

혼합 호산성 박테리아를 이용한 광미로부터 비소의 Column Bioleaching : 기술적 평가

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Column Bioleaching of Arsenic from Mine Tailings Using a Mixed Acidophilic Culture: A Technical Feasibility Assessment

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요 약

광미의 처리를 위한 heap bioleaching은 유망 기술이나 이 과정을 오랜 기간 수행한 연구는 부족한 상황이다. 본 연구는 약 436 일 동안의 column bioleaching 실험을 통해, 광미에서 고농도 비소의 제거특성을 평가하였다. 높이 350 mm의 플라스틱 column에 750 g의 광미와 *A. thiooxidans* 및 *A. ferrooxidans*로 구성된 호산성 박테리아를 접종하여 실험을 진행하였다. 비소 제거 효율을 확인하고, 그 관련 기작을 이해하고자 침출액의 산화·환원전위와 pH, 액중 철 이온(ferrous와 ferric)의 생성 및 비소 농도를 측정하였다. 실험 436일 후, 비소의 제거율을 약 70%까지 달성 할 수 있었다. 하지만, 부분적으로 침출율이 감소하는 경향을 확인할 수 있었고, 이는 비소의 독성으로 인한 박테리아 군집의 활성도 저하에 의한 것으로 판단되었다. 본 연구의 결과는 향후 광미의 heap bioleaching 연구를 위한 기초 자료로서 활용될 수 있을 것으로 기대된다.

주제어 : 광미, column bioleaching, heap bioleaching, 비소 독성

Abstract

Heap bioleaching for detoxification of mine tailings is a promising technology; however, long-term studies that aim to understand the potential of this process are scarce. Therefore, this study assesses the feasibility of column bioleaching as an alternative technology for treatment of mine tailings with high concentrations of arsenic during a long-term experiment (436 days). To accomplish this objective, we designed a 350-mm plastic column that was packed with 750 g of mine tailings and inoculated with an acidophilic bacterial culture composed of *A. thiooxidans* and *A. ferrooxidans*. Redox potential, pH, ferric ion generation, and arsenic concentration of the off-solution were continuously monitored to determine the efficiency of the technology. After

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436 days, we obtained up to 70% arsenic removal. However, several drops in removal rates were observed during the process; this was attributed to the harmful effect of arsenic on the bacteria consortium. We expect that this article will serve as a technical note for further studies on heap bioleaching of mine tailings.

Key words : mine tailings, column bioleaching, heap bioleaching, arsenic toxicity

1. Introduction

Heap bioleaching is the most accepted technique for microbial processing of metals because it requires few complex processes and operation costs are low. This technique is primarily based on the recovery of metals from agglomerated mineral ores that are irrigated with an acid solution containing leaching microorganisms. The role of these microorganisms, primarily iron oxidizers such as acidophilic and mesophilic bacteria, is to produce strong oxidizing agents by transforming the available ferrous iron into ferric iron. The leaching solution solubilizes the metals while it percolates through the heap. In industrial applications, the principal target metal recovered using this technology is copper. Statistics show that approximately 7% of the copper produced worldwide is obtained through heap bioleaching.¹⁾ This process has been proven to achieve extraction efficiencies comparable to other technologies that use chemical leaching for metal extraction. Other metals, such as nickel, cobalt, and zinc, are also recovered using this technology.²⁻⁵⁾

The potential application of heap bioleaching to recover metals from unconventional sources is part of the scientific conversation worldwide because this technique responds to the need to optimize a reliable and cost effective remediation technology. For example, heap bioleaching is being studied as a way to decontaminate mine tailings with high concentrations of heavy metals, particularly arsenic. In this attempt, some studies have claimed recovery rates greater than 60% of arsenic after approximately 100 days of experiments. However, the available literature regarding long-term experiments to purify mine tailings with high arsenic concentrations is scarce.⁶⁻⁸⁾

Therefore, we tested the potential application of heap bioleaching for the detoxification of mine tailings with high arsenic content by studying the removal rates during

long-term experiments. We designed and built a lab-scale column that was monitored over 436 days. The column was packed with mine tailings with approximately 12,000 mg/kg of arsenic and was inoculated with an unadapted mesophilic bacterial consortium composed of *A. thiooxidans* and *A. ferrooxidans*. Scholars have proven that a consortium composed of iron and sulphur oxidizing bacterial species increment the leaching efficiency of pyritic minerals.⁹⁻¹⁰⁾ The role of iron oxidizing bacteria is to produce the necessary ferric ion to oxidize the mineral. Whereas, sulphur oxidizing bacteria avoid the formation of a passivation layer on the mineral surface through the accumulation of elemental sulphur.^{3,11)} The column was aerated and irrigated at constant rates during the entire process. Ferrous to ferric oxidation rates, solution pH, sulfate production, and arsenic removal were analyzed to obtain insights into the phenomena involved in column bioleaching of mine tailings. To the best of our knowledge, this is the first attempt made to study long-term column bioleaching of arsenic from mine tailings in South Korea.

2. Materials and Methods

2.1. Microorganisms and Medium

A consortium composed of *A. thiooxidans* (KCTC-4515) and *A. ferrooxidans* (KCTC-4516) was selected for the bioleaching experiments. The strains were provided by the Korea Research Institute of Bioscience and Biotechnology. Each bacterial strain was independently cultivated under aerobic conditions in 500 ml flasks with 200 ml of culture medium. The flasks were incubated in a shaking incubator at 150 rpm at 25°C. DSMZ medium 882 was selected as the culture medium and formulated with (NH₄)₂SO₄, 132.0 mg/L; MgCl₂·6H₂O, 53.0 mg/L; KH₂PO₄, 27.0 mg/L; CaCl₂·2H₂O, 147.0 mg/L; FeSO₄·7H₂O, 20.0 g/L; MnCl₂·2H₂O, 62.0 µg/L; ZnCl₂,

68.0 µg/L; CoCl₂·6H₂O, 64.0 µg/L; H₃BO₃, 31.0 µg/L; Na₂MoO₄, 10.0 µg/L; CuCl₂·2H₂O, 67.0 µg/L; and deionized water, 1000 ml. The solution was sterilized by autoclaving at 121°C for 30 min and set at pH 1.8 using 5 M H₂SO₄. Preliminary oxidation of ferrous ions by the autoclaving process was disproved by analyzing the ferrous ions concentration before and after the process.¹²⁻¹³⁾

2.2. Sample

The mine tailings used in this study were collected from the Tereksai mine, Kyrgyzstan. The chemical composition of the tailings (Table 1) was determined by inductively coupled plasma (ICP) analysis (Optima 7300DV, PerkinElmer, USA), and the mineralogy (Fig. 1) was determine from X-ray diffraction (XRD) spectra within 2 theta ranges (Bruker HRXRD, Germany). The results confirmed a high concentration of arsenic, i.e., higher than tolerable levels, in the form of arsenopyrite (Table 1). The primary gangue mineral was quartz. The samples were screened to obtain a mean particle size greater than 4 mm. The morphology and structure of the mine tailings were determined by scanning electron microscopy energy dispersive X-ray spectrometry (SEM-EDS; JSM-6701F, JEOL, Japan) before and after the experiments to monitor the impact of the leaching process on the surface of the mine tailings.

2.3. Column Apparatus

To determine the feasibility of column bioleaching of mine tailings, our research group designed a lab-scale prototype (Fig. 2) following the experimental set up proposed in Seh-Bardan, et al. (2012).¹²⁾ The apparatus has two main components: a 350-mm-high hermetic plastic column with an internal diameter of 62.8 mm and a 2-liter continuous stirred reactor (CSTR) containing the off-solution draining from the column. An air pump ensures proper aeration of the system from the bottom

of the column, and a peristaltic pump transports the leaching solution from the CSTR to the top of the column. The solution is recirculated to the tank by gravity. Several valves allow control of the air flux and solution throughout the system.

2.4. Bioleaching experiments

The preparatory process prior to the bioleaching experiment was divided into three stages. First, the mine tailings with a particle size greater than 4 mm were

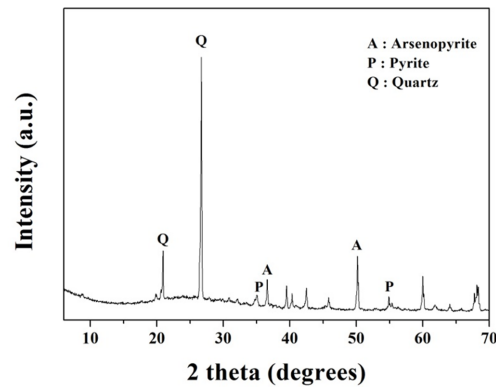


Fig. 1. XRD patterns of mine tailings: (A) Arsenopyrite, (P) Pyrite, and (Q) Quartz.

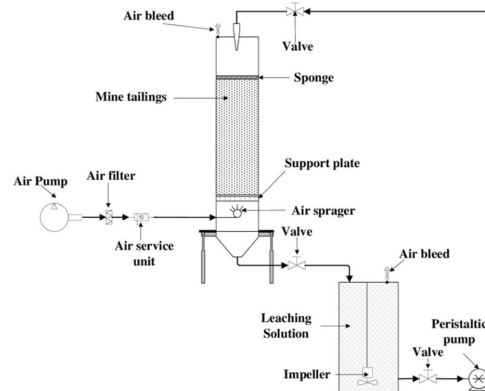


Fig. 2. Schematic and flowchart of the column apparatus.

Table 1. Chemical composition of mine tailings obtained by ICP analysis

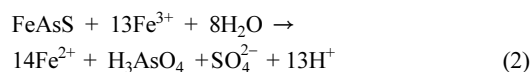
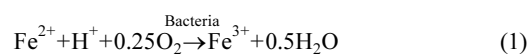
Chemical composition (mg/kg)							
As	Cd	Cu	Fe	Ni	Pb	S	Zn
11153.17	0.37	20.42	32381	19.44	8.37	17283.60	44.20

packed up to 80% of the capacity of the column, which was approximately 750 g. Second, the mine tailings were acid conditioned with H₂SO₄ (95% solution in water, ACROS Organics) by recirculation of acid through the column until the discharge solution pH was stabilized at 1.8. Finally, the system was inoculated through the CSTR with an initial bacterial concentration of approximately 1×10^9 cells/mL. Re-inoculation of the system with similar bacterial concentration was required several times during the experiment; on day 225, additional nutrients were added to the solution. The experiment was performed at room temperature. The irrigation and air input rates were set to 33.6 and 200 ml/min, respectively.

The off-solution was continuously sampled from the bottom of the column to monitor redox potential and solution pH. In addition, arsenic removal efficiency and total iron concentration were determined by ICP analysis. Ferrous ion concentration and sulfate concentrations were determined by *o*-phenanthroline¹⁴⁾ and gravimetric¹⁵⁾ methods, respectively. Ferric ion concentration was obtained by the difference between total iron and ferrous ion concentration. After 436 days, the column was unpacked and sampled for SEM-EDS analysis.

3. Results and Discussion

Bioleaching is primarily governed by an electrochemical process by which cells suspended in fluid and/or attached to the mineral surface generate an oxidizing agent¹⁶⁾; in heap bioleaching, the generated ferric ion percolates through the column while dissolving the metals. Consequently, analysis of the arsenic leaching kinetics could be understood by focusing on the behavior of the oxidation rates from ferrous to ferric ions observed in the column system over time. The reactions proposed by Nagpal et al. (1994)¹⁷⁾ are expected to represent the bioleaching reaction of arsenic from arsenopyrite that is present in the mine tailings used in the present study according to the XRD results (Fig. 1). The reactions are as follows:



Where reaction (1) represents the ferrous-to-ferric oxidation promoted by bacterial metabolism, and reaction (2) shows the dissolution of arsenic to arsenic acid. Both reactions highlight the importance of studying the redox potential of the solution over time because it is sensitive to the changes in ferrous and ferric concentrations in a given system. Redox potential is calculated by the Nernst equation.¹⁸⁾

$$E = E^0 + \frac{RT}{ZF} \ln \frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]} \quad (3)$$

Where, E^0 is the potential difference, R is the ideal gas constant, T is the temperature in kelvin, Z is the quantity of electrons transferred in the reaction, and F is the Faraday constant.

This equation clarifies the changes in the concentration of the iron species in the solution; thus, we considered the oxidation–reduction potential (ORP) trend as one of the most important parameters for our analysis. The redox potential trend showed several drastic changes over the duration of the experiment (436 days). To elucidate the reasons for this behavior, we split the trend into five phases considering the drastic changes in value: each drastic change yields a new phase (Fig. 3). Note that the acid conditioning phase is not considered in the analysis. As expected, the trends observed in our analyses were closely related to the trend of redox potential. A decrease of redox potential value was consistently reflected in a decrease of arsenic removal (Fig. 4) and ferric and sulfate generation rates as well as pH variations (Fig. 5). Table 2 contains the average rates observed in each phase.

Phase 1 began on day 32, after acidic conditioning, and finished on day 116. This phase was characterized by a constant decrease of redox potential, reaching the lowest value in the overall process. In terms of the ferrous-to-ferric oxidation rate, a peak in ferric concentration on day 75 provoked an increase in the overall oxidation rate in this phase. This peak is also reflected in the pH trend, indicating acceleration of acid consumption.

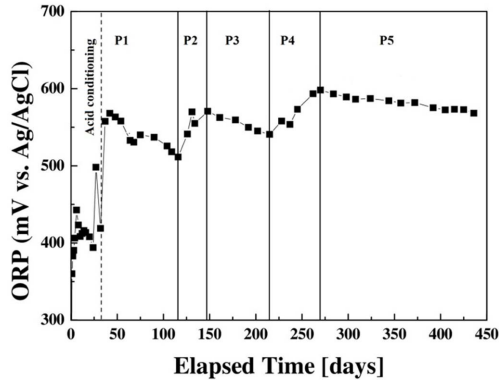


Fig. 3. Redox potential variation (mV vs. Ag/AgCl) over time.

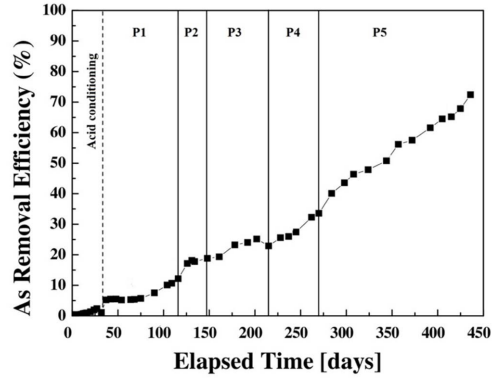


Fig. 4. Solubilization of As during the column bioleaching experiment over time.

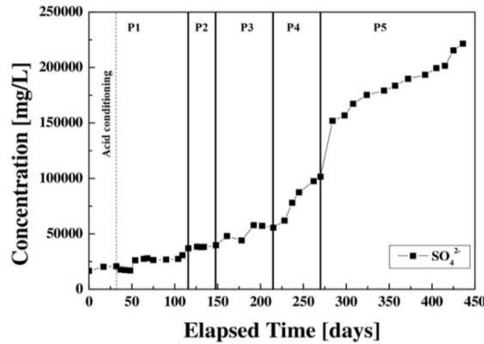
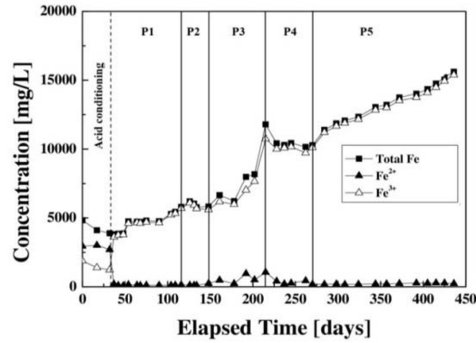
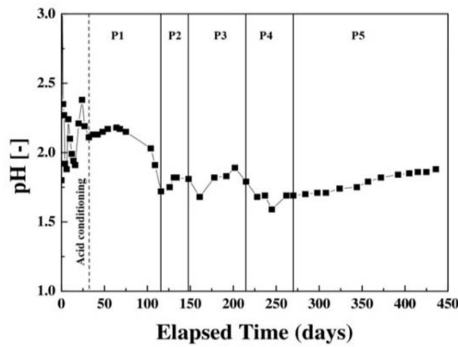


Fig. 5. pH, iron, and sulfate generation trends in the off-solution over time.

Table 2. Ferric and sulfate generation rate, arsenic removal rates, and initial and final arsenic concentrations for each phase

Phase	Fe ³⁺ ppm/day	SO ₄ ppm/day	As ppm/day	[As] _{initial} ppm	[As] _{final} ppm
1	56.47	406.42	10.78	94.46	999.77
2	11.80	87.81	17.99	999.77	1575.32
3	77.88	236.87	5.07	1575.32	1915.14
4	11.66	834.90	16.26	1915.143	2809.55
5	31.80	723.13	18.56	2809.55	5890.08

The second phase presented the second highest arsenic leaching efficiency during the experiment. ORP results suggest that this phase was characterized by high iron oxidation. The SO_4^{2-} generation rate indicated an incremental increase in acid production, which is reflected in a considerable increase of arsenic leaching efficiency. Note that the second phase started on day 116 and finished on day 148. A slight decrease of redox potential was observed in the phase 3, indicating deceleration of sulfuric acid production in the system according to sulfate and pH measurements. Nevertheless, a sudden increment of iron ion concentration was observed at the end of phase 3 (Fig. 5), responding to the addition of nutrients on day 225 while the arsenic leaching rate was not significantly affected by this addition (Fig. 4). Indeed, the arsenic leaching rate reached its minimum value in the whole process (Table 2). However, the effects of the addition of nutrients on day 225 on the arsenic leaching rate were observed in phase 4. A constant increment of the ferrous-to-ferric oxidation rate, acid generation and, most importantly, the arsenic removal rate were observed. Finally, in phase 5, which was the longest phase, constant iron oxidation and arsenic leaching rates were observed until the last day of the experiment. At the end of the process, approximately 70.0% of arsenic was leached into solution, implying the potential of this technology for mine tailings treatment if compared with the results obtained using different approaches and microorganisms.¹⁹⁻²⁰⁾

After a deep analysis of the trends and a literature review, we primarily attribute the aforementioned constant variations of oxidation and removal rates to the rapid ferric consumption observed in the first phase and the inhibitory effect of heavy metals (presumably arsenic) on the bacterial consortium. Factors, such as temperature, aeration rates, and irrigation rates, were fixed parameters and were not considered in the analysis. The constant decline of the redox potential in the first phase might be attributed to the rapid ferric ion consumption to leach the metals in the mine tailings. Ferric ions are expected to be consumed as soon as they are generated, which reduces their measurability in the solution²¹⁾. However, the slow leaching rate

observed during the first 35 days after inoculation can be attributed to the time required by the bacteria to be fully effective in the system. Consequently, the availability of ferric ions in the system is expressed only from phase 2 when the microorganisms are oxidizing the available iron in solution and the mine tailings, which corresponds to the increasing ORP (Fig. 3). In phase 3, bacterial activity seems to be affected by the high concentration of arsenic in the solution. As can be seen in Table 2, arsenic reaches concentrations of approximately 1,500 ppm, which we speculate would be harmful to the bacteria consortium. The inhibitory effect of arsenic on bacteria is reflected in a decrease of ferrous to ferric oxidation rate and acid generation according to the ORP and pH measurements. Several studies have demonstrated that arsenic inhibits bacterial activity and the inhibition varies from species to species.²²⁾ To prove this, we performed a bacteria toxicity assessment on a mesophilic bacterial culture and observed that bacteria were inhibited in presence of arsenic even in concentrations as low as 2 ppm.²³⁾ A similar observation was also found by Leng et al, (2009),²⁴⁾ demonstrating that a mixed culture of *A. ferrooxidans* and *A. thiooxidans* is inhibited by the presence of arsenic at 1,400 ppm, which is similar to the concentration found at the end of phase 2. This explains the drop in the ferric ion generation rate and the subsequent deceleration of the leaching efficiency in phase 3. Nevertheless, after 67 days of exposure to arsenic, the bacteria appear to have adapted to the metal concentrations in the solution, which is reflected by the increase of the ferrous-to-ferric oxidation rate during phase 4. From this hypothesis, it can be speculated that, after phase 3, the bacteria consortium progressively adapted over time without experiencing any major inhibition provoked by the presence of the arsenic in the percolated solution until the end of the process. The higher ferric ion generation during phases 4 and 5 supports this speculation. Adaptation mechanisms and their effect on bioleaching kinetics have been widely discussed.²⁵⁻²⁸⁾ We consider that the generation of a passivation layer due to the high ferric ion concentration could also be a potential inhibitor of bacterial leaching. The presence of a passivation layer affects leaching

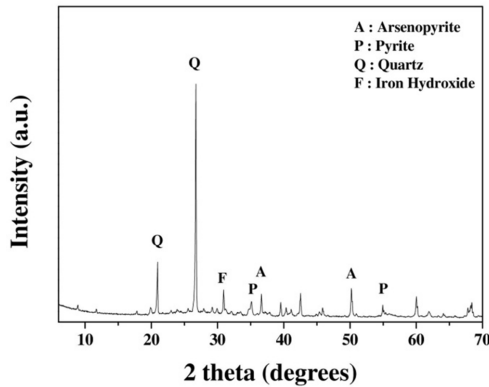


Fig. 6. XRD results for solid residues after the bioleaching process.

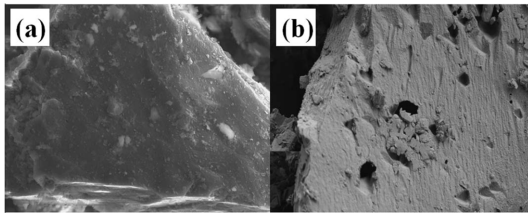


Fig. 7. SEM images of mine tailings a) before bioleaching and b) after bioleaching.

efficiency because the bacteria consortium would be prevented from attaching to the surface of the mine tailings, thus inhibiting oxidation, and the leachate agent cannot contact the mineral.^{12, 29-30)} Therefore, to further support our speculations, XRD analysis of the solid residues was performed and the result is presented in Fig. 6. The XRD results showed that iron hydroxide was the only precipitate and was present in minimum concentrations. Similarly, comparison of SEM images before and after the process showed no accumulation of precipitates that could form a passivation layer on the mineral surface; no jarosite formation was observed (Fig. 7).³⁰⁾ These results underline the crucial role of *A. thiooxidans* in the oxidation of elemental sulfur, which might produce a passivation layer.

4. Conclusion and Final Remarks

The objective of this study was to determine the potential of heap bioleaching technology to remove

arsenic from mine tailings using a mesophilic bacteria consortium composed of *A. ferrooxidans* and *A. thiooxidans*. The final extraction efficiency achieved was as high as 70%, which supports the potential application of this technology for such a purpose. We observed the sudden decrease in the ferrous-to-ferric oxidation rates, which was mainly attributed to the effects of arsenic toxicity on the bacterial consortium. This assumption was supported by the absence of a passivation layer, which is known as a common leaching inhibitor. Additional efforts to clarify the influence of other factors, such as particle size, temperature, aeration rates, and irrigation rates as well as microbiological and hydrological factors, during heap bioleaching of mine tailings are required.

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學會誌 投稿 安內

種 類	內 容
論 說	提案, 意見, 批判, 時評
展望, 解說	現況과 將來의 견해, 研究 技術의 綜合解說, Review
技 術 報 告	實際的인 試驗, 調查의 報告
技術, 行政情報	價値있는 技術, 行政情報를 간결히 解說하고, comment를 붙인다.
見 聞 記	國際會義의 報告, 國內外의 研究 幾關의 見學記 등
書 評	
談 話 室	會員相互의 情報交換, 會員 自由스러운 말, 隨霜 등
Group 紹介	企業, 研究幾關, 大學 등의 紹介
研究論文	Original 研究論文으로 本 學會의 會誌에 揭載하는 것이 適當하다고 보여지는 것

수시로 원고를 접수하오니 많은 투고를 바랍니다.