Available online at www.sciencedirect.com
ScienceDirect
Hydrometallurgy 92 (2008) 95–101
hydrometallurgy
www.elsevier.com/locate/hydromet

Mechanism of enhanced bioleaching efficiency of *Acidithiobacillus ferrooxidans* after adaptation with chalcopyrite

Lexian Xia, Xinxing Liu, Jia Zeng, Chu Yin, Jian Gao, Jianshe Liu *, Guanzhou Qiu

School of Resources Processing and Bioengineering, Central South University, Changsha 410083, PR China

Received 28 October 2007; received in revised form 23 December 2007; accepted 10 January 2008
Available online 17 January 2008

Abstract

To clarify the role and mechanism of bacterial adaptation in bioleaching, the leaching of chalcopyrite by adapted and unadapted *Acidithiobacillus ferrooxidans* was compared. Three extrinsic factors (adsorption, tolerance to shearing stress and copper tolerance) in relation to bioleaching were investigated. The results showed that there were significant differences in bacterial attachment and tolerance to shearing stress of unadapted and adapted cells due to the variation of cellular wall component and structure. Consequently, there was significant difference in bioleaching rate between unadapted and adapted bacteria. In addition, some differences of copper accumulation and distribution in adapted and unadapted cells also existed, but this was not one of the key factors that affected their bioleaching rates.

Keywords: *Acidithiobacillus ferrooxidans*; Bioleaching; Adaptation; Attachment; Chalcopyrite; Copper tolerance; Shearing stress

1. Introduction

Adaptation to environment is a natural phenomena that takes place in many animals, plants and microorganisms. These adapted organisms achieve stronger applicability than unadapted organisms after habitation in a specific environment for a long time. In the biohydrometallurgical industry, adaptation to special environment conditions by selective culturing is the most popular method for improving bioleaching activity of strains — although that is time consuming (Li and Ke, 2001a,b). Microorganisms used for bioleaching are generally pre-adapted repeatedly so as to further enhance the dissolution rates of metals from minerals before entering into bioleaching systems (Olson et al., 2003a,b).

Attia et. al achieved successes on pre-treating refractory Au ore tailings containing auriferous pyrite with adapted *Acidithiobacillus ferrooxidans* (Attia and Zeky, 1989). They also found that adaptation for bacteria prior to bioleaching enhanced the degree of sulfide leaching by 2–4 fold over unadapted bacteria in bioleaching pyrite (FeS_2), chalcopyrite ($CuFeS_2$), and arsenopyrite (FeAsS). Similarly, the bacterial activity was increased 2–4.8 fold. Moreover, the activity of adapted bacteria was in close relation to the adaptation time of bacteria (Elzeky and Attia, 1995). It was also reported that the adapted *A. ferrooxidans* showed higher bioleaching efficiency than the unadapted *A. ferrooxidans* on bioleaching refractory pyrite concentrates (Shahverdi et al., 2001). Similarly, adapted *A. ferrooxidans* markedly increased the dissolution rate of marmatite compared with that of the original *A. ferrooxidans* (Shi and Fang, 2004). The study by Li and Ke (2001a) testified Ni^{2+}-adapted strains exhibited better leaching efficiency in the bioleaching of nickel from nickel-bearing pyrrhotite compared with that of unadapted strains. Moreover, Ni^{2+} -adapted strains showed high tolerance to Ni^{2+} but not to Cu^{2+} and Mg^{2+} (Li and Ke, 2001b). Further studies by Das et al. (1998) showed proteinaceous differences between surface of Cu^{2+}-adapted bacteria and that of unadapted bacteria and a high tolerance property of Cu^{2+}-adapted bacteria to Cu^{2+} which disappeared when cell surface of Cu^{2+}-adapted bacteria was treated by protein hydrolyzing enzymes.

Although adaptation was very early realized, few studies were carried out to investigate the mechanism for adaptation. In

* Corresponding author. Tel.: +86 731 8836372; fax: +86 731 8879815.
E-mail address: ljscsut@126.com (J. Liu).

0304-386X/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.hydromet.2008.01.002

this report, some exterior factors such as bacterial adsorption to mineral surface, shearing stress and copper tolerance were studied, and the results showed that bacterial attachment to the mineral surface and shearing stress played important roles in enhancing the bioleaching rates, which greatly increased our insight on the mechanism for bacterial adaptation.

2. Materials and methods

2.1. Harvest of adapted and unadapted bacteria

A pure strain of *A. ferrooxidans* isolated from Dexin copper ore (Jiangxi, China) was used in all the studies, which utilized ferrous ion or elemental sulfur as energy source. All bacteria were incubated at 180 rpm and 30 °C in sterile 9 K basic salt medium containing different energy substrates as follows (Silverman and Lundgren, 1959).

The unadapted bacteria were cultured in 9 K medium with ferrous ion as an energy source. After incubation for three days, the unadapted bacteria were harvested by filtrating with 0.45 μm Whatman paper and the filtrate was centrifuged at 10000 rpm for 15 min.

Adaptation of *A. ferrooxidans* was performed by repeated sub-culturing for the bacteria in iron-free 9 K medium containing increased pulp densities of chalcopyrite: 1%, 2%, 3%, 4% and 5%. The mineral samples in this study were processed by ultrasonic washing for 30 min to refresh the surface of ore particles (average size ~0.074 mm). The pulp density was 1% in the initial sub-culturing. At each step, when the bacterial concentration reached 10^7cells/ml, they were re-inoculated into fresh iron-free 9 K medium with a higher pulp density. The bacterial concentration in the media with a pulp density of 5% reached 10^7cells/ml, as was considered to be adapted to the mineral environment. The subsequent procedures for treating adapted bacteria were similar to that of unadapted bacteria.

2.2. Leaching with unadapted and adapted bacteria

Leaching experiments were carried out in 500 ml flasks with 200 ml iron-free 9 K medium and 10 g chalcopyrite (5%w/v pulp density). Flasks were separately inoculated with unadapted and adapted bacteria suspension at 10% (v/v) concentration and incubated at 180 rpm and 30 °C. The copper concentration was determined by a Thermo Jarell SVIDEO 11E atomic absorption spectrophotometer.

2.3. Bacterial adsorption on the mineral surface

The concentration of adapted and unadapted bacteria in culture was adjusted to 10^9cells/ml with iron-free 9 K medium. The adsorption experiment was carried out in 50 ml flask with total volume of 30 ml iron-free 9 K medium containing 2% (w/v) of chalcopyrite particles and 2 ml the above adjusted bacterial suspension in an orbital shaker (160 r/min) incubated at 30 °C. Samples were analyzed at regular intervals, and the medium was filtrated through 0.45 μm Whatman paper to prepare for the following procedures. The bacterial populations before and after adsorption on the mineral surface were determined by blood cell counting chambers under a phase contrast microscope. Three or more counts were carried out for each system until a variation equal to or less than 5% was found. The difference of cell population before and after adsorption was assumed to be the number of adsorbed cells on the mineral surface.

The percentage of attached cells on mineral surface was calculated according to the following equation, B was the cell concentration of suspension before attachment, and A was the cell concentration of filtrated suspension.

$$\%\text{Attachment} = \left[1 - \frac{A}{B}\right] \times 100.$$

2.4. Measurements on electrokinetic behavior of bacterial cells

Electrokinetic behaviors of unadapted and adapted *A. ferrooxidans*, as well as chalcopyrite particles (0.074 mm or so), were determined using a Zeta-meter (Zetaplus, corp.). These tests were carried out at 25 °C under the required pH by using dilute HNO_3 or NaOH until the pH stabilized. Chalcopyrite particles and bacteria were dispersed in 10^{-2} mol/l KCl and their concentrations were 2% (w/v) and 3×10^8 cells/ml, respectively.

2.5. Contact angle measurement

Polar liquids of water and formamide, and apolar liquids of di-iodomethane were used to measure the contact angles on mineral surfaces and bacterial lawns. The mineral samples were first molded in epoxy resin with one flat surface being exposed for contact angle measurements. The surface of each sample was polished using 1000 and 4000 grit SiC paper. Final polishing was carried out with 0.05 μm alumina suspension. The samples were thoroughly washed with distilled water before carrying out the measurement. The cultured mixture containing the microorganism was filtered using a 0.2 μm Millipore filter paper. Adequate bacteria were necessary and filtered so that a uniform bacterial lawn was formed on the filter paper. This bacterial lawn was used for contact angle measurements. The contact angles of three liquids on bacterial lawns and solid sulphide surfaces were measured using the sessile drop technique with FIBRO 1100 DAT dynamic absorption tester and the contact angles and calculated surfaced energy were shown in Table 1.

2.6. Diffused reflectance infrared Fourier transform spectroscopy

The infrared spectra of unadapted and adapted cells were recorded on a Nexus-470 Fourier transform spectroscope with diffuse reflectance and a TGS detector against a non-absorbing KBr matrix, used as a reference. The samples (10 mg) were prepared by dispersing in 100 mg of KBr. Typical measurement time was 4 min (60 scans) at a resolution of 4 cm^{-1}. The cells were dried by a vacuum freeze drier for an hour. After obtaining the spectra, the peak areas of several peaks were analyzed by using software available with the instrument.

2.7. Effect of shearing stress on bacterial growth

The adapted and unadapted bacteria were inoculated into 9 K medium respectively. Meantime, SiO_2 powders with the particle size of about 0.074 mm were added to the 9 K medium instead of chalcopyrite particles until the finial pulp density reached 5%. The redox potential, which indicated the changes in concentration of Fe(III) and the ratio of Fe(III)/Fe(II), was measured with a Pt electrode (ref. saturated calomel electrode). The growth rates of adapted and unadapted bacteria could be optimally described by measuring the redox

Table 1
Contact angle and surface energy data for mineral and bacterial cells

System	Contact angle, θ (°)			Surface energy (mJ.m^{-2})			
	Water	Formamide	Diiodomethane	γ^{LW}	γ^{+}	γ^{-}	γ^{AB}
Adapted cell	17.21(3)	24.2(4)	68.23(3.5)	40.83	7.84	50.4	39.76
Unadapted cell	45.32(4.5)	48(2.5)	61(2.5)	37.47	2.13	59.3	32.82
Chalcopyrite	53(3.5)	59.24(3)	34.2(4)	30.8	0.02	5.7	0.67

Means of three replicates. In brackets standard deviation.