Possibility of bacterial leaching of Antimony, Chromium,
Copper, Manganese, Nickel, and Zinc from contaminated
sediment

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Abstract

The environmental risk of antimony (Sb) becoming a major pollutant may soon be real due to the quick increase in production of Sb industry. However, the investigations on interaction between Sb and microorganisms and bioremediation of Sb are still rare. To our knowledge, this paper is the first investigation on bioleaching of Sb from contaminated sediment which is collected in the vicinity of a Sb processing plant using an iron-oxidizer, *Acidithiobacillus ferrooxidans*. The bioleaching of Chromium, Copper, Manganese, Nickel, and Zinc in the sediment was also investigated to compare with that of Sb. Duplicated batch-type experiments based on different incubation time, media with and without ferrous ion and in the presence and absence of silver ion, 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 an element which is definitely difficult to solubilize by bacterial leaching. The catalytic effect of silver ion was not observed when compared to a set-up in its absence, which could possibly be due to the inhibition of silver ion on the activity of *A. ferrooxidans* and formation of silver jarosite precipitation on the surface of sediment particles. Though the extraction of Sb was not efficient, this study contributed a meaningful literature on the response of Sb to the bioleaching and generated necessary motivation in looking for a feasible technique in remediation of Sb.

Keywords: Antimony; Metal partitioning; Contaminated sediment; *Acidithiobacillus ferrooxidans*

1. Introduction

Antimony (Sb) is a metalloid that is grouped into Group 15 of the periodic table of elements, together with nitrogen (N), phosphorus (P), arsenic (As) and bismuth (Bi), because of the identical s^2p^3 outer orbital electron configuration. It occurs ubiquitously in the environment as stibnite (Sb_2S_3), which is the most common mineral form (Ehrlich and Newman, 2009). It was known to be a clastogenic agent, and has carcinogenic properties as well. There were also claims that it is teratogenic, but not much research has been done on this effect to prove that it is true (Léonard and Gerber, 1996). The World Health Organization has set the acceptable concentration for Sb in drinking water at $5 \mu\text{g}\cdot\text{L}^{-1}$ (W. H. O., 2003). For years, Sb has mostly been used in large quantities as a flame retarding additive and therapeutic agents against several tropical protozoan diseases, particularly leishmaniasis. Heavy metals have been used by humans for thousands of years. Their effects on human health have been intensively studied (Järup, 2003; Martin and Griswold, 2009). Antimony and heavy metals released from Sb-producing factories could deposit in soil, sediment, and water, resulting in the increase of the concentration of Sb in surrounding environment of processing plant, especially in the sediments. It is, therefore, necessary to develop a suitable and economical technology for the treatment of sediments contaminated with Sb and heavy metals.

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 Fe^{2+} and sulfur compound. Thereby, the availability of Fe^{3+} and proton, the leaching agents, serves as a significant rationale for bioleaching (Vera et al., 2013). Diverse groups of microorganisms were found to be the microbial agents in bioleaching process. *Acidithiobacillus ferrooxidans* is one of the most studied leaching bacteria belong to the genera of *Acidithiobacillus*.

A. ferrooxidans mostly derives energy from the oxidation of Fe^{2+} which serves as an electron donor. The bacterium is also able to grow on reduced inorganic S compounds under anaerobic condition, using Fe^{3+} 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 Fe^{2+} to produce Fe^{3+} which serves as a strong oxidizing agent of sulfide minerals. The dissolved Fe^{3+} attacks sulfide minerals following thiosulfate and polysulfide pathway. Overall non-contact mechanism can be described by the following equations (Boon et al., 1998; Fowler and Crundwell, 1998; Vera et al., 2013):

Thiosulfate pathway:

Polysulfide pathway:

where MS is a metal sulfide

In the case of 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 to other leaching processes. However, it has been reported that some catalytic ions have potential application 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, 2007b, 2007c; Yuehua et al., 2002). Among the listed metal ions above, it was noted that Ag ions 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 ions as a catalyst in a bioleaching process (Chen and Lin, 2009; Gómez et al., 1999; Juárez et al., 2006; López-Juárez et al., 2006; Muñoz et al., 2007a, 2007b, 2007c; 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 sulphide minerals using *A. ferrooxidans* in the presence of Ag ion have been reported previously (Gómez et al., 1999; Zhang et al., 2015). The effect of different concentrations of Ag ion 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 ions were used (Chen and Lin, 2009). These positive results were explained by the following reactions (Eqs. (7) to (10) (Juárez et al., 2006; López-Juárez et al., 2006; Wang et al., 2004)):

where MeS is a metal sulphide.

However, a higher concentration of Ag ions 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, 2007b, 2007c).

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 ions. 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 was 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 litters 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 Thermo Orion pH

& mV Meter model 230A+ (Thermo Electron Corp., USA) according to manufacturer's instructions. 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 hours 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 ($\text{HCl} : \text{HNO}_3 = 3 : 1$, 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 9K medium (Silverman and Lundgren, 1959) in a 1-L Erlenmeyer flask incubated in a shaking incubator at 30°C and 200 rpm. The 9K 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 $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 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 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 2-week incubation.

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 500 hours. Twelve flasks, each with a working volume of 200 mL containing 9K and a sterile sediment sample, were inoculated with 10% of inoculum (7×10^5 CFU·mL⁻¹). The solid concentration (5%, w/v) was chosen in the range which was suggested by Chen and Lin (2010). Silver ion (30 mg·L⁻¹) was added as an enhancement agent (Chen and Lin, 2009). The experimental sets were designed as shown in Table 1.

During the experiment, aliquot samples (2 mL) were periodically taken from each flask for determining pH, redox potential, Fe²⁺, total Fe, soluble Sb, Cr, Mn, Ni, Cu, and Zn. For experimental sets with added Ag ion, ABfAg and ABaAg, filtered sample were additionally diluted for determining Ag ion concentration during the bioleaching process. The redox potential and pH were measured by using a combination electrode connected to Thermo Orion pH & mV Meter model 230A+ (Thermo Electron Corp., USA). The Fe²⁺ concentration was

determined by a modified ferrozine method (Stookey, 1970). Total Fe, Ag, Sb, Cr, Mn, Ni, Cu, and Zn were determined using inductively coupled plasma mass spectrometry (ICP-MS 7700 series; Agilent Technologies, USA) calibrated with blank solution and 5 standard solutions of metals (10 µg/L, 50 µg/L, 100 µg/L, 500 µg/L, and 1000 µg/L).

2.4 Metal partitioning

A selective sequential extraction procedure proposed by Tessier et al. (1979) were performed to determine the partitioning of Sb, Cr, Mn, Ni, Cu, and Zn in the sediment before and after the leaching. The details of extraction steps are as follows.

For exchangeable fraction, the sediment sample was extracted by MgCl₂ (0.5 M; pH 7.0), and was continuously agitated at 150 rpm for 1 hour at room temperature. For carbonate and specifically adsorbed fraction, the residue from the last step was extracted by CH₃COONa (1 M) buffered to pH 5.0 with CH₃COOH and was continuously agitated at 150 rpm for 5 hours at room temperature. For Fe-Mn oxides bound fraction, the residue from the earlier step was leached by NH₂OH·HCl (0.04 M) in CH₃COOH (25%; v/v), and was occasionally agitated for 6 hours at 95°C. For organic matter and sulfides bound fraction, the residue from the previous step was extracted by HNO₃ (0.02 M) and H₂O₂ (30%; adjusted to pH 2.0 with HNO₃) and was occasionally agitated for 2 hours at 85°C. Then, H₂O₂ (30%; adjusted to pH 2.0 with HNO₃) was added into the same tube and occasionally agitated for 3 hours at 85°C. Finally, CH₃COONH₄ (3.2 M) in HNO₃ (20%) was added into the tube. For residual fraction, finally, the residue from the last step was digested by aqua-regia (HCl : HNO₃ = 3 : 1, v/v).

The procedure started with 1 g of a dry sediment sample. Between each successive extraction, separation was done by centrifugation at 15,000 rpm for 15 minutes. The supernatant was filtered by 0.45-µm Whatmann nylon syringe filter, and then introduced to

ICP-OES for determining Sb and heavy metal concentrations. Before carrying out the next extraction, the residue was washed twice with distilled water and centrifuged for 15 minutes, and this supernatant was discarded.

3. Results and discussion

3.1 Physicochemical properties of the sediment

The general physiochemical properties of the sediment are given in Table 2. The pH measurements confirm the neutral conditions of the sediment. The water content was determined as 29.1%. The organic matter content of the sediment is quite low, i.e., approximately 1.9%. To assess the level of contamination, total concentrations of Sb and heavy metals were compared to the value of soil samples which was collected by Aydinalp and Marinova (2003) and Korean uncontaminated soil used as controls (Jung, 2008; Jung et al., 2002). As shown in Table 2, all concentrations of heavy metals in this sediment are above natural value and uncontaminated soil, especially in the case of Sb. Although no legal regulations exist at the present time for limiting the Sb concentration in sediments, it is noted that contamination of Sb in this sediment is very high compared to natural values and uncontaminated soils.

3.2 Variation of pH and redox potential during bioleaching

The pH values in all experimental sets reached steady state after 200 hours (Fig. 1a). The experimental set ABf, normal bioleaching experiment, obtained the lowest pH at pH 1.71 after 500-hour incubation. The pH decrease during experiment can be explained by the equations (2) – (6). In the case of polysulfide pathway, though proton was consumed through Eq (3), the total process still produces proton which controls acidification of bioleaching

system. In addition, proton was also released by the co-precipitation of Fe^+ , a product from chemical and biological oxidation of Fe^+ , in various forms of jarosites and insoluble products (Fonti et al., 2013). The pH of experimental set without Fe^{2+} addition was higher compared to other sets and quite stable during incubation. It revealed that the addition of Fe^{2+} is a key factor for acidification of bioleaching system. This kind of results was also observed in the previous study (Fonti et al., 2013). The similarity of pH values of experimental sets inoculated with Ag ion, and the distinct difference between those of systems with and without Ag ion exposed the inhibition of Ag ion with *A. ferrooxidans* activity or acidification during incubation time.

Besides the pH reduction, the oxidation of Fe^{2+} caused an increase in the redox potential of the media during the bioleaching process due to the production of highly oxidative Fe^{3+} . The variations of redox potential during the experiment for various experimental sets are shown in Fig. 1b. In the relation to pH propagation, the redox potential also reached steady state after 200-hour incubation. The maximum redox potential value of set ABf reached 570 mV after 164 hours, and then decreased slightly. The high redox potential of bioleaching systems with and without inorganic nutrients was mainly due to bacterial oxidation of Fe^{2+} to Fe^{3+} and the maintenance of the dissolved state of Fe^{3+} and high ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ in the solution (Lee et al., 2005). In the case of experimental set Af, Fe^{2+} was not provided during the experiment but the initial redox potential value was quite high and it was stable during incubation time. That might be due to the readily redox-controlling chemical pairs in the solution (Lee et al., 2005). The similarity in the redox potential values of the abiotic controls with and without Ag ion, which were not initially inoculated with the bacterium, to those of bioleaching system with Ag ion once again substantiated the suppression of Ag ion with the bacterial growth.

3.3 Fe^{2+} oxidation by *A. ferrooxidans*

As shown in Eqs. (2) and (4), Fe^{3+} , a strong oxidant, can remarkably mediate metal solubilization from sediment, especially from metal sulfides. The aqueous Fe^{3+} is extremely unstable in every condition because it promptly formed Fe oxides or hydroxides and is removed from solution by precipitation. Therefore, the production and sustentation of dissolved Fe^{3+} in the leaching media are the essential conditions for effective bioleaching processes. In Fig. 2, the disparity between the input amount of Fe^{2+} ($9\text{ g}\cdot\text{L}^{-1}$) and the total dissolved Fe which was observed at the given time stands for the amount of precipitated Fe. Likewise, the difference between total dissolved Fe in solution and aqueous Fe^{2+} represents the amount of aqueous Fe^{3+} . In the case of normal bioleaching (experimental set ABf), the Fe^{2+} initially added to flask appeared to be rapidly oxidized to Fe^{3+} and approximately $2\text{ g}\cdot\text{L}^{-1}$ of Fe^{3+} was maintained in the medium for a long period. In comparison, when the bacteria were not inoculated into the media (experimental set ABa and AB), a significant amount of Fe^{2+} (approximately $4\text{ g}\cdot\text{L}^{-1}$) still existed in the media until the end of incubation. Those results indicated a lower capacity of those cases than set ABf for extracting heavy metals from the sediment. An amount of Fe^{2+} in the abiotic controls could be oxidized to Fe^{3+} by oxygen in the air without a bacterial catalyst under the experimental condition. The bioleaching system without inorganic nutrients showed the highest production of dissolved Fe^{3+} (approximately $4\text{ g}\cdot\text{L}^{-1}$) and it was maintained until the end of this experiment. Such a result revealed that the reduction of inorganic nutrients supplied to bioleaching of the sediment is likely possible. In the case of bioleaching system to which Ag ion was added, the given results showed the similar trend to the experimental sets without bacterial inoculation.

The presence of Ag ion might inhibit the oxidation of Fe^{2+} by *A. ferrooxidans*. This kind of inhibition was reported previously (De et al., 1997, 1996; Imai et al., 1975). The oxidation of Fe^{2+} by *A. ferrooxidans* could be delayed 3 days in the presence of $10\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ (1.08

mg·L⁻¹) Ag ion (Imai et al., 1975). A concentration of 100 µmol·L⁻¹ (10.8 mg·L⁻¹) Ag ion could inhibit 80% Fe²⁺ oxidizing activity of *A. ferrooxidans* and the increase of Ag concentration to 1 mmol·L⁻¹ (108 mg·L⁻¹) could inhibit up to 90% Fe²⁺ oxidizing activity of *A. ferrooxidans* (De et al., 1997, 1996; Imai et al., 1975). The inhibition of Ag ion on Fe²⁺ oxidation activity by *A. ferrooxidans* was attributed to a mixed mechanism in which Ag⁺ replaced Fe²⁺ in the active site of the oxidizing enzyme (De et al., 1996; Dopson, 2003).

3.4 Bacterial extraction of Sb, Cr, Mn, Ni, Cu, and Zn

The removal of Sb, Cr, Mn, Ni, Cu, and Zn from the sediment has been expressed, at each sampling time, as an increase of metal solubilization efficiencies in the media from the beginning of the experiment to the last sampling time (Fig. 3). The greatest solubilization efficiency was obtained from standard bioleaching system without Ag ion. It was observed that the highest removal efficiencies (>95%) was obtained for Mn and Ni, whereas the extraction efficiency of Sb was very low (2.2%) compared to other metals. The extraction efficiency of metals from the sediment was in the decreasing order as follows, Ni > Mn > Cr > Cu > Zn > Sb. All the heavy metals including Cr, Mn, Ni, Cu and Zn obtained the highest solubilization from the standard bioleaching system without Ag ion, and the lowest from the leaching system which was not added with Fe²⁺. Such results again affirmed the importance of Fe²⁺ addition to metal solubilization in bioleaching processes. The high pH value of the bioleaching system without Fe addition also inhibited the solubilization of metals though its redox potential was higher than the others. It is because Fe plays an important role in acidification of bioleaching system. These conclusions of this study entirely coincided with the results of an investigation on metal bioleaching of contaminated marine sediment (Fonti et al., 2013). In fact, pH directly affects metal solubilization during bioleaching processes of contaminated sediment (Rohwerder et al., 2003). The significance of pH to metal

solubilization in bioleaching of river sediment was quantitatively investigated previously (Chen and Lin, 2001a). However, the linear relationship between pH and metal solubilization was not recommended. Metal solubilization efficiencies are not only mediated by pH, but they are also affected by other factors such as oxidative condition, metal partitioning, and specific metal properties, etc. The influence of metal partitioning on metal solubilization efficiency would be discussed in the next part of this report.

The behavior of Sb under various leaching condition was totally different with other metals. The lowest solubilization of Sb occurred in experimental set Bf which was not supplied with inorganic nutrients (solution A). The solubilization of Sb in the experimental set ABf reached the maximum value after approximately 100 hours, had the stationary phase in a short time, and then decreased continuously. The decrease of Sb concentration was most likely due to the re-precipitation of aqueous Sb under low pH, high redox potential condition (Filella et al., 2002). However, further investigations in the future are necessary to figure out these mechanisms. The extraction of Sb in the absence of Ag ion increased continuously from the beginning of the experiment to the end of incubation time and obtained the highest extraction efficiency compared to other experiment sets. These result demonstrated a contrast in the bioleaching mechanism of Sb with that of other metals.

The similarity between the solubilization curves of Mn and Ni during leaching processes indicated that the extraction of Mn and Ni could be occurred with the same mechanism. The extraction efficiency of Mn and Ni was virtually equivalent in all the experimental sets except the set Af, to which no Fe^{2+} was supplied. Without Fe^{2+} addition, 45.3% of Mn was extracted, whereas only 27.8% of Ni was solubilized from sediment. It could be inferred that Mn and Ni was effectively solubilized at a pH under 3.0 (Fig. 1).

The addition of Ag ion to the leaching process did not significantly enhance the metal extraction efficiencies (Fig. 3). In the abiotic controls (experimental sets ABAg and AB), the

addition of Ag ion only enhanced the Sb extraction, but not significantly. In contrast, in the biotic leaching with *A. ferrooxidans* (experimental sets ABfAg and ABf), the addition of Ag ion inhibited the extraction of Cr, Cu, and Zn. The difference in extraction efficiency of Sb, Cr, Cu, and Zn between the biotic leaching system with Ag ion and abiotic leaching with Ag ion during the incubation time indicated that the bacterial activities were not totally disabled in the presence of Ag ion. A detailed monitoring of bacterial growth, i.e. bacterial cell concentration, during bioleaching experiments should be conducted to comprehensively understand the inhibition of Ag ion on the growth of *A. ferrooxidans*. Indeed, Silver ion was reported to be toxic to *A. ferrooxidans* (De et al., 1996; Guay et al., 1989; Imai et al., 1975; Norris and Kelly, 1978). The growth of *A. ferrooxidans* was delayed 2 days in the presence of $10\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ($1.08\text{ mg}\cdot\text{L}^{-1}$) Ag ion (Imai et al., 1975). The addition of $100\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ($10.8\text{ mg}\cdot\text{L}^{-1}$) Ag ion to 9K medium could inhibit 90% of *A. ferrooxidans* growth, and the increase of Ag concentration to $1\text{ mmol}\cdot\text{L}^{-1}$ ($108\text{ mg}\cdot\text{L}^{-1}$) could almost completely inhibit the growth of *A. ferrooxidans* (De et al., 1997, 1996; Imai et al., 1975). In contrast, *A. ferrooxidans* was observed to be tolerant to $1\text{ mmol}\cdot\text{L}^{-1}$ ($108\text{ mg}\cdot\text{L}^{-1}$) Ag ion after acclimation (Ehrlich, 1984). The addition of Ag ion to the bioleaching process might even cause a slight enhancement of bacterial growth (Gómez et al., 1997, 1999). The bioleaching experiment in this study was conducted without any procedure to acclimate *A. ferrooxidans* to Ag ion. This could be a potential limitation that caused an adverse effect of Ag ion on bioleaching. In addition, the sludge of sediment in bioleaching system was usually supposed to have an inhibiting effect on the growth of acidophilic chemoautotrophic bacterial strains. The total of free Ag ion in the media during the experimental process was lower than $10\text{ }\mu\text{g}\cdot\text{L}^{-1}$. It might suggest that Ag jarosite ($\text{AgFe}_3(\text{SO}_4)_2\cdot(\text{OH})_6$) formed and precipitated on the surface of particles in the bioleaching system (Chen and Lin, 2009; Gómez et al., 1999). Thus, the removal of heavy metals from sediment was inhibited. The bioaccumulation of Ag by *A. ferrooxidans* was

observed previously by Pooley (Pooley, 1982). Nevertheless, this process was believed not to be a potential reason for the significant decrease of Ag ion in the medium because the bacterial accumulation of metal ion is considerably low.

3.5 Variation of metal partitioning in the sediment before and after bioleaching

The sequential extraction study revealed that most of the studied metals including Cr, Ni, Cu, and Sb exist in the sediment as residual fraction (over 50% of mass total), especially in the case of Sb with 97.1 % of its total as residual fraction (Fig. 4). It could be said that Sb was the most immobile metal in the sediment. The metals investigated in the sediment can be ranked according to a decreasing mobility order as follows, $Mn > Zn > Cu > Ni > Cr > Sb$. This ranking is little different with the ranking of solubilization efficiencies. The residual fraction reflects the natural geochemical background of the sediment (Guvén and Akinci, 2008; Yuan et al., 2004; Zhang et al., 2014). The predominance of a metal in the sediment could be explained by the metal loading. The higher and the former were the metal disposed into surrounding environment, the greater is the tendency to have high residual metal fraction. Manganese seemed to accumulate mostly as Fe-Mn oxides bound fraction (47.8% of mass total), whereas most of the Zn (52.4% of mass total) was found as the organic matter/sulfides bound fraction and Fe-Mn oxides bound fraction. Most of the Cu (57.6% of mass total) was also found in the residual fraction but the amount of Cu in the organic matter and sulfide bound fraction was in the highest ratio when compared with the other studied metals. The residual fraction of Cr represented 75.0%, whereas that of Ni represented 66.7%.

Generally, the metal solubilization efficiency in the bioleaching process appears to be depending on the specific partitioning of metals in the sediment. The metals in exchangeable fraction, carbonate bound and specifically adsorbed fraction, and Fe-Mn oxides bound fraction, are considered to be easier to solubilize and bioavailable. On the contrary, the

organic matter and sulfides bound and residual fraction are stable and non-bioavailable (Perin et al., 1997). The removal efficiency of Sb was the lowest because only 2.6% of Sb was defined as labile fraction. In this study, the bioleaching process could solubilize Cr, Cu, Mn, Ni, and Zn in labile fraction and a part of stable fraction. The stable fraction of metals in the sediment was most likely to be solubilized through the edge dissolution of some acid-sensitive minerals and the release of soluble metal sulfate complexes, of which the ionic spheres are subjected to a minor less steric hindrance (Fonti et al., 2013). The results also revealed that bioleaching could not extract any Sb from residual Sb fraction. It indicated that the residual fraction of Sb might be more sustainable under bioleaching condition than the residual fraction of other metals. In this regard, the behavior of Sb in bioleaching system was entirely different from that of As. A great amount of As was reported to be able to release from residual fraction in a bioleaching with *A. ferroxidans* (Nguyen et al., 2015).

In order to confirm the efficiency of bioleaching, the sequential extraction was conducted to investigate the partition of metals in final residues. The distribution of metals after bioleaching represents not only the efficiency of bioleaching but also the stability and bioavailability of metals remained in the sediment after bioleaching. The sequential extraction results of the final residues indicated that the bioleaching had a significant impact on changes in partitioning of Sb, Cr, Mn, Ni, Cu, and Zn in the residues (Fig. 4). After bioleaching, the metal contents in the residual sediment are relatively low, except for Cu, Zn and Sb. The remainders of Cr, Mn, Ni, Cu, and Sb in residues of standard bioleaching were indirectly evaluated to represent as stable and non-bioavailable forms, whereas an amount of Zn (8.8%) which represents as Fe-Mn oxides bound fraction could still not be removed by bioleaching. Notably, the abiotic control could even extract all amounts of Cr, Mn, Ni, Cu, and Sb which existed in sediment as mobile forms.

In comparison to the previous studies on bioleaching of heavy metals from sediments (Chen and Lin, 2009, 2004, 2001a, 2001b), the extraction efficiencies of Cr, Cu, and Zn were relatively low. This is due to the partitioning of Cr, Cu, and Zn in the sediments. The residual fractions of these heavy metals in the sediment of this study represented more than 50% of their total concentration, which are much more than those in the sediment of previous studies (Chen and Lin, 2009, 2004).

4. Conclusions

Even though Sb occurs in the same group with As in the periodic table of elements, the response of Sb during the bioleaching process was totally different. Only 2.2 % of total Sb was extracted, which is the lowest compared to the solubilization of other metals, such as Ni (97%), Mn (95.2%), Cr (65.8%), Cu (36.2%), and Zn (34.8%) in this sediment. Besides the specific partitioning of metals in the sediment, other factors such as acidification, oxidative condition maintenance, and specific metal characteristics are the important factors that directly influence the metal solubilization in this bioleaching system. The application of Ag ion which is well-studied as a strong catalytic agent for enhancing extraction efficiency of Sb has not lived up to our expectation. The result indicated that Sb contaminated in the sediment is definitely difficult to extract by microbial leaching. Therefore, the study to find another method for extracting Sb from the sediment is necessary. Though Zn, Cu, and Sb were not extracted efficiently by bioleaching, this study is a meaningful contribution to the literature on the behavior of Sb in the bioleaching condition.

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Figure captions

Fig. 1 Variation of pH (a) and redox potential (b) in different experimental sets during the bioleaching processes. ABfAg: standard bacterial leaching with Ag ion; ABf: standard bacterial leaching without Ag ion; Af: bacterial leaching without Fe^{2+} ; Bf: bacterial leaching without inorganic nutrients; ABAg: abiotic control with Ag ion; AB: abiotic control without Ag ion.

Fig. 2 Variation of total dissolved Fe and Fe^{2+} concentrations during leaching processes. (a) standard bacterial leaching with Ag ion (experimental set ABfAg); (b) standard bacterial leaching without Ag ion (experimental set ABf); (c) bacterial leaching without inorganic nutrients (experimental set Bf); (d) abiotic control with Ag ion (experimental set ABAg); (e) abiotic control without Ag ion (experimental set AB).

Fig. 3 Solubilization efficiencies of Sb (a), Cr (b), Cu (c), Mn (d), Ni (e), and Zn (f) in the different experimental sets during the bioleaching processes. ABfAg: standard bacterial leaching with Ag ion; ABf: standard bacterial leaching without Ag ion; Af: bacterial leaching without Fe^{2+} ; Bf: bacterial leaching without inorganic nutrients; ABAg: abiotic control with Ag ion; AB: abiotic control without Ag ion.

Fig. 4 Variation in partitioning of Sb (a), Cr (b), Cu (c), Mn (d), Ni (e), and Zn (f) in the initial sediment and the residues after bioleaching. Initial: the sediment before leaching experiment; ABfAg: standard bacterial leaching with Ag ion; ABf: standard bacterial leaching without Ag ion; Af: bacterial leaching without Fe^{2+} ; Bf: bacterial leaching without inorganic nutrients; ABAg: abiotic control with Ag ion; AB: abiotic control without Ag ion.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Tables

Table 1

The experimental sets employed in this study

Run No.	Sediment	Inorganic nutrient [*]	Ferrous ion ^{**}	Bacterial inoculum	Silver Ion
ABfAg	5%	Yes	Yes	Yes	30 mg·L ⁻¹
ABf	5%	Yes	Yes	Yes	No
Af	5%	Yes	No	Yes	No
Bf	5%	No	Yes	Yes	No
ABAg	5%	Yes	Yes	No	30 mg·L ⁻¹
AB	5%	Yes	Yes	No	No

^{*}Inorganic nutrient was supplied by Solution A containing 3 g·L⁻¹ of (NH₄)₂SO₄, 0.5 g·L⁻¹ of MgSO₄·7H₂O, 0.1 g·L⁻¹ of KCl, 0.02 g·L⁻¹ of Ca(NO₃)₂·4H₂O, and 0.5 g·L⁻¹ of K₂HPO₄.

^{**}Ferrous ion was supplied by Solution B containing 45 g·L⁻¹ of FeSO₄·7H₂O (equivalent to 9 g·L⁻¹ of Fe²⁺)

Table 2

Characteristics of the sediment used in this study

Parameters	Value (mean $\pm$ S.D [*] , n=3)	Natural soil (Aydinalp and Marinova, 2003)	Uncontaminated soil in Korea (Jung, 2008; Jung et al., 2002)
Water content (%)	29.13 $\pm$ 2.17	-	-
Loss-on-ignition (%)	3.15 $\pm$ 0.32	-	-
Organic matter (%)	1.94 $\pm$ 0.27	-	-
Organic carbon (%)	1.13 $\pm$ 0.16	-	-
pH	6.98 $\pm$ 0.19	-	-
Fe (%)	4.84 $\pm$ 0.07	2.1	-
Cr (mg·kg ⁻¹)	65.95 $\pm$ 3.69	6.3	6.3 - 54
Mn (mg·kg ⁻¹)	789.32 $\pm$ 6.99	320	320 - 850
Ni (mg·kg ⁻¹)	52.32 $\pm$ 2.28	17	17 - 40
Cu (mg·kg ⁻¹)	65.93 $\pm$ 0.90	15	20 - 38
Zn (mg·kg ⁻¹)	366.91 $\pm$ 3.52	36	83 - 111
Sb (mg·kg ⁻¹)	3056.25 $\pm$ 52.30	2	0.9 - 1.5

^{*}S.D.: Standard Deviation