



# Bioleaching of arsenic and heavy metals from mine tailings by pure and mixed cultures of *Acidithiobacillus* spp.



Van Khanh Nguyen, Mu Hyun Lee, Hyung Jun Park, Jong-Un Lee \*

Department of Energy and Resources Engineering, Chonnam National University, Gwangju 500-757, Republic of Korea

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## ABSTRACT

Microbial removal of heavy metals in mine tailings from a Cu–Ag mine in the Philippines was investigated. Effect of bacterial strains on bioleaching and fractionation of heavy metals were also studied. Single and mixed cultures of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* were used separately to compare efficiency of metal solubilization. The results showed that mixed cultures were more efficient than each bacterium for Cu and As, while *A. ferrooxidans* demonstrated faster extraction efficiency for Mn and Zn. The results of sequential extraction for residues indicated that pure bacteria and mixed cultures mediated occurrence mode of heavy metals in different ways.

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## 1. Introduction

The bioleaching technique has been increasingly applied to the extraction and recovery of heavy metals from metallic ores and sediment, soil and sludge contaminated with heavy metals. It is an environmentally cleaner process when compared with physical and chemical processes [1] and rarely emits atmospheric contaminants. Bioleaching operations are relatively inexpensive and simple to operate [2].

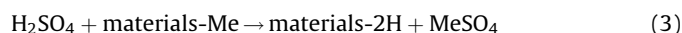
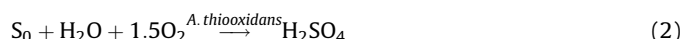
The bacteria commonly employed in the bioleaching processes are found to be of genus *Acidithiobacillus* and *Thiobacillus*, such as *Acidithiobacillus ferrooxidans*, *A. thiooxidans* and *Thiobacillus thio-parus* [3]. Among them, *A. thiooxidans* uses reduced forms of inorganic S but not  $\text{Fe}^{2+}$  as an energy source. In addition, they are highly acidophilic (pH 0.5 to 5.5, optimum pH 2 to 3.5), and can decrease the pH of the leaching medium to 1.5 to 1.0 and even lower [4]. Metal solubilization mechanisms by *A. thiooxidans* can be direct or indirect. In the direct mechanism, metal sulfides can be oxidized into  $\text{SO}_4^{2-}$  directly by these acidophilic bacteria. In the indirect mechanism,  $\text{H}_2\text{SO}_4$ , a strong leaching agent, is generated from the oxidation of elemental S or reduced S compounds by

*A. thiooxidans*. The direct and indirect mechanism can be described by the following reactions (Eqs. (1)–(3)) [5].

Direct mechanism:



Indirect mechanism:



where Me is a bivalent metal.

On the other hand, *A. ferrooxidans* differs from *A. thiooxidans* by the fact that they derive energy from the oxidation of  $\text{Fe}^{2+}$  as an electron donor in addition to reduced S compounds. In the absence of oxygen, *A. ferrooxidans* is still able to grow on reduced inorganic S compounds using  $\text{Fe}^{3+}$  as an alternative electron acceptor [6]. The mechanisms of bioleaching by *A. ferrooxidans* are usually discussed in terms of direct bacterial attack on sulfide minerals and indirect oxidative dissolution of minerals by  $\text{Fe}^{3+}$  [7]. The direct dissolution of minerals is caused by the attack on sulfide minerals by the enzymatic system of the bacteria situated at the mineral surface (Eq. (4)) [8–10].

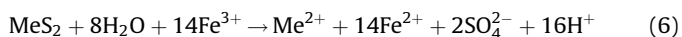
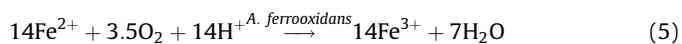


where  $\text{MeS}_2$  is an insoluble metal sulfide and  $\text{Me}^{2+}$  is a metal ion.

\* Corresponding author. Tel.: +82 62 530 1728; fax: +82 62 530 1729.

E-mail address: [jongun@chonnam.ac.kr](mailto:jongun@chonnam.ac.kr) (J.-U. Lee).

In the indirect mechanism, the bacteria regenerate  $\text{Fe}^{3+}$  through the oxidation of  $\text{Fe}^{2+}$  in solution and  $\text{Fe}^{3+}$  serves as a strong oxidizing agent of sulfide minerals (Eqs. (5) and (6)) [11,12]



To date, many studies on the bioleaching of heavy metals from mine tailings using *A. thiooxidans* are reported [13,14]. Some investigations on bioleaching employed a pure or mixed cultures; for example, bioleaching of chalcopyrite by pure and mixed cultures of *Acidithiobacillus* spp. and *Leptospirillum* spp. [15,16], bioleaching of As from medicinal realgar by pure and mixed cultures of *A. thiooxidans* and *A. ferrooxidans* [17], and bioleaching of sediment contaminated with heavy metals by pure and mixed cultures of *Acidithiobacillus* spp. [4].

The objective of this research was to elucidate the microbial removal efficiencies for Cu, Mn, Zn, and As from mine tailings taken from a Cu–Ag mine in the Philippines. A set of batch experiments was conducted to compare the efficiency of metal solubilization in the bioleaching processes which used both pure and mixed cultures of two *Acidithiobacilli* species, *A. ferrooxidans* and *A. thiooxidans* as microbial agents for the same mine tailings and under the same condition. The partition of heavy metals which represents the mode of occurrence in the mine tailings was also investigated through sequential extraction experiments.

## 2. Materials and methods

### 2.1. Sample characterization

The mine tailings used in this study were collected from a floatation plant of Dizon Mine located in San Marcelino, Luzon, Philippine. The target product of the mine is Cu and accompanied precious metals are Ag and Au. The particle size of the sample was lower than 0.180 mm in diameter. Prior to experiments, an amount of mine tailings was air-dried for chemical analysis and bioleaching experiments. The mine tailing samples were digested by aqua regia and then analyzed for their total Fe, Mn, As, and heavy metals (Cu, Zn, Co, Cr, Ni, Mo, and Pb) by an inductively coupled plasma optical emission spectrometer (ICP–OES). For the digestion, 0.25 g of an air-dried sample was digested with a concentrated  $\text{HNO}_3$ –HCl (1:3 v/v) mixture according to the Standard Methods of Korean Ministry of Environment [18]. Then, the suspension was filtered through a 0.45  $\mu\text{m}$  nylon syringe filter, serially diluted and introduced to the ICP–OES. The mine tailing sample was also subjected to X-ray fluorescence (XRF) analysis to determine both major and trace elements (Si, Al, Fe, Mg, Na, K, Ca, S, Ti, Cu, P, Cl, Mn, Zn, Sr, Cr, and As).

### 2.2. Microorganisms and acclimation tests

The cultures of *A. ferrooxidans* and *A. thiooxidans* were obtained from Microbial Geochemistry Laboratory, Chonnam National University in Korea and used throughout this study. Bacterial cells were cultivated in 250 mL of Medium 271 [19] in 500 mL flasks located in a shaking incubator at 30 °C and 200 rpm. The Medium 271 contains 2 g  $(\text{NH}_4)_2\text{SO}_4$ , 0.5 g  $\text{K}_2\text{HPO}_4$ , 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g KCl, and 0.02 g  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in 1000 mL distilled water. In addition, 45 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 10 g elemental S were used as energy sources for *A. ferrooxidans* and *A. thiooxidans*, respectively. The Medium 271,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and elemental S were sterilized separately, cooled and mixed subsequently.

The acclimation tests were performed prior to bioleaching experiments. In the acclimation tests, 25 mL subcultures of *A.*

*ferrooxidans* and *A. thiooxidans* were separately added in 250 mL of the mine tailing suspension in the Medium 271 (1% solid contents, w/v) in 500 mL Erlenmeyer flasks. The mine tailing samples were autoclaved at 121 °C for 30 min before being suspended in the Medium 271. Then 1% of sterilized elemental S and  $\text{Fe}^{2+}$  were added as energy sources for *A. thiooxidans* and *A. ferrooxidans*, respectively. The flasks were shaken at 200 rpm in a shaking incubator at 30 °C. The pH and redox potential of acclimation tests were monitored periodically and continued until pH of the media dropped below 2.0 and then the inoculums for bioleaching experiments were obtained.

The number of bacterial cells in inoculums was defined by enumerating the colony forming unit (CFU) using the plate count method on a sterile agar in a Petri dish. For *A. thiooxidans* enumeration, *Acidithiobacillus* agar medium [20] was prepared separately and poured into a Petri dish. The *Acidithiobacillus* agar medium contains 0.1 g  $\text{NH}_4\text{Cl}$ , 3 g  $\text{KH}_2\text{PO}_4$ , 0.1 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.1 g  $\text{CaCl}_2$ , 5 g  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , and 20 g technical agar (Becton, Dickinson and Company Sparks, Maryland, USA) in 1000 mL of distilled water, and pH was adjusted to 4.2 before it was autoclaved at 121 °C for 15 min. For *A. ferrooxidans* enumeration, a modified thiobacillus solid medium (TSM1) was prepared [21]. The TSM1 medium is composed of three solutions; Solution A, basal salts: 3 g  $(\text{NH}_4)_2\text{SO}_4$ , 0.1 g KCl, 0.05 g  $\text{K}_2\text{HPO}_4$ , 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.015 g  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , and 0.25 g tryptone soya broth dissolved in this order in 600 mL of distilled water, acidified with 1 M  $\text{H}_2\text{SO}_4$  to pH 2.5 and autoclaved at 121 °C for 15 min and cooled to 60 °C. Solution B: 22 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  dissolved in 150 mL of distilled water acidified to pH 2.5, filter-sterilized and warmed to 60 °C. Solution C: 5 g agarose Biorad High mr 162–0001 in 250 mL distilled water, autoclaved at 121 °C for 15 min and cooled down to 60 °C. Three solutions were mixed and poured on a Petri dish. The inoculums were diluted serially and spread on an agar plate, then the cultures were incubated at 25 °C and the CFUs were counted at the end of the incubation time.

### 2.3. Bioleaching experiments

The bioleaching experiments were conducted in duplicate in 500 mL flasks incubated in a shaking incubator at 30 °C and 200 rpm for 600 h. Eight flasks, each with a working volume of 200 mL containing the Medium 271 with filter-sterilized 45 g/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (9 g/L of  $\text{Fe}^{2+}$ ) for *A. ferrooxidans*, 1% (w/v) sterilized elemental S for *A. thiooxidans* and mixed cultures, and the 5% (w/v) sterile mine tailing sample was inoculated with 10% (v/v) bacterial inoculums. The solid content was chosen in the range which was suggested by Chen and Lin [22]. The inoculums of two bacterial cultures (*A. thiooxidans* and *A. ferrooxidans*) were obtained from an acclimation process with cell concentrations of  $6 \times 10^6$  cells/mL and  $8 \times 10^5$  cells/mL, respectively. An abiotic control experimental set was also carried out without bacterial inoculation. The experimental sets were designed as shown in Table 1. During the experiments, aliquot samples were periodically taken from each flask and pH, redox potential,  $\text{Fe}^{2+}$ , total Fe,  $\text{SO}_4^{2-}$ , soluble As and heavy metals were determined.

### 2.4. Sequential extraction of heavy metals

The sequential extraction procedures proposed by Tessier et al. [23] were performed to determine the binding forms of heavy metals in the mine tailings. The details of extraction steps and target phases are described in Table 2. In order to examine the chemical forms of As solid phases in the mine tailings, a modified procedure suggested by Ahn et al. [24] was employed. The modes of As occurrence in the mine tailings were separated into an ionically bound fraction, strongly adsorbed fraction, amorphous

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