



Possibility of bacterial leaching of antimony, chromium, copper, manganese, nickel, and zinc from contaminated sediment



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ABSTRACT

There is an environmental risk of Sb becoming a major pollutant due to the quick increase in the production of Sb industry. However, there have been few investigations on interactions between Sb and microorganisms and bioremediation of Sb this paper presents an investigation on bioleaching of Sb from contaminated sediment which is collected in the vicinity of a Sb processing plant using the iron-oxidizer, *Acidithiobacillus ferrooxidans*. The bioleaching of Cr, Cu, Mn, Ni, and Zn in the sediment was also investigated to compare with that of Sb. Duplicated batch-type experiments based on different incubation time, on the presence or absence of Fe²⁺ and/or Ag⁺, were conducted to compare the efficiency of metal extraction. After bioleaching, 97.4% of Ni, 95.2% of Mn, 65.8% of Cr, 36.2% of Cu, 34.8% of Zn, and 2.2% of Sb were extracted from sediments. The extraction efficiency of Sb was the lowest in comparison to those of Cr, Cu, Mn, Ni, and Zn. This result indicated that Sb is difficult to solubilize by bacterial leaching. The catalytic effect of Ag⁺ was not observed, which could be due to the inhibition of Ag⁺ on the activity of *A. ferrooxidans* and formation of Ag jarosite precipitation on the surface of sediment particles. Though the extraction of Sb was not efficient, this study demonstrates the potential for Sb to be bioleached and a feasible technique in remediation of Sb.

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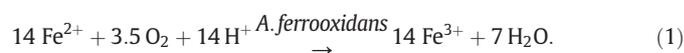
1. Introduction

Antimony is a metalloid that is placed into Group 15 of the periodic table of elements, together with N, P, As, and Bi, because of the identical s²p³ outer orbital electron configurations. It occurs in the environment as several hundred minerals including stibnite (Sb₂S₃), which is the most common mineral form of Sb (Ehrlich and Newman, 2009). It is known to be a clastogenic agent and has carcinogenic properties. There are also claims that it is teratogenic (Léonard and Gerber, 1996). The World Health Organization has set the acceptable concentration for Sb in drinking water at 5 µg·L⁻¹ (W. H. O., 2003). In the past, Sb has been used in large quantities as a flame retarding additive and therapeutic agents against several tropical protozoan diseases, particularly leishmaniasis. The effects of Sb and other heavy metals on human health have been extensively studied (Järup, 2003; Martin and Griswold, 2009). Due to the toxicity of Sb, it is necessary to develop a suitable and economical technology for the treatment of soils and sediments contaminated with Sb and heavy metals from industrial activities.

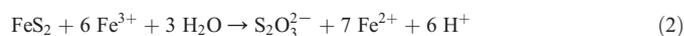
Bioleaching is a process described as the mobilization of elements from solid materials mediated by bacteria and fungi. In bioleaching processes, the solubilization of metals is triggered by microbial oxidation of

Fe²⁺ and sulfur compound. Thereby, the availability of Fe³⁺ and proton, the leaching agents, serves as a significant rationale for bioleaching (Vera et al., 2013). Diverse groups of microorganisms may be the microbial agents in bioleaching process. Of these, *Acidithiobacillus ferrooxidans* is one of the most studied leaching bacteria.

A. ferrooxidans mostly derives energy from the oxidation of Fe²⁺ which serves as an electron donor. The bacterium is also able to grow on reduced inorganic S compounds under anaerobic conditions, using Fe³⁺ as an alternative electron acceptor (Bosecker, 1997). The bacterial cells affect the dissolution of sulfide minerals by two mechanisms which are “contact” and “non-contact” mechanism (Rohwerder et al., 2003). In the non-contact mechanism, the bacteria oxidize dissolved Fe²⁺ to produce Fe³⁺ which serves as a strong oxidizing agent of sulfide minerals. The dissolved Fe³⁺ attacks sulfide minerals following thiosulfate and polysulfide pathways. The non-contact mechanism can be described by the following equations (Boon et al., 1998; Fowler and Crundwell, 1998; Vera et al., 2013):



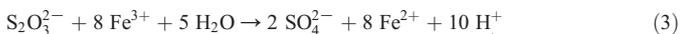
Thiosulfate pathway:



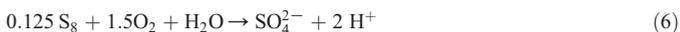
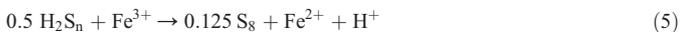
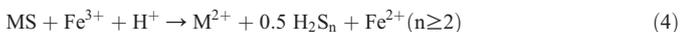
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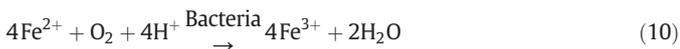
Polysulfide pathway:



where MS is a metal sulfide.

In the case of the contact mechanism, the exopolymers excreted by *A. ferrooxidans* contain complexed Fe^{3+} which mediates bacterial cell attachment onto sulfide minerals and plays the function of oxidative dissolution of sulfide minerals, similar to the role of free Fe^{3+} in the non-contact mechanism (Rohwerder et al., 2003).

Bioleaching is easy to manage and rather inexpensive but the time taken to achieve high metal solubilization under this process is longer when compared with other leaching processes. However, it has been reported that some catalytic ions have potential applications in the bioleaching process. The combination of the addition of various metal ions, such as Ag^+ , Hg^{2+} , Co^{2+} , Bi^{3+} , As^{5+} , Mn^{2+} , and Ru^{3+} , with the use of acidophilic mesophiles could reduce the operation time of bioleaching (Muñoz et al., 2007a,b,c; Yuehua et al., 2002). Among the listed metal ions above, it was noted that Ag^+ had the most positive effect, accelerating the bioleaching process (Yuehua et al., 2002). Accordingly, there have been some studies on the effects of the use of Ag^+ as a catalyst in a bioleaching process (Chen and Lin, 2009; Gómez et al., 1999; Juarez et al., 2006; López-Juárez et al., 2006; Muñoz et al., 2007a,b,c; Sato et al., 2000; Wang et al., 2004). It was known that Ag^+ is toxic to microorganisms; however, *Acidithiobacillus* spp. are reported to tolerate most heavy metals at high concentrations (Gholami et al., 2011). A few hydrometallurgical studies on bioleaching of sulfide minerals using *A. ferrooxidans* in the presence of Ag^+ have been reported previously (Gómez et al., 1999; Zhang et al., 2015). The effect of different concentrations of Ag^+ on the bioleaching of heavy metals from contaminated sediments has also been investigated. The results showed that the high solubilization efficiencies of above 95% were obtained with Cu, Zn, and Ni when $30 \text{ mg} \cdot \text{L}^{-1}$ of Ag^+ s was used (Chen and Lin, 2009). These positive results were explained by the following reactions (Eqs. (7) to (10) (Juarez et al., 2006; López-Juárez et al., 2006; Wang et al., 2004)):



where MeS is a metal sulfide.

However, a higher concentration of Ag^+ than $30 \text{ mg} \cdot \text{L}^{-1}$ might act as an inhibitor instead of a catalyst. In another investigation on bioleaching of low-grade Cu ores, it was shown that smaller additions of Ag^+ dissolved higher amounts of Cu and Fe. The study also mentioned that the adverse toxic effect of Ag^+ acting on the microorganisms delayed the appearance of oxidizing conditions (Muñoz et al., 2007a,b,c).

To date, the bioleaching of As and heavy metals from various contaminated sediments has been intensively studied (Akinci and Guven, 2011; Fonti et al., 2013; Guven and Akinci, 2013; Sabra et al., 2011; Zhu et al., 2014). However, not much information on bioleaching

of Sb has been clarified yet. This study aims to investigate the microbial extraction of Sb and heavy metals, such as Cr, Cu, Mn, Ni, and Zn, from contaminated sediments collected in the vicinity of an Sb processing plant. A set of batch experiments was conducted to compare the efficiency of metal solubilization during the bioleaching processes which used *A. ferrooxidans* as a microbial agent either with or without Fe^{2+} , inorganic nutrients, and Ag^+ . The partitioning of Sb, Cr, Cu, Mn, Ni, and Zn in the sediment and the residues after bioleaching was also elucidated by a scheme of sequential extraction.

2. Materials and methods

2.1. Sediment characterization

The sediments used in this study were collected at a point where the water is 0.5-m deep at the junction of the drainage channel of an Sb processing plant and natural freshwater stream in Chungnam, Korea. Prior to experiments, all gravels and litter were removed from these sediments by screening, and then they were transported to laboratory and kept at 4 °C. The pH of the sediments was measured using a combination electrode connected to a Thermo Orion pH & mV Meter model 230A+ (Thermo Electron Corp., USA). For routine work, 5 g of sediment and 25 mL of distilled water (1:5, sediment to water ratio) were added to a 50 mL beaker and stirred vigorously. The mixture was kept standing without agitation for 2 h and then it was stirred well immediately before the electrodes were immersed and the pH was measured. The water content and organic matter content of the sediment were determined by loss-on-ignition analysis (Dean, 1974; Heiri et al., 2001; Santisteban et al., 2004). Briefly, the sediment was heated at 105 °C for 4 h and 450 °C for 6 h to estimate the water and organic matter content, respectively. An amount of sediment was air-dried and screened with an 80-mesh (0.18 mm) sieve for chemical analysis and for bioleaching experiments. The sediment samples were digested by aqua-regia ($\text{HCl}:\text{HNO}_3 = 3:1, \text{v/v}$), and then analyzed for their total Sb and heavy metal contents by inductively coupled plasma-optical emission spectrometry (ICP-OES 7000 series; Thermo Scientific Corp., USA). Prior to bioleaching experiments, the sediments were sterilized by autoclaving at 121 °C for 30 min.

2.2. Microorganisms and inocula

A culture of *A. ferrooxidans* (KCTC 2677) was obtained from the Microbial Geochemistry Laboratory, Chonnam National University, Korea. This bacterial culture was found to be able to obtain the early stage of exponential phase after 30 h from the start of incubation. Bacteria cells were cultivated in 500 mL of a 9 K medium (Silverman and Lundgren, 1959) in a 1-L Erlenmeyer flask incubated in a shaking incubator at 30 °C and 200 rpm. The 9 K medium consisted of two solutions. Solution A contained 3 g $(\text{NH}_4)_2\text{SO}_4$, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g KCl, 0.02 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and 0.5 g K_2HPO_4 in 700 mL of distilled water. Solution B contained 45 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 300 mL of distilled water. Before the two solutions were mixed, solution A was autoclaved while solution B was filter-sterilized. The solution A and solution B provided inorganic nutrients and energy sources to the bacteria growth, respectively. The bacterial cultures were subcultured every week by inoculating 50 mL of inocula into 450 mL of culture media. The bacterial inocula for bioleaching experiments were obtained from the flask after a 48-hour preculture.

The bacterial growth in the inocula was quantified by counting the viable colony-forming units (CFUs) on agar plate. A modified thiobacillus solid medium (TSM1) was prepared upon the procedure described by Visca et al. (1989). The inocula were diluted serially and spread on TSM1 agar plate, and then the plates were incubated at 25 °C. The CFUs were counted after a 2-week incubation.

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