-neck Erlenmeyer flasks, each containing 150 mL M88 medium at pH 2 supplemented with one or more energy sources (1.0 g/L elemental sulfur, 3.0 g/L potassium tetrathionate, 1.0 g/L ferrous iron and/or 1.0 g/L yeast extract), pulp density of 2.0% (w/v) and an initial cell population of 1×10^8 cell/mL. Cells of *A. copahuensis* cultured mixotrophically with potassium tetrathionate and yeast extract were used as inoculum and it was prepared as described in Section 2.2. Flasks were incubated at 120 r/min on an orbital shaker in darkness at 65 °C. Each bioleaching condition was conducted in duplicate. Sterile controls were performed replacing the inoculum by the same volume of a thymol 2.0% (w/v) in methanol solution. Periodically, distilled water was added to the flasks in order to compensate for evaporation losses.

To determine leaching efficiency, sample solutions were routinely withdrawn from each flask to analyze iron, zinc and pH at regular intervals. The amounts of

zinc and total soluble iron released during bioleaching were determined on filtered samples by atomic absorption spectrophotometry (AAS). Ferrous iron concentrations were quantified by spectrophotometric *o*-phenanthroline method [18].

Mineralogical analysis of the leached residues was performed using X-ray diffraction (XRD). Solids were retrieved by filtering leach solution samples through Whatman No. 42 cellulose filter paper. The residues were washed with distilled water (pH 2) and dried at 65 °C and then analyzed with a Philips 3020 X-ray diffractometer using Cu K α radiation. The operational conditions were 50 kV and 80 mA. Step scans were conducted at 2θ from 5° to 70° in 0.02° increments using 1 s count time.

2.5 Visualization of biofilms on mineral or elemental sulfur

1 g of Hualilan mineral or six sulfur coupons were placed in 100 mL Erlenmeyer flasks containing 50 mL of MAC medium at pH 2 and an initial cell density of 1×10^8 cell/mL. Flasks were incubated at 65 °C and 150 r/min. Mineral grains or sulfur coupons were withdrawn for microscopic observation and washed with filter-sterilized tap water. Samples were covered with a 10 mmol/L bicarbonate solution containing 50 μ g/mL TRITC-labeled Concanavaline A (ConA) (Invitrogen) for 30 min. Stained samples were washed three times with filter-sterilized tap water to remove unbound lectins. Then, cell staining was done by covering the samples with 6 μ mol/L SYTO 9 (Invitrogen) or 0.01% 4,6-diamidino-2-phenylindole (DAPI) in 2% formaldehyde for 20 min. Finally, samples were washed twice with filter-sterilized tap water, dried at room temperature and mounted on glass slides using an anti-fading agent to prolong the fluorescence of the dyes. Samples were visualized by epifluorescence microscopy (EFM) (Axiovert–100 MBP microscope, Zeiss) or by confocal laser scanning microscopy (CLSM) (using a Leica TCS SP5 microscope or a LSM 510 Carl Zeiss microscope).

2.6 Scanning and transmission electron microscopy (SEM/TEM)

A. copahuensis cells pregrown on tetrathionate were fixed with 2.5% glutaraldehyde (pH 7.2) at 4 °C for 1 h, post-fixed with 2.0% osmium tetroxide and dehydrated in a graded series of alcohol (25%–100%). Dehydrated samples were dried in a critical point chamber, coated with a thin layer of gold and visualized by a LEO EVO 40 XVP scanning electron microscope (SEM).

Microorganism ultra-structures and morphological characteristics of *A. copahuensis* were studied by transmission electron microscopy (TEM). Planktonic cells pregrown on tetrathionate were negatively stained

with phosphotungstic acid and samples were viewed in a JEOL JEM 1200EX II TEM.

2.7 Zeta-potential measurements

The zeta-potential measurements of *A. copahuensis* cells as well as the mineral were determined using a Brookhaven 90 Plus/Bi-MAS conditioning at a specified pH value.

Measurements were performed on microbial suspensions of 1×10^8 cell/mL. The zeta-potentials were conducted at ionic strength (KCl) of 1×10^{-3} .

3 Results and discussion

The kinetics of cell attachment to the mineral is shown in Fig. 1(a). In all conditions, the nonspecific

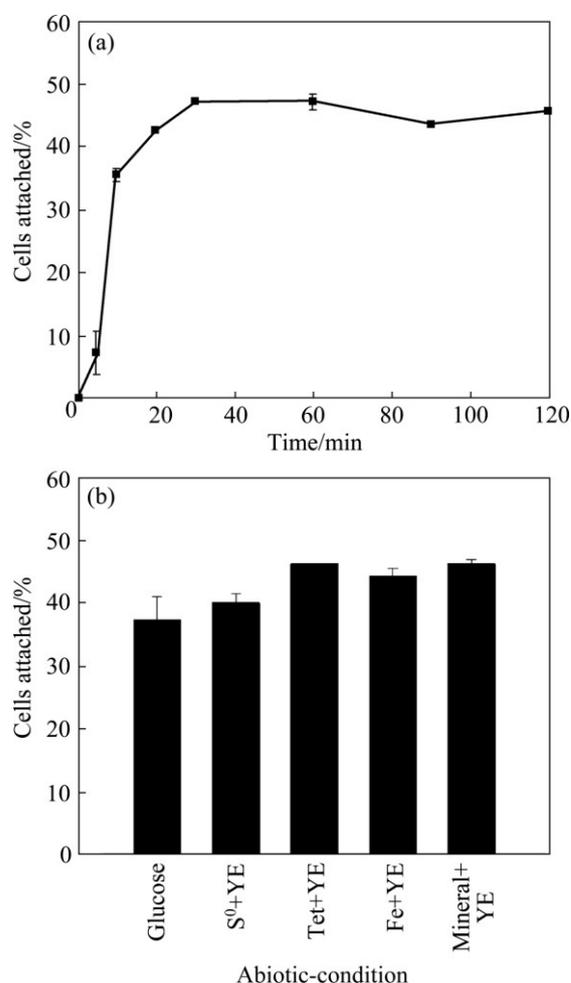


Fig. 1 Representative attachment kinetics of *A. copahuensis* cells to Hualilan ore during the first 120 min (a) and attachment of *A. copahuensis* cells to Hualilan ore, cultured on five different growth conditions: glucose (Glu), elemental sulfur and yeast extract (S⁰+YE), Hualilan ore and yeast extract (Mineral+YE), ferrous iron and yeast extract (Fe+YE), potassium tetrathionate, and yeast extract (Tet+YE) (b) (Error bars show standard deviation from three independent experiments (n=3))

attachment to the glass wall was between 10% and 15% (data not shown). The main attachment to the mineral was during the first 30 min of contact between the cells and the mineral particles. The levels of attachment attained after 30 min of incubation increased only marginally, with the equilibrium phase established 120–180 min after incubation. Similar results were reported by other researchers, in studies carried out with other acidophilic microorganisms and minerals [2,19–22]; and also a comparable trend of rapid attachment was shown by *A. copahuensis* in other tests performed to analyze the attachment to pyrite and a chalcopyrite concentrate [23,24].

Also in Fig. 1(b) the maximum percentages of cell attachment to the mineral can be observed. The attachment seems to be slightly dependent on the growth history of the cells. The extent of attachment for cells pregrown in mixotrophic conditions using potassium tetrathionate or Hualilan ore as energy sources, reached approximately 46% of microbial adsorption. Cell attachment to the mineral was a bit lower (45%, 40%, and 37%) when cells were pregrown with ferrous iron, sulfur, and glucose as electron donors, respectively. Although the behavior of zeta potential in function of pH (2–7) and the isoelectric points differed according to the growth history of the cells (data not shown), at pH 2.0, surface zeta potentials were 14.05, 7.29, 6.87, and 5.78 mV for cells pregrown on tetrathionate and yeast extract, glucose, sulfur and yeast extract, and ferrous iron and yeast extract, respectively. Since the mineral showed a negative surface zeta potential (–5.94 mV) at the same pH value, it seems that surface zeta potential provides an explanation for the fact that cells pregrown on any substrate adhered easily to the surface of the mineral although those grown on tetrathionate achieved the maximum adherence.

The attachment to the ore and biofilms facilitate the contact mechanism, which plays an important role in bioleaching processes since most of the reactions can occur directly at the mineral surface/interface [4]. Since the maximum percentage of attachment reached using tetrathionate as energy source, the inoculum used for bioleaching experiments was previously grown on that substrate.

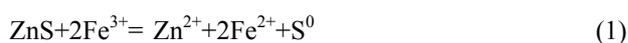
It is well known that the attachment process for many bioleaching microorganisms is mediated by extracellular polymeric substances (EPS) surrounding the cells. Usually, the production of EPS creates a less negative, more hydrophilic surface increasing the adhesion to the mineral and favoring the bioleaching. Although this phenomenon is quite well known for mesophilic bacteria [4], very little information has been reported for thermophilic archaea. Figure 2 seems to confirm that *A. copahuensis* also secretes EPS.

Figure 2(a) shows a detail of a cell of *A. copahuensis* surrounded by an amorphous slime, while Fig. 2(b) shows EPS aggregates over the cell surface mediating the contact with the surface and between cells. Confocal microscopy shows the colonization of mineral surface (after 5 d of incubation) using SYTO 9 (Fig. 2(c)) which is green-fluorescent stain for live and dead bacteria. Finally, Fig. 2(d) shows the spread of EPS on the same surface indicating its correlation with the colonization. It can be seen from Fig. 2(d) that cells were stained with ConA which is a lectin able to interact with EPS matrix.

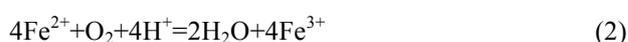
Figure 3 illustrates the zinc extraction from Hualilan ore by the extremely thermophilic *A. copahuensis* archeon at 65 °C. Chemical leaching in abiotic controls reached 15%–25% of zinc recovery after 42 d. Cultures under all conditions achieved zinc extractions higher than 40%. External addition of tetrathionate allowed reaching the maximum percentages of zinc recovery (90%–100%) although the presence of one additional energy source (yeast extract) allowed a faster kinetics. Zinc extraction was not significantly improved by the addition of sulfur.

Figure 4 shows the effect of the external and initial addition of ferrous iron on the bioleaching. The presence of iron improved zinc extraction (approximately 60% or higher) in all cultures although the best performance (100%) was again reached by adding tetrathionate. However, the simultaneous addition of yeast extract provoked a decrease in the final zinc extraction.

The effect of the external addition of iron can be interpreted through the attack of ferric iron [8]. Zinc extraction from sphalerite (main zinc mineral species present into the ore) due to ferric iron attack can be represented by



Similar to other iron-oxidizing microorganisms, *A. copahuensis* can regenerate ferric iron by the catalysis of the oxidation of ferrous iron:



Like some other thermophilic archaea [25], *A. copahuensis* utilizes other substrates (like tetrathionate) ahead of ferrous iron and in addition this species oxidizes iron at slow but existent rates [15].

However, this indirect mechanism of bioleaching does not explain the improvement in zinc extraction by adding tetrathionate. Metal–sulfur bonds in acid-soluble sulfides (like sphalerite) can also be broken by protons (instead of ferric iron), liberating hydrogen sulfide which may then be oxidized to sulfur [2]. This oxidative dissolution of sphalerite initiated by protons can be described as follows:



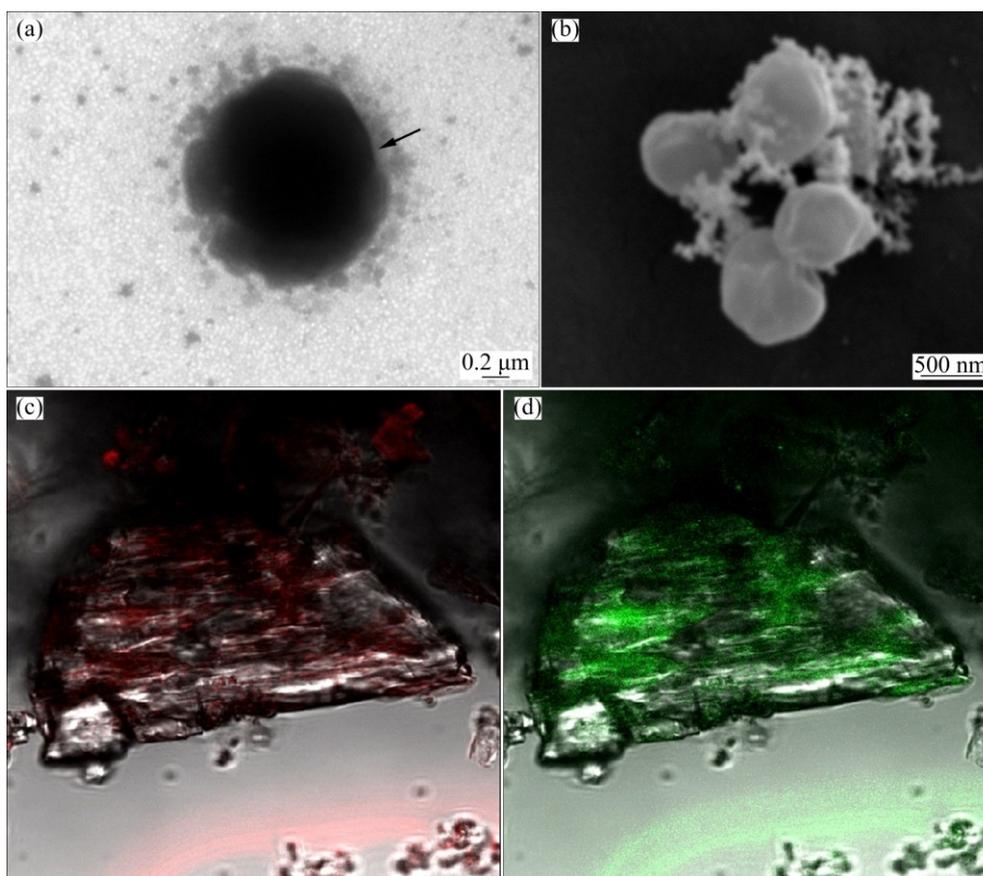


Fig. 2 EPS production and biofilm formation by *A. copahuensis* cells visualized by TEM (a), SEM (b) and CLSM (c, d) (Cells were stained with SYTO 9 (green) (c) and their EPS with lectin ConA (red) (d). Arrows point EPS production)

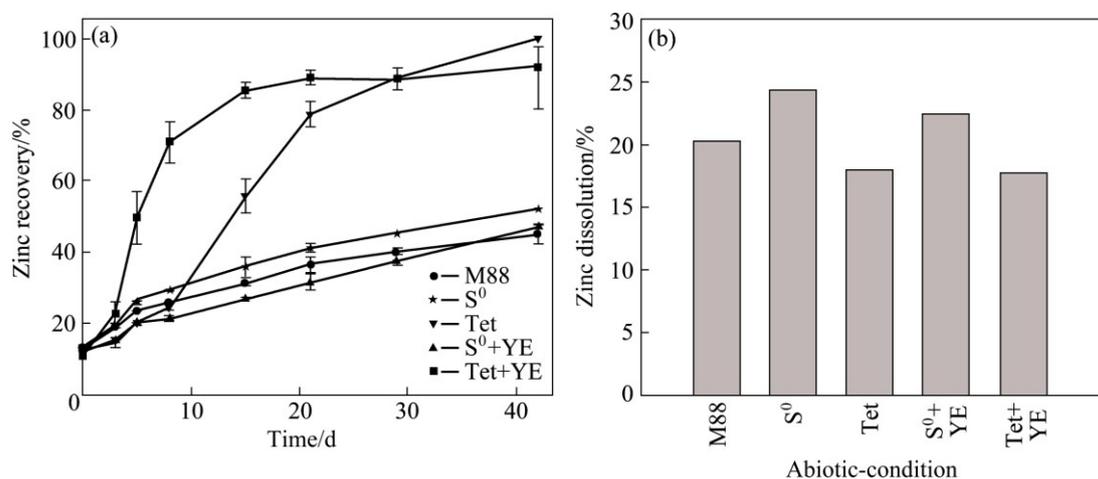


Fig. 3 Kinetics of zinc solubilization from Hualilan ore by *A. copahuensis* in media without ferrous iron supplementation (a) and zinc dissolution in abiotic controls (b) (Zinc recovery was tested in shake flask cultures with 2.0% (w/v) pulp density incubated at 65 °C during 42 d. Error bars show standard deviation from two independent experiments ($n=2$))

Protons are consumed during the dissolution of sphalerite, indicating that this mechanism can only be maintained if there is an independent acid production.

Figure 5 shows the variation of pH in the cultures during bioleaching of the ore without (Figs. 5(a) and (b)) and with (Figs. 5(c) and (d)) the external addition of

ferrous iron, respectively. Except for cultures containing tetrathionate, pH values increased from the beginning in all the systems (including abiotic controls). The upward trend of pH was surely due to the acid dissolution of sphalerite plus the dissolution of other alkaline species present in the ore. The final pH values were lower when

iron was supplemented probably because ferric iron precipitation buffers pH.

Since it can be seen in the figures, the oxidation of tetrathionate by the cells produced the decrease of the pH. The equations are as follows:

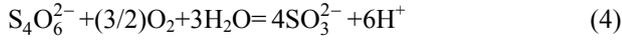


Figure 6 shows growth kinetics when *A. copahuensis* cells are cultured on tetrathionate (MAC medium, 3.0 g/L potassium tetrathionate, 120 r/min, 65 °C, initial pH 2.5) with and without the external addition of 1.0 g/L yeast extract. It is clear that

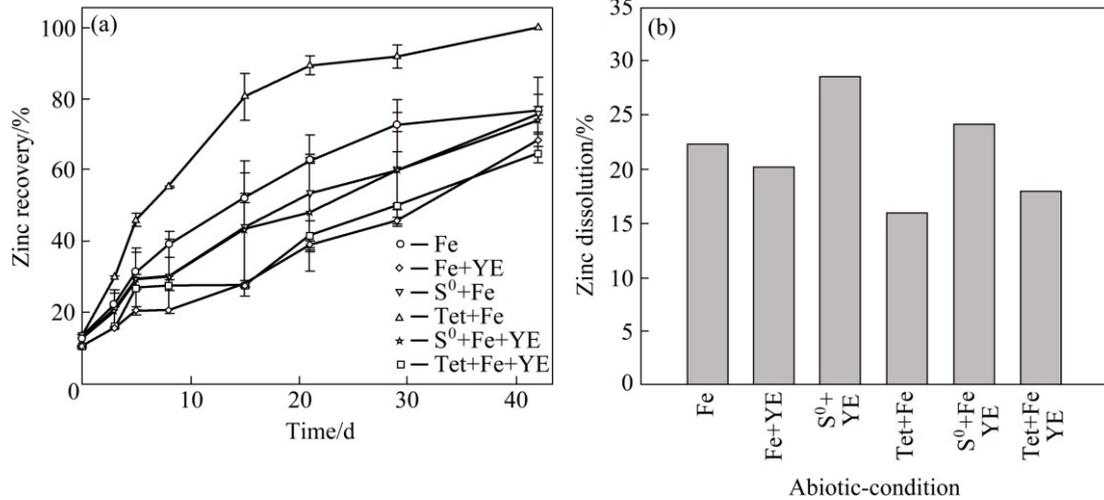


Fig. 4 Kinetics of zinc solubilization from Hualilan ore by *A. copahuensis* in media with ferrous iron supplementation (a) and zinc dissolution in abiotic controls (b) (Zinc recovery was tested in shake flask cultures with 2.0% (w/v) pulp density incubated at 65 °C during 42 d. Error bars show standard deviation from two independent experiments ($n=2$))

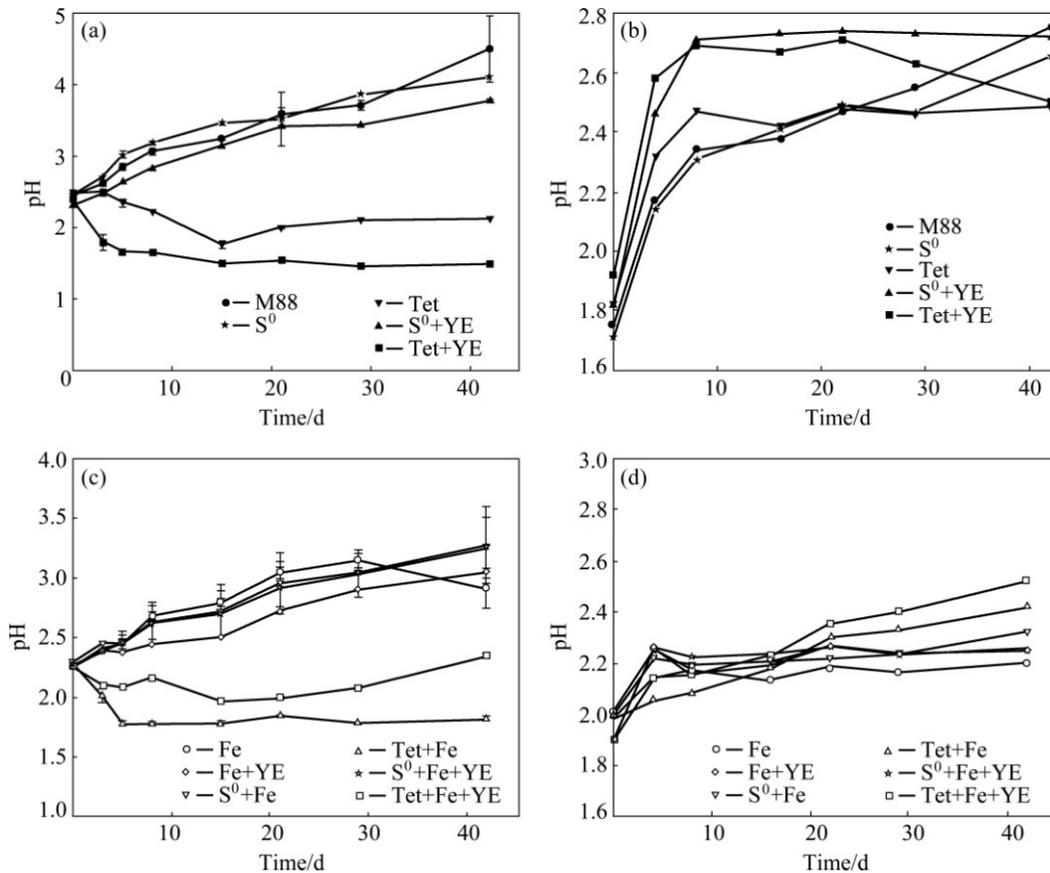


Fig. 5 Evolution of pH values during bioleaching tests with *A. copahuensis* in cultures without (a) and with (c) ferrous iron supplementation, and abiotic controls without (b) and with (d) ferrous iron supplementation (Error bars show standard deviation from two independent experiments ($n=2$))

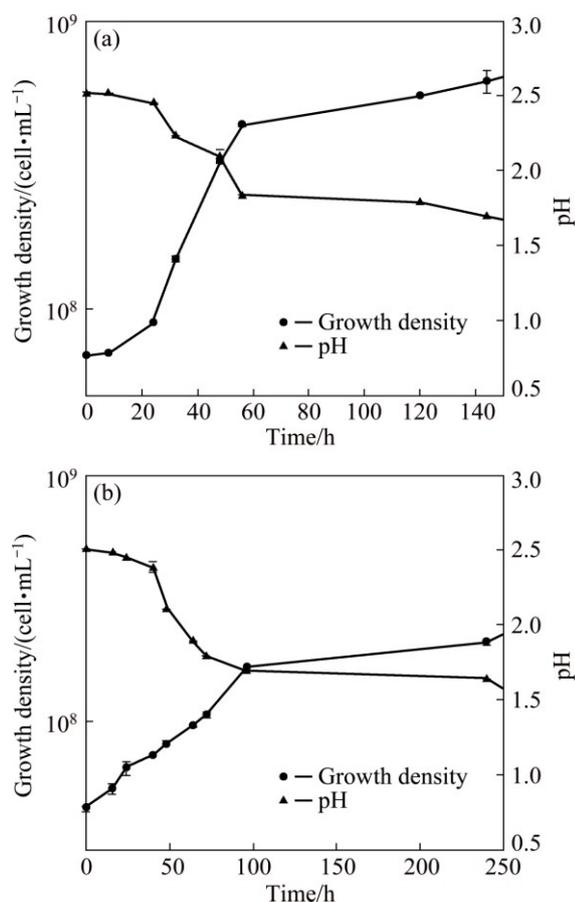


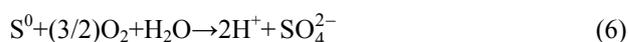
Fig. 6 Growth of *A. copahuensis* in tetrathionate media and pH evolution over time (Error bars show standard deviation from two independent experiments ($n=2$)): (a) Mixotrophic conditions; (b) Autotrophic conditions

A. copahuensis is able to grow oxidizing tetrathionate and producing acid. Although pH reached a similar value (about 1.6), the growth rate was faster and better in the presence of yeast extract (Fig. 6(a)) than that in the absence of yeast extract (Fig. 6(b)).

The higher zinc extractions in the cultures with the external addition of tetrathionate are explained by Reaction (4) which maintains the pH below 2 and promotes the continuation of Reaction (3). XIANG et al [26] reported a similar conclusion in a bioleaching of marmatite using *Sulfobacillus thermosulfidooxidans* although in a different experimental design. MEHRABANI et al [27] reported a different behavior when mesophilic and thermophilic consortia of iron and sulfur-oxidizing bacteria were used to bioleach lead–zinc tailings. They found that changing pH from 1.5 to 2.0, the percentage of zinc extraction increased. The high content of pyrite of those tailings could have produced a drastic decrease of pH (much higher than that of our experiments), partially inhibiting the bacterial action. Also, AHMADI and MOUSAVI [10] observed something similar in a bioleaching of a zinc sulfide

concentrate using a mixed culture of iron- and sulfur-oxidizing moderately thermophilic microorganisms when they compared the results in experiments with different initial pH values. However, the lowest pH value (where less zinc extraction was obtained) was 1.2 where these microorganisms are mostly inhibited. Similar explanation was reported by DEVECI et al [11] in the bioleaching of complex zinc sulfides using mesophilic and thermophilic microorganisms.

It can be seen in Fig. 5 that, surprisingly, the addition of elemental sulfur did not generate the same trend as tetrathionate (a soluble substrate) even when its oxidation produced more moles of protons per sulfur-mol.



Moreover, it seemed clearly that sulfur externally added was not oxidized in the cultures. However, the decrease trend in pH values correlated with the increase of cells in the growth kinetics of *A. copahuensis* on sulfur (MAC medium, 10 g/L sulfur, 120 r/min, 65 °C, initial pH 2.5) with and without the external addition of 1.0 g/L yeast extract (Fig. 7), demonstrating that the

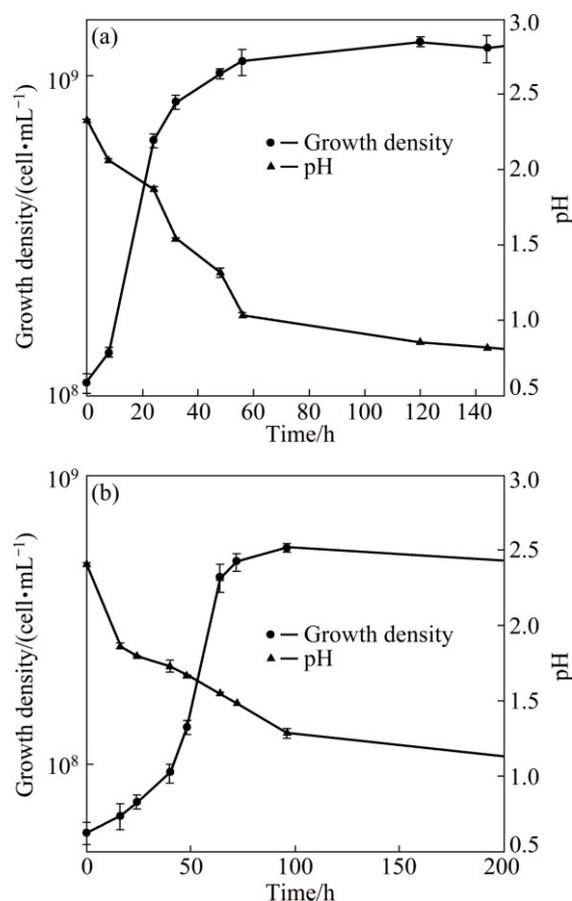


Fig. 7 Growth of *A. copahuensis* in elemental sulfur media and pH evolution over time (Error bars show standard deviation from two independent experiments ($n=2$)): (a) Mixotrophic conditions; (b) Autotrophic conditions

ability of *A. copahuensis* to oxidize sulfur coupled to growth. The addition of yeast extract allowed better growth and faster kinetics of biooxidation and consequently the culture achieved lower pH values [15].

It should be noted that the growth on sulfur results in a lower pH and higher cell population than in cultures on tetrathionate. However, in the bioleaching systems, *A. copahuensis* cells did not oxidize sulfur. Figure 8 shows images of sulfur surfaces colonized by *A. copahuensis* when it grows with sulfur as the only substrate; cell attachment and formation of microcolonies (stained by DAPI) over the surface and EPS production (stained with ConA) were detected. Since attachment is the first and indispensable step in the oxidation of sulfur in bioleaching systems, the absence of sulfur oxidation in the presence of ore can be attributed to preferential attachment to ore particles when sulfur was added.

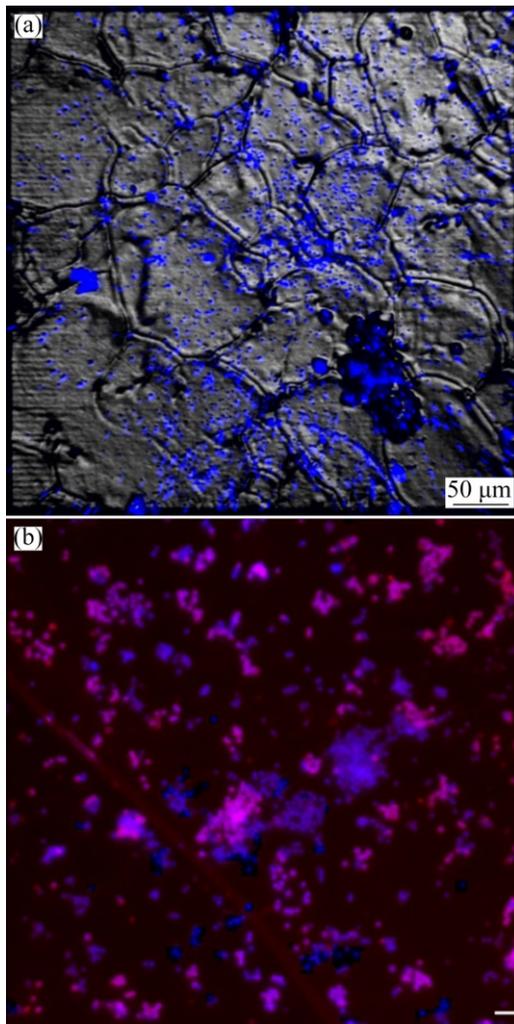


Fig. 8 *A. copahuensis* biofilms on sulfur after 6 d of incubation: (a) CLSM image (biofilms cells were stained by DAPI (blue) and sulfur surface was shown in reflection mode); (b) EFM image (cells attached on sulfur surface were stained with DAPI (blue) and their EPS with lectin ConA (red))

As attachment to ore particles is comparatively high, fewer cells were available to oxidize sulfur in those cultures when both substrates were present.

The kinetics for the bioleaching of zinc was analyzed by applying the shrinking core model [7,9,28]. If bioleaching kinetics is controlled by diffusion through these products, the kinetics might be correlated graphically as Eq. (7):

$$K_p t = 1 - (2/3)x - (1-x)^{2/3} \quad (7)$$

where K_p is the parabolic rate constant (d^{-1}); t is the time (d); and x is the mole fraction of reacted zinc.

However, if the chemical reaction was the controlling step of the reaction, the following rate equation would be applied [29]:

$$K_p t = 1 - (1-x)^{1/3} \quad (8)$$

Plots of $1 - (2/3)x - (1-x)^{2/3}$ versus time are shown in Fig. 9 for the experimental results reported here for the bioleaching of sphalerite under various conditions. Plots $1 - (1-x)^{1/3}$ versus time are presented in Fig. 10 using experimental values.

Taking into account the values of the linear correlation coefficient (R^2), the model of zinc dissolution

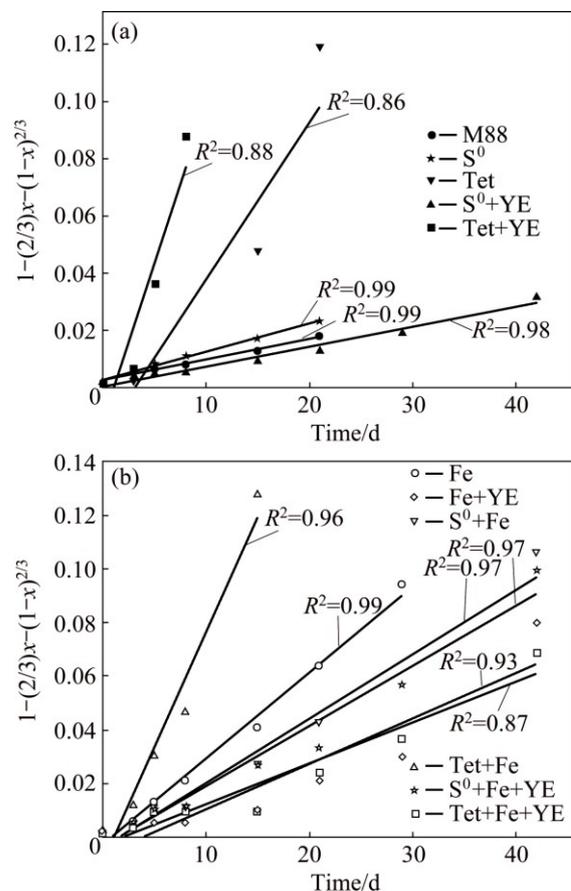


Fig. 9 Plots of $1 - (2/3)x - (1-x)^{2/3}$ versus time for microbial leaching data: (a) Media without ferrous iron supplementation; (b) Media with ferrous iron supplementation

kinetics controlled by chemical reaction correlates better than the kinetics limited by diffusion model for cultures supplemented with tetrathionate. In contrast, most of the other cultures and mainly those with external addition of iron show better linear correlation coefficients with the diffusion model. This result means that there is a product layer over the mineral surface. Although sulfur and/or polysulfide layers have been reported and could reduce leaching efficiencies [7,8,30,31], in our experiments, iron precipitation was extensive and it surely formed diffusion barriers. Jarosite was identified in the formed precipitates by X-ray diffraction (XRD) (data not shown).

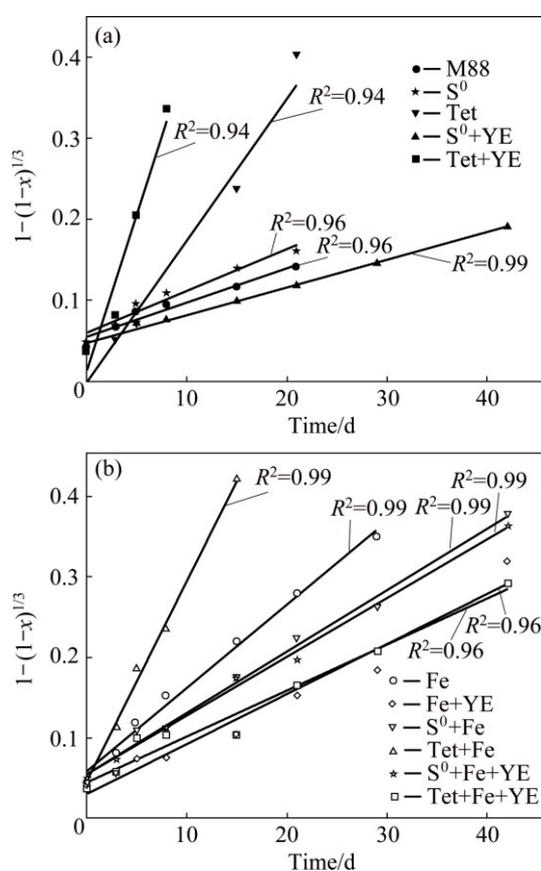


Fig. 10 Plots of $1-(1-x)^{1/3}$ versus time for microbial leaching data: (a) Media without ferrous iron supplementation; (b) Media with ferrous iron supplementation

Figure 11 shows the final soluble iron concentration in cultures without external addition of iron. From these data, it is possible to conclude that a greater concentration of ferric iron remains soluble in the presence of tetrathionate than that in the case of other cultures. That, together with the low pH values, has limited the formation of ferric iron precipitates, thus maximizing microbial access to sulfide surfaces as well as the diffusion of key soluble species to and from mineral surfaces during zinc extraction from sphalerite. Similar iron effects were reported on sphalerite

bioleaching by mesophilic, and also by moderately and extremely thermophilic microorganisms [9–11,22].

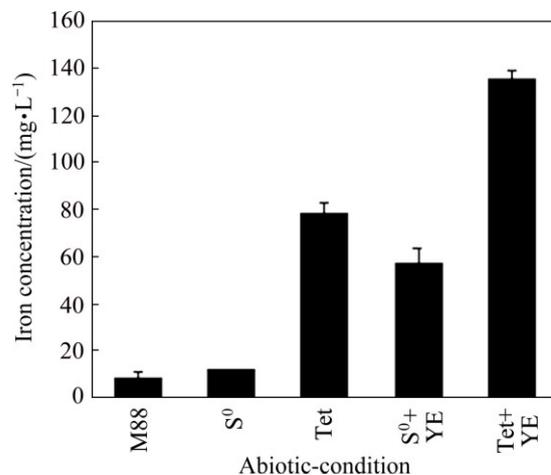


Fig. 11 Final iron concentrations during bioleaching tests with *A. copahuensis* in media without ferrous iron supplementation (Error bars show standard deviation from two independent experiments ($n=2$))

4 Conclusions

A. copahuensis achieved a successful bioleaching of Hualilan ore allowing high zinc recoveries. This bioprocess could be simultaneously used to increase the gold recovery from this ore where gold is dispersed in a sulfidic matrix. The external addition of tetrathionate as energy source in *A. copahuensis* cultures increased the capability of attachment to Hualilan ore and such attachment seems to be mediated by EPS production. Furthermore, the presence of tetrathionate significantly improved the zinc recovery in the bioleaching experiments reaching 100% of zinc recovery. The dissolution is produced through the action of protons generated by the oxidation of tetrathionate. The decreased pH promoted the increased acid dissolution of sphalerite, while also diminishing ferric iron precipitation with the concomitant formation of inhibiting surface layers. The simultaneous addition of yeast extract and tetrathionate allowed increasing the zinc extraction rate. Zinc dissolution kinetics seems to be controlled by chemical reaction in cultures with the external addition of tetrathionate but by diffusion through a product layer of jarosite in other cultures.

Acknowledgments

The authors gratefully acknowledge support from ANPCyT (PICT 2012 0623 and PICT 2013 0630). The authors also acknowledge to Dr. Wolfgang Sand and Dr. Mario Vera (Biofilm Centre, Universität Duisburg-Essen, Germany), who kindly allowed the use of EFM and CLSM to get the images shown in Fig. 8.

References

- [1] LIAO M X, DENG T L. Zinc and lead extraction from complex raw sulfides by sequential bioleaching and acidic brine leach [J]. *Minerals Engineering*, 2004, 17: 17–22.
- [2] RODRÍGUEZ Y, BALLESTER A, BLÁZQUEZ M L, GONZÁLEZ F, MUÑOZ J A. New information on the sphalerite bioleaching mechanism at low and high temperature [J]. *Hydrometallurgy*, 2003, 71: 57–66.
- [3] JOHNSON D B. Biomining-biotechnologies for extracting and recovering metals from ores and waste materials [J]. *Current Opinion in Biotechnology*, 2014, 30: 24–31.
- [4] SCHIPPERS A, HEDRICH S, VASTERS J, DROBE M, SAND W, WILLSCHEER S. Biomining: Metal recovery from ores with microorganisms [J]. *Advances in Biochemical Engineering/Biotechnology*, 2014, 141: 1–47.
- [5] BRIERLEY C L, BRIERLEY J A. Progress in bioleaching. Part B: Applications of microbial processes by the minerals industries [J]. *Applied Microbiology and Biotechnology*, 2013, 97: 7543–7552.
- [6] RAWLINGS D E. Heavy metal mining using microbes [J]. *Annual Review of Microbiology*, 2002, 56: 65–91.
- [7] GIAVENO A, LAVALLE L, CHIACCHIARINI P, DONATI E. Bioleaching of zinc from low-grade complex sulfide ores in an airlift by isolated *Leptospirillum ferrooxidans* [J]. *Hydrometallurgy*, 2007, 89: 117–126.
- [8] HAGHSHENAS D F, ALAMDARI E K, BONAKDARPOUR B, DARVISHI D, NASERNEJAD B. Kinetics of sphalerite bioleaching by *Acidithiobacillus ferrooxidans* [J]. *Hydrometallurgy*, 2009, 99: 202–208.
- [9] MARCHEVSKY N, URBIETA M S, BERNARDELLI C, MAS M, DONATI E R. Zinc recovery during refractory ore biooxidation by an indigenous consortium [J]. *International Journal of Mineral Processing*, 2015, 138: 30–37.
- [10] AHMADI A, MOUSAVI S J. The influence of physicochemical parameters on the bioleaching of zinc sulfide concentrates using a mixed culture of moderately thermophilic microorganisms [J]. *International Journal of Mineral Processing*, 2015, 135: 32–39.
- [11] DEVECI H, AKCIL A, ALP I. Bioleaching of complex zinc sulphides using mesophilic and thermophilic bacteria: Comparative importance of pH and iron [J]. *Hydrometallurgy*, 2004, 73: 293–303.
- [12] XIA J L, ZHAO X J, LIANG C L, YANG Y, NIE Z Y, TANG L, MA C Y, ZHENG L, ZHAO Y D, QIU G Z. Sulfur speciation transformation during bioleaching of pyrite-containing sphalerite concentrate by thermophile *Sulfolobus metallicus* at 65 °C [J]. *Journal of Central South University of Technology*, 2012, 19: 1961–1966.
- [13] WATLING H R. The bioleaching of sulphide minerals with emphasis on copper sulphides—A review [J]. *Hydrometallurgy*, 2006, 84: 81–108.
- [14] WHEATON G, COUNTS J, MUKHERJEE A, KRUIH J, KELLY R. The confluence of heavy metal biooxidation and heavy metal resistance: Implications for bioleaching by extreme thermoacidophiles [J]. *Minerals*, 2015, 5: 397–451.
- [15] GIAVENO M A, URBIETA M S, ULLOA J R, GONZÁLEZ TORIL E, DONATI E R. Physiologic versatility and growth flexibility as the main characteristics of a novel thermoacidophilic *Acidianus* strain isolated from Copahue geothermal area in Argentina [J]. *Microbial Ecology*, 2013, 65: 336–346.
- [16] URBIETA M S, RASCOVAN N, CASTRO C, REVALE S, GIAVENO M A, VAZQUEZ M, DONATI E R. Draft genome sequence of the novel thermoacidophilic archaeon *Acidianus copahuensis* strain ALE1, isolated from the Copahue volcanic area in Neuquén, Argentina [J]. *Genome Announcements*, 2014, 2(3): e00259–14.
- [17] MACKINTOSH M E. Nitrogen fixation by *Thiobacillus ferrooxidans* [J]. *Journal of General Microbiology*, 1978, 105: 215–218.
- [18] KOLTHOFF I M, SANDELL E B, MEEHAN E J, BRUCKENSTEIN S. Quantitative chemical analysis [M]. 6th ed. Nigar: Buenos Aires, 1998.
- [19] AFZAL GHOURI M, OKIBE N, JOHNSON D B. Attachment of acidophilic bacteria to solid surfaces: The significance of species and strain variations [J]. *Hydrometallurgy*, 2007, 85: 72–80.
- [20] BROMFIELD L, AFRICA C J, HARRISON S T L, van HILLE R P. The effect of temperature and culture history on the attachment of *Metallosphaera hakonensis* to mineral sulfides with application to heap bioleaching [J]. *Minerals Engineering*, 2011, 24: 1157–1165.
- [21] HARNEIT K, GOKSEL K, KOCK D, KLOCK J H, GEHRKE T, SAND W. Adhesion to metal sulfide surfaces by cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* [J]. *Hydrometallurgy*, 2006, 83: 245–254.
- [22] KONISHI Y, NISHIMURA H, ASAI S. Bioleaching of sphalerite by the acidophilic thermophile *Acidianus brierleyi* [J]. *Hydrometallurgy*, 1998, 47: 339–352.
- [23] CASTRO C, VERA M, DONATI E, SAND W. Visualization of attachment and colonization of pyrite surfaces by a novel species of *Acidianus* [J]. *Advanced Materials Research*, 2013, 825: 70–73.
- [24] CASTRO C, DONATI E. Effects of different energy sources on cell adhesion and bioleaching of a chalcopyrite concentrate by extremophilic archaeon *Acidianus copahuensis* [J]. *Hydrometallurgy*, 2016, 162: 46–59.
- [25] SHIERS D W, RALPH D E, BRYAN C G, WATLING H R. Substrate utilization by *Acidianus brierleyi*, *Metallosphaera hakonensis* and *Sulfolobus metallicus* in mixed ferrous ion and tetrathionate growth media [J]. *Minerals Engineering*, 2013, 48: 86–93.
- [26] XIANG Xiang-xue, GU Guo-hua, BAN Jin-rong, LI Shuang-ke. Bioleaching and electrochemical property of marmatite by *Sulfobacillus thermosulfidooxidans* [J]. *Transactions of Nonferrous Metals Society of China*, 2015, 25: 3103–3110.
- [27] MEHRABANI J V, SHAFAEI S Z, NOAPARAST M, MOUSAVI S M, RAJAEI M M. Bioleaching of sphalerite sample from Kooshk lead–zinc tailing dam [J]. *Transactions of Nonferrous Metals Society of China*, 2013, 23: 3763–3769.
- [28] FOWLER T A, CRUNDWELL F K. Leaching of zinc sulfide by *Thiobacillus ferrooxidans*: Bacterial oxidation of the sulfur product layer increases the rate of the sulfide dissolution at high concentrations of ferrous ions [J]. *Applied and Environmental Microbiology*, 1999, 65: 5285–5292.
- [29] GÓMEZ C, BLÁZQUEZ M L, BALLESTER A. Bioleaching of a Spanish complex sulphide ore bulk concentrate [J]. *Minerals Engineering*, 1999, 12: 93–106.
- [30] PECINA T, FRANCO T, CASTILLO P, ORRANTIA E. Leaching of a zinc concentrate in H₂SO₄ solutions containing H₂O₂ and complexing agents [J]. *Minerals Engineering*, 2008, 21: 23–30.
- [31] WEISENER C G, SMART R S C, GERSON A R. A comparison of the kinetics and mechanism of acid leaching of sphalerite containing low and high concentrations of iron [J]. *International Journal of Minerals Processing*, 2004, 74: 239–249.

连四硫酸盐存在下利用嗜酸热古菌 *Acidianus copahuensis* 提高锌回收率

Camila CASTRO, Edgardo R. DONATI

CINDEFI (CCT La Plata—CONICET, UNLP), Facultad de Ciencias Exactas,
Universidad Nacional de La Plata (UNLP), Calle 47 y 115, (1900) La Plata, Argentina

摘 要: 通过吸附和生物浸出实验考察利用嗜酸热古菌 *Acidianus copahuensis* 从 Hualian 矿中回收锌。经过在连四硫酸盐表面预处理的菌种具有比经其他能量供给剂预处理的菌种更强的矿物吸附能力, 且此吸附能力可由所产生的体外聚合物调节。当加入连四硫酸盐时, 用 *A. copahuensis* 生物浸取 Hualian 矿中的锌, 其浸出率达 100%。同时添加酵母和连四硫酸盐不仅能保持较高的锌浸出率, 而且能加快浸出速率。添加连四硫酸盐后, 培养基中锌的溶解动力学受化学反应控制; 而在未添加连四硫酸盐培养基中, 锌的溶解动力学受经过黄钾铁矾反应层的扩散控制。

关键词: 生物浸出; 锌; 连四硫酸盐; 微生物吸附; *Acidianus copahuensis*; 嗜热生物

(Edited by Wei-ping CHEN)