

A feasibility study on bioelectrokinetics for the removal of heavy metals from tailing soil

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Abstract The combination of bioremediation and electrokinetics, termed bioelectrokinetics, has been studied constantly to enhance the removal of organic and inorganic contaminants from soil. The use of the bioleaching process originating from Fe- and/or S-oxidizing bacteria may be a feasible technology for the remediation of heavy metal-contaminated soils. In this study, the bioleaching process driven by injection of S-oxidizing bacteria, *Acidithiobacillus thiooxidans*, was evaluated as a pre-treatment step. The bioleaching process was sequentially integrated with the electrokinetic soil process, and the final removal efficiency of the combined process was compared with those of individual processes. Tailing soil, heavily

contaminated with Cd, Cu, Pb, Zn, Co, and As, was collected from an abandoned mine area in Korea. The results of geochemical studies supported that this tailing soil contains the reduced forms of sulfur that can be an energy source for *A. thiooxidans*. From the result of the combined process, we could conclude that the bioleaching process might be a good pre-treatment step to mobilize heavy metals in tailing soil. Additionally, the electrokinetic process can be an effective technology for the removal of heavy metals from tailing soil. For the sake of generalizing the proposed bioelectrokinetic process, however, the site-specific differences in soil should be taken into account in future studies.

Keywords Tailing soil · Sulfur-oxidizing bacteria · Bioleaching · Electrokinetics · Heavy metal · Remediation

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Introduction

Mine tailing soil, which is a detrimental by-product from mining activities, contains several types of toxic contaminants including As and heavy metals. Recently, As and heavy metal contaminations of agricultural soils and crops surrounding the tailing-dumping area have been noted as one of the most serious environmental problems in South Korea (Kim et al. 2003). Therefore, the proper management scheme and remediation technology for such a problem are actively being developed.

The bioleaching process driven by Fe- and S-oxidizing bacteria in aerobic conditions originated from the practical processes for extracting valuable metals from low-grade ores (Rohwerder et al. 2003). Commercial approaches of bio-oxidation have advanced considerably, owing their success to favorable cost-effectiveness and reduced environmental problems compared to conventional methods such as smelting (Olson et al. 2003). In addition, in the field of environmental technology, it is well known that this technique is effective in removing metal contaminants from soil, sediment, and sludge (White et al. 1998; Seidel et al. 2004).

The bioelectrokinetics, which is defined by the combination of bioremediation and electrokinetics, has been studied constantly to enhance the removal of various contaminants from soil (Wick et al. 2007; Lee et al. 2009a). While this has been actively studied to enhance the biodegradation of organic pollutants, little study has focused on the remediation of heavy metal contaminants. The sequential coupling of bioleaching and electrokinetics has been applied for the removal of Cu from contaminated soil (Maini et al. 2000). This study used the technique of soil amendment with elemental S as a biostimulation process. Currently, we have reported the combination of anaerobic bioleaching and electrokinetics for the removal of As (Lee et al. 2009a) and multiple heavy metals (Lee et al. 2009b) from mine tailing soil. Anaerobic bioleaching was performed with the stimulation of indigenous bacteria by the injection of carbon sources into the soil, which was mainly associated with the microbial Fe and Mn reduction. Such previous studies on bioelectrokinetics demonstrated its cost-effectiveness.

In the present study, the bioleaching process driven by active injection of S-oxidizing bacteria, *Acidithiobacillus thiooxidans*, was evaluated as a pre-treatment step. The bioleaching process was sequentially integrated with the electrokinetic soil process, and the final removal efficiency of the bioelectrokinetic process was compared with that of the electrokinetic process.

Materials and methods

Soil

Tailing soil, heavily contaminated with multiple heavy metals, was collected from the abandoned

Nakdong mine, which is located in the northeastern region of South Korea. This abandoned mine was actively operated for the mining of Fe, As, and Bi until 1989. The tailing soil sample was carefully collected at depths of 0 ~ 30 cm from the surface of the tailing-dumping area. The representative physicochemical properties, such as soil texture (based on particle size distribution), organic matter content (calculated from measured loss on ignition at 400°C for 24 h), and soil pH (5 g soil in 50 mL of water), were investigated. To investigate the initial concentrations of 11 heavy metals (As, Cd, Cu, Pb, Zn, Ni, Co, Cr, Al, Fe, and Mn) in the tailing soil sample, it was digested with aqua regia, which consisted of concentrated HNO₃ and HCl (1:3), and heated at 70°C for 1 h (Ure 1995). The heavy metal concentrations in all samples were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer, USA).

Speciation analyses

To examine the chemical fractions of heavy metals in the tailing soil, the sequential extraction technique was used, as suggested by Tessier et al. (1979). Each of the 5 steps of the sequential extraction was referred to in terms of the exchangeable, weak acid soluble, reducible, oxidizable, and residual fractions, respectively (Table 1).

For the purpose of mineralogical investigations, the tailing soil sample was embedded in epoxy resin and then polished for analyses using reflection microscopy as well as scanning electron microscopy–energy-dispersive spectrometry (SEM–EDS, JEOL JSM-6380LV, Japan). In addition, the back-scattered electron (BSE) images and EDS spectra were used to characterize the elemental composition of individual grains within the sample.

Cultivation of bacteria

A strain of *Acidithiobacillus thiooxidans* (ATCC 8085), S-oxidizing bacteria, was used in the experiments. The strains were pre-cultivated in modified ATCC 125 Thiobacillus liquid medium that contained the following compositions in 1 l of tap water: 0.2 g of (NH₄)₂SO₄, 0.5 g of MgSO₄·7H₂O, 0.33 g of CaCl₂·2H₂O, 3 g of KH₂PO₄, and 0.01 g of FeSO₄·7H₂O. Inoculums of *A. thiooxidans* were grown at 30°C in

Table 1 The sequential extraction procedure for heavy metals (modified from Tessier et al. 1979)

Step (Target phase)	Extractant	Step time
I. Exchangeable fraction	0.5 M MgCl ₂ , pH 7, 25°C, continuous agitation	2 h
II. Weak acid soluble fraction	1 M NaOAc, pH 5 (adjusted with HOAc), 25°C	6 h
III. Reducible fraction	0.04 M hydroxyl-ammonium hydrochloride (NH ₂ OH HCl) in 25% HOAc, 95°C, occasional agitation	7 h
IV. Oxidizable fraction	0.02 M HNO ₃ and 30% H ₂ O ₂ (adjusted to pH 2), 85°C, 2 h, occasional agitation 30% H ₂ O ₂ (adjusted to pH 2) added, 85°C, 3 h, occasional agitation after cooling, 3.2 M ammonium acetate (NH ₄ OAc) in 20% (v/v) HNO ₃ added	6 h
V. Residual fraction	HCl and HNO ₃ (3:1), 70°C, occasional agitation	1 h

250-ml erlenmeyer flask with 100 ml of medium and 1 g of sterilized elemental sulfur. By measuring the sulfate concentration and pH level, the activities of *A. thiooxidans* during bacterial growth were monitored. Finally, after pre-cultivation for 10 days, which was the log-phase of bacterial growth (data not shown), the bacterial solution was used in the further bioleaching step.

Bioleaching experiment

Four sets of experiments are summarized in Table 2. In the bioleaching experiments, 100 g of the tailing soil sample was mixed with 100 mL of pre-cultivated bacterial solution. This mixture was placed in a small reactor and incubated at 25°C under static conditions (without agitation and aeration). Such an experimental setup simulated the in situ application of bacteria in the contaminated field site, where the active treatment using a slurry-phase bioreactor after excavation of soil is impossible. Also, it was assumed that the oxygen in soil and pore water was enough to

maintain an aerobic environment in the reactor. Such bioaugmentation experiments were performed for 15 and 80 days in parallel along with the abiotic controls that used only sterilized ATCC 125 medium as a leaching solution. Following these experiments, soil samples were taken directly from the soil bed for the total digestion and sequential extraction. The removal of heavy metals and the altered binding strengths of heavy metals in soil were evaluated.

Electrokinetic experiment

A schematic diagram of the device for the electrokinetic process is represented in Fig. 1, and the combination scheme is summarized in Table 2. Two sets of electrokinetics were performed for 8 days after the abiotic and 15-day biotic pre-treatment, which were regarded as short-term EK and BioEK, respectively. Pre-treated soil was transferred into the electrokinetic soil cell by discarding the supernatant in the bioleaching reactor. The applied voltage was sequentially changed (3 → 2 → 1 → 2 V/cm) (see Fig. 5a).

Table 2 Summary of experimental conditions for the bioleaching and the electrokinetic processes

Experiment	Bioleaching		Electrokinetics		Comments
	Type	Duration (days)	Voltage (V/cm)	Duration (days)	
EK8	Abiotic control ^a	15	3 → 2 → 1 → 2 ^b	8	Short-term EK
BioEK8	Bioaugmentation ^c	15	3 → 2 → 1 → 2	8	Short-term BioEK
EK20	Abiotic control	80	1 ^d	20	Long-term EK
BioEK20	Bioaugmentation	80	1	20	Long-term BioEK

^a The sterilized ATCC 125 medium was injected into the tailing soil

^b The applied voltage was changed sequentially

^c Sulfur-oxidizing bacteria, *A. thiooxidans*, pre-cultivated in ATCC 125 medium were injected into the tailing soil

^d Constant voltage was applied

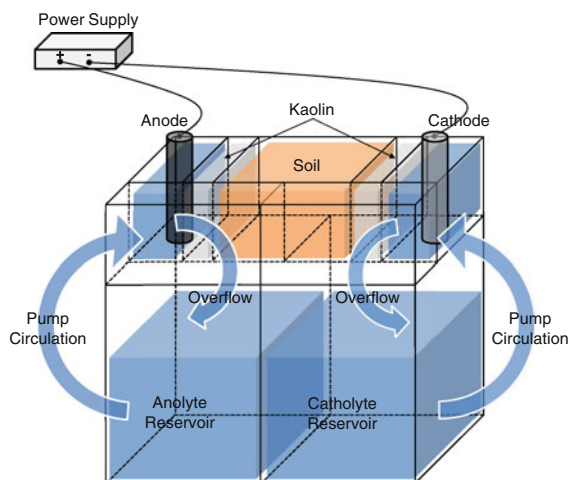


Fig. 1 Schematic diagram of the experimental device

On the other hand, the other two electrokinetic experiments were conducted for 20 days after the 80-day pre-treatment, which were regarded as long-term EK and BioEK, respectively. Constant voltage (1 V/cm) was applied. Apart from these, all other conditions of the experiments on electrokinetics were identical. A rectangular-type electrokinetic cell (14 cm in length, 4 cm² of cross-sectional area) was used, and approximately, 50 g of soil with 20% water content was used. The cathode was a titanium rod, while an iridium-coated titanium rod was used for the anode. In addition, mesh-type titanium electrodes, which were bordered by a filter paper to allow ions and water to pass, were placed in the soil cell, the kaolin cell, and electrode compartments. Both electrode compartments were connected to each electrolyte reservoir, which were placed beneath the electrokinetic cell; the electrolyte solutions were circulated continuously. In all experiments, 0.01M HNO₃ was used as an anolyte. The catholyte solution was 0.1 M HNO₃ and was used to neutralize the hydroxide ions generated by cathodic water electrolysis and to prevent alkaline conditions from developing within the soil bed; this process is a conventional electrolyte conditioning used to decrease soil pH. In order to keep the pH below 2 in the catholyte, the appropriate amount of concentrated HNO₃ was added to the catholyte reservoir.

The overall current of the soil cell was measured during the experiments. Electroosmotic flow was also observed by measuring the volume of water transported across the soil cell. The variations in soil pH

were monitored regularly during the electrokinetic experiments. After the electrokinetic process, soil samples were obtained directly from three sections of the soil bed and two sections of the kaolin bed. To investigate the residual concentrations of 11 heavy metals, collected soil samples were digested and analyzed by the equivalent methods as mentioned previously.

Results and discussion

Characteristics of tailing soil

The tailing soil was predominantly sand (soil texture according to USDA) with an organic matter content of 2.635% and pH of 5.58. Table 3 represents the initial concentrations of heavy metals in the tailing soil. The chemical analysis revealed that the tailing soil was highly contaminated with multiple heavy metals such as Cd, Cu, Pb, Zn, Co, and As in comparison with natural concentrations presented by the USGS. The dominant chemical fractions of most heavy metals were residual fractions, step V in the sequential extraction method, except for Co and Mn (Fig. 2). Although each corresponding fraction cannot be exactly defined by each step in this method, the results can be used to evaluate the relative binding strengths of each heavy metal. High proportions of strongly bound fractions of most heavy metals indicate that they primarily originated from ore minerals. Co and Mn appeared in relatively high amounts in adsorbed and oxides fractions, reflecting weakly bound fractions in soil. The minerals containing S in the tailing soil were determined by SEM-EDS analysis as primary sulfides, e.g. pyrite (FeS₂) and arsenopyrite (FeAsS) (Fig. 3). Such a result suggests that this tailing soil contained the reduced forms of S, which can be an energy source of S-oxidizing bacteria. Heavy metals may coexist with these minerals as adsorbed, co-precipitated, or mineralized forms in the tailing soil.

Effect of the bioleaching process

The S-oxidizing bacteria, *A. thiooxidans*, were introduced into the tailing soil as a pre-treatment step of the bioelectrokinetic process. Contrary to the normal bioleaching process dominated by the injection of elemental S into soil, only the S-oxidizing bacteria

Table 3 Total concentrations of heavy metals in the initial tailing soil (unit: mg/kg)

	As	Cd	Cu	Pb	Zn	Ni	Co	Cr	Al	Fe	Mn
Natural conc. ^a	7.2 (0.1 ~ 97) ^b	0.35 (0.01 ~ 2)	25 (1 ~ 700)	19 (10 ~ 700)	60 (5 ~ 2900)	19 (5 ~ 700)	9.1 (3 ~ 70)	54 (1 ~ 2000)	72000 (700 ~ 100000)	26000 (100 ~ 100000)	550 (2 ~ 7000)
Tailing soil	5664.3	142.6	163.9	73.4	218.9	21	24.3	36.2	25663	86082	743.5

^a Natural concentration of each species in soils suggested in the U.S. Geological Survey Professional Paper 1270 (1984)

^b Median (range)

were used because the oxidizable S already exists in the tailing soil. The major mechanisms to mobilize heavy metals in soil can be summarized as follows: First, heavy metals coexisted with the primary sulfide minerals lose their stability by the bacterial oxidation of S. Second, the final product of this oxidation process is H₂SO₄, which also increases the metal mobility by decreasing soil pH.

Both mechanisms are feasible for this bioaugmentation process. The initial soil pH of 5.58 was decreased to 3.94 and 3.91 after 15 and 80 days of bioleaching, respectively, whereas that of the abiotic control was not changed significantly. After the bioleaching experiments, the total concentrations and overall chemical fractions of heavy metals were analyzed to determine the effect of this step. There was no significant difference between the total concentrations before and after bioleaching (data not shown). Although the heavy metals could be mobilized in soil, they were not effectively separated from soil and pore water in the static reactor. Therefore, there was no variation in the total concentrations, even if the supernatant was discarded after the bioleaching step. However, we observed that the exchangeable fractions of all heavy metals increased after the bioleaching process (Fig. 4). Also, the effect of bioleaching was more significant in the long-term process, in contrast to the short-term process, which supports the idea that the mobilization of heavy metals by S-oxidizing bacteria requires sufficient time to do so. In the result of 80-day bioleaching experiment (Fig. 4b), only the exchangeable concentration of Pb was lower than that of the abiotic control. This might be caused by the formation of an insoluble PbSO₄ complex that shows a solubility product of 1.58×10^{-8} , which is significantly lower than the other target metals (Morel and Hering 1993).

Results of the (bio)electrokinetic process

All tailing soils that were pre-treated by abiotic and biotic processes were transferred in the electrokinetic step (see Table 2). The current gradually increased in all of the experiments (Fig. 5a), which reflects the increase in ionic migration caused by the applied electric field. In the short-term (bio)electrokinetic experiments, the current was mainly varied by an applied voltage gradient; there was no significant difference between the electrokinetic (EK8) and

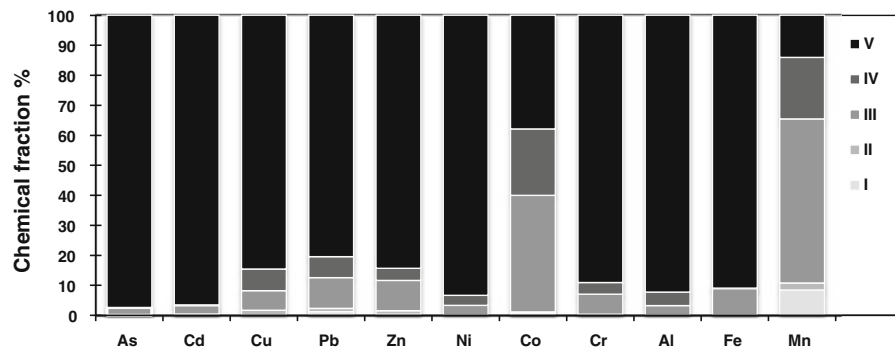


Fig. 2 Chemical fractions (%) of heavy metals in the initial tailing soil sample by sequential extraction. Each step of the sequential extraction was in terms of the exchangeable (I), weak acid soluble (II), reducible (III), oxidizable (IV), and residual (V) fractions

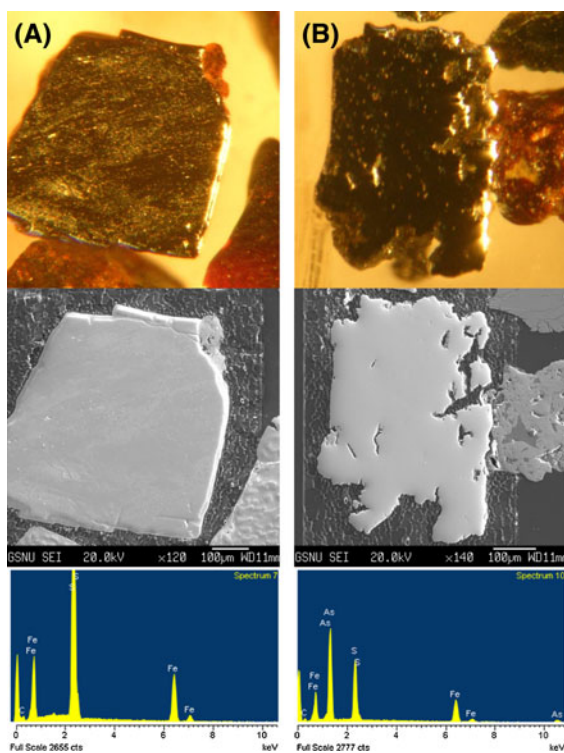


Fig. 3 Microscopic photograph, back-scattered electron (BSE) images, and energy-dispersive spectroscopy (EDS) spectra of tailing soil sample. **a** pyrite (FeS_2) and **b** arsenopyrite (FeAsS)

bioelectrokinetic (BioEK8) experiments. This result again supports that the bioleaching process could not effectively enhance the mobility of ions in bioelectrokinetics if constricted by a limited time frame. On the other hand, in the long-term experiments with a constant voltage gradient of 1 V/cm, the bioelectrokinetic (BioEK20) experiment showed a higher current level than electrokinetic (EK20) experiment

for the entire duration. This strongly suggests that the ionic migration was enhanced and the electric resistance in the soil cell was reduced by the bioelectrokinetic process.

The transported water volume in the cathode reservoir decreased in all experiments (Fig. 5b), which means the reverse electroosmosis (from cathode to anode) was dominant in all cases. The direction of electroosmotic water flow is mainly dependant on the soil pH. When the soil pH is lower than the point of zero charge (PZC) of soil, the zeta potential of the soil surface changed to a positive value, resulting in the opposite direction of electroosmotic flow. Therefore, the reverse electroosmosis generally occurred in the extremely acidic conditions of the electrokinetic process (Kim et al. 2002). Occasionally, such a reverse water flow can interfere with the electromigration of cations, but this effect has been considered as a minor one (Acar and Alshawabkeh 1993). For this reason, most heavy metals in this experiment primarily migrated toward the cathode compartment via electromigration, while a little transport of heavy metals toward the anode was also observed (data not shown).

Figure 6 represents the variation in soil pH at each section during the (bio)electrokinetic experiments. The rate of soil acidification in the short-term experiments was extremely high (Fig. 6a, b). The soil pHs at all sections were less than 1 within 2 days. This was caused by the rapid migration of hydrogen ions from the anode compartment to the soil cell, which was driven by the applied high-voltage gradient. On the other hand, in the long-term experiments, the soil acidification rate was, relatively, not very fast (Fig. 6c, d). Although the constant voltage gradient (1 V/cm) was applied in both experiments, the soil

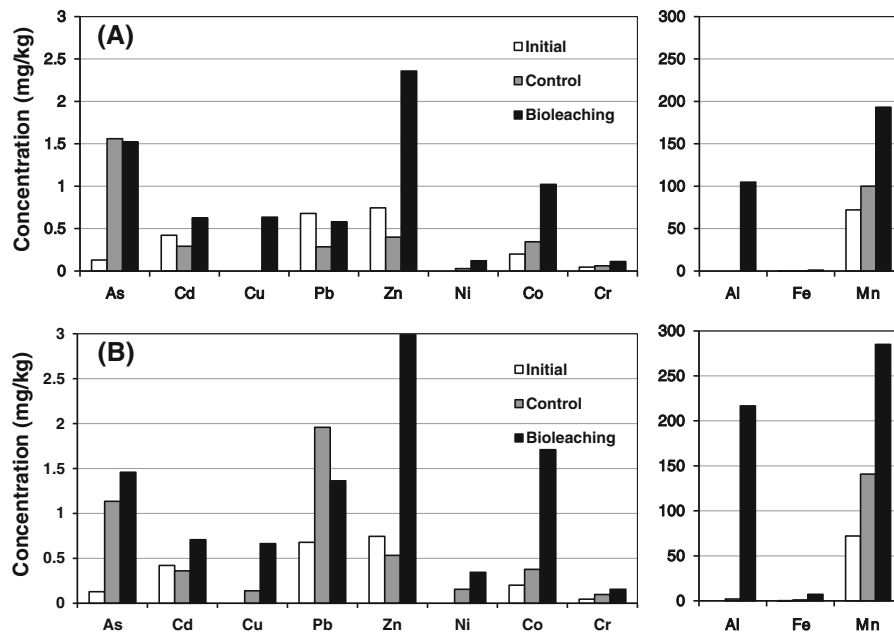


Fig. 4 Exchangeable concentrations of heavy metals in tailing soil before and after bioleaching for **a** 15 days and **b** 80 days

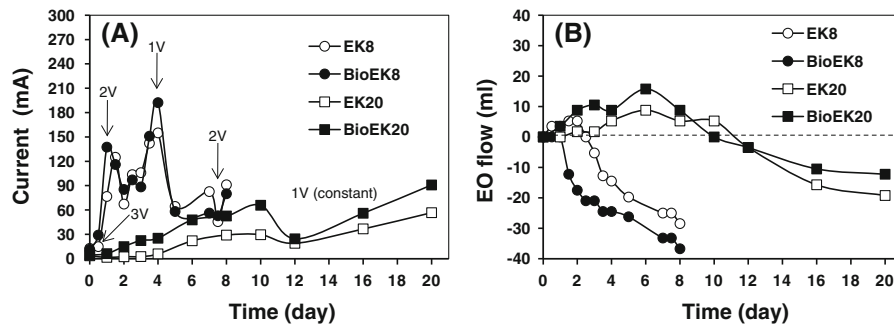


Fig. 5 **a** Current change measured and **b** transported water volume measured in the cathode reservoir during electrokinetic and bioelectrokinetic experiments

acidification was faster in the bioelectrokinetics experiment, while slower in the electrokinetics experiment. This result can be explained by: First, the initial soil pH decreased after the bioleaching process. Second, sulfur-oxidizing bacteria still exist in the soil bed, so they could continue oxidizing S and generating hydrogen ions. Third, due to the enhanced current level, a large amount of hydrogen ions was generated by the electrolysis of water at the anode compartment.

The results of the current and soil pH measurements reflected the synergistic effects of the bioelectrokinetic process to achieve the enhanced

removal efficiency of heavy metals. Table 4 represents the average removal efficiencies of heavy metals after all experiments. Because the target tailing soil contained heavy metals existing as relatively strongly bound chemical forms, the process resulted in a poor removal overall. However, the removal efficiencies of almost all of the heavy metals were relatively higher in the bioelectrokinetic experiment than in the electrokinetic experiment. Especially in the long-term experiments, the difference in heavy metal removal was obvious. This study demonstrated that the application of S-oxidizing bacteria

Fig. 6 Variation in pH at each section; anodic kaolin section (S1), soil section (S2, S3, S4), cathodic kaolin section (S5). **a** EK8, **b** BioEK8, **c** EK20, and **d** BioEK20

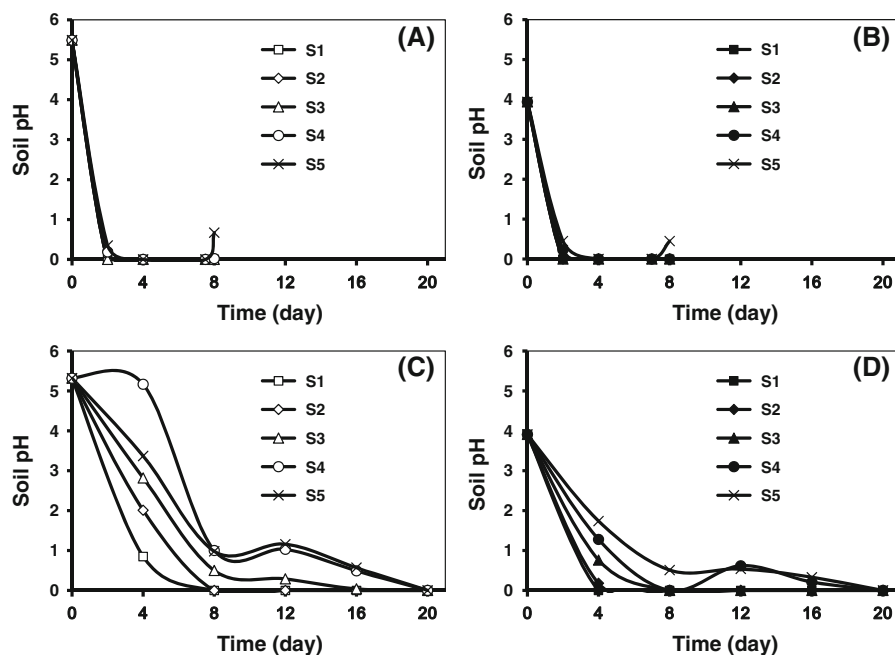


Table 4 Average removal efficiencies of heavy metals after experiments

	Removal efficiency (%) ^a										
	As	Cd	Cu	Pb	Zn	Ni	Co	Cr	Al	Fe	Mn
EK8	11.8	17.3	23.9	16.3	19.9	– ^b	58.9	28.5	27.2	3.4	82.0
BioEK8	11.9	23.5	28.0	19.6	22.1	–	60.5	32.4	31.9	1.1	84.5
EK20	2.9	29.2	11.1	7.7	33.8	9.6	61.6	21.8	22.9	56.4	76.8
BioEK20	4.8	33.9	24.9	29.7	49.3	32.1	71.6	47.8	41.1	60.0	86.5

^a The ratio of the initial concentration to the final concentration after experiment

^b No difference between before and after the experiment

in bioelectrokinetics was feasible for the removal of heavy metals from contaminated soil. Compared with the single electrokinetic process, enhanced removal efficiency of heavy metal removal can be expected in the combined process with the identical conditions such as electrolytes, voltage gradient, and processing time.

The highest removal efficiency of most heavy metals was observed in the long-term bioelectrokinetic experiment. Among the heavy metal contaminants targeted, Co and Mn, which exist as the weakly bound forms in soil, were effectively removed (71.6 and 86.5% of best removal, respectively). However, other heavy metals showed poor removal efficiency due to its initial condition as strongly bound forms in soil. The initial fraction and mobility of heavy metal

contaminants are predominant factors of final removal efficiencies of them. Notably, As also showed poor removal efficiency in all cases due to its strongly bound forms in soil. Moreover, due to As dominantly existing as various anions in the natural environment, alkaline conditions appear the most favorable for As electromigration (Lee et al. 2009a). An acid-enhanced bioelectrokinetic process driven by S-oxidation might be effective for normal heavy metals, but not for As.

The power consumption (kWh/ton) of each bioelectrokinetic process was calculated. Bioelectrokinetics for the short-term experiment with high voltage was 585.6 kWh/ton, and the power consumption for the long-term experiment with low voltage was 357.6 kWh/ton. Although the higher electrical

energy was consumed in the former case, it showed lower removal efficiency than the latter case. This strongly suggests that longer times are required for heavy metals in soil to be desorbed and dissolved; such a mobilization step is more important for heavy metal removal than an electromigration step in the electrokinetic process.

Conclusion

From the overall results of the experiments, we could conclude that the bioleaching process by S-oxidizing bacteria might be a good pre-treatment step to mobilize heavy metals in tailing soil. Additionally, the bioelectrokinetic process is an effective technology for the remediation of tailing soil. In order to prove the generalization of the bioelectrokinetic process proposed, however, the site-specific discrepancy in the soil should be considered in the future studies.

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