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

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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 facilities 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 Caviahue-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.

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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 $FeSO_4\cdot7H_2O$, 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