

Bioremediation of Antimony and Arsenic Co-contamination from Antimony Mining Area with Sulfate-reducing Bacteria

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Abstract: The contamination of antimony (Sb) and arsenic (As) in the soil of antimony mining area is the most common pollutant. Metal sulfide precipitation by sulfate-reducing bacteria (SRB) is considered to be a promising method. In this work, antimony and arsenic contaminated soil from an antimony mining area was treated by the application of a mixed culture of SRB. The soil samples initially contained the concentration of 17550 mg/kg Sb and 3231 mg/kg As, and the leaching concentration exceeds the groundwater IV standard. Changes of Eh, pH, aqueous Sb and as were monitored over 50 days in this experiment. The results indicated that SRB was able to increase the pH value and decrease the redox potential of the solution. When the SRB was growing well in the system, the high concentration of Sb in the solution was reduced from 7.8 mg/L to less than 0.5 mg/L. However, As showed a completely opposite trend to antimony. The presence of SRB can promote the release of as from contaminated soil, and the concentration of As will gradually decrease when the activity of SRB decreased. This work demonstrates that SRB can trigger the release of Sb, while as caused the opposite effect.


1 INTRODUCTION


Antimony (Sb) and Arsenic (As) are toxic and carcinogenic metalloids of global concern and listed as priority control pollutants by the European Union and the United States Environmental Protection Agency (Fu, et al., 2016). As the largest antimony producer in the world, China risks more severe of antimony pollution far than other countries. In addition to high concentration of antimony pollution, high arsenic pollution usually accompanies in


antimony mining area and surrounding environment due to the mineral oxidation and arsenic alkali slag leaching. Antimony and arsenic are non-essential toxic elements for human body, which could be absorbed and accumulated by plants when they existed in dissolved state, and potentially entered the human body and caused various diseases such as cardiovascular diseases, skin lesion, reproductive disorders, diabetes, and even cancer in the skin, bladder, kidney, and lung (Alam, McPhedran, 2019; Singh, et al., 2015).


In the past 20 years, significant progress has been made in the treatment of antimony/arsenic pollution. These technologies fall into two main categories, directly remove antimony and arsenic from contaminated media and reduce its biotoxicity in the environment, both of which can reduce the health risks of antimony and arsenic to human body (Feng, et al., 2017). At present, remediation technologies are mainly including biological


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oxidation/reduction, electrokinetics, phytoremediation, coagulation–flocculation, ion exchange, and solidification/stabilization (Wan, et al., 2019). Biological methods have been widely used in the treatment of heavy metal pollution due to their advantages of low remediation cost, environmental friendliness and simple operation. In particular, sulfide precipitation generated by sulfate reducing bacteria (SRB) is considered to be an effective method for the removal of heavy metal pollution. Under anaerobic conditions, SRB can reduce sulfate to sulfide, which is able to precipitate metals and metalloids, decreasing its solubility and pervasiveness (Zacarias-Estrada, et al., 2020).

Microbial redox reactions can significantly affect the conversion of valence states between Sb (III) and Sb (V). Although there are relatively few studies on bioreduction of Sb (V), it has been shown that microorganisms have the ability to reduce Sb(V). Sb is a strong chalcophile element, thus it can be easily reacted with the dissolved sulfide produced by SRB to form antimony sulfide. Sb (V) usually exists as $\text{Sb}(\text{OH})_6^-$ and can be reduced to $\text{HSb}_2\text{S}_4^{4-}$, $\text{Sb}_2\text{S}_4^{2-}$, Sb_2S_3 , and Sb (III)-sulfide complexes by the dissolved sulfide under anoxic conditions (Xi et al., 2020). Sb and As belong to the same main group and their properties are similar. As(V) usually exists in the form of H_2AsO_4^- and HASO_4^{2-} and the sulfide produced by SRB can potentially precipitate As(V) as arsenic sulfides (realgar (AsS) and orpiment (As_2S_3)), or form soluble thio-As species when As concentration reaches equilibrium, thus promoting or reducing As migration and immobilization (Fan, et al., 2018).

There are many reports of As remediation by SRB, and the Sb biotransformation has also been studied in recent years. However, few studies have investigated the bioremediation of Sb and As co-contamination by SRB. Therefore, this study investigated the potential of As/Sb removal and immobilization ability mediated by SRB in the soil of antimony mining area. The objectives of this study are (1) investigate the bioremoval efficiency of As/Sb during the growth of a mixed culture of SRB, and (2) evaluate the long-term stability of the remediation process.

2 MATERIALS AND METHODS

2.1 Site Location and Soil Sampling

Soil samples were derived from an antimony mining area located in Qinglong County, Guizhou Province,

Southwest China. The soil samples were collected from the upper surface (5-15 cm) in April, 2021. The collected samples were transferred with ice to the laboratory within two days, and stored at 4°C before the incubation experiments. To determine the main elemental compositions in the soil, samples were freeze-dried, sieved through 100 mesh and then measured using X-ray fluorescence (XRF, PANalytical BV).

2.2 Bacterial Culture

The SRB consortium used in this study was originated from National Engineering Laboratory of Biohydrometallurgy. Prior to the experiments, the bacteria were cultured in liquid medium containing per liter: 0.01 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mL sodium lactate, 1.0 g Yeast extract, 0.5 g KCl, 0.5 g K_2HPO_4 . The SRB were incubated for 3 days to a late exponential phase at 30°C in an anaerobic reactor.

2.3 Bioreduction Experiment

The microcosm experimental system was performed simultaneously in 500 mL serum bottles, containing 200 g contaminated soil, 375 mL of liquid medium, with 10% v/v of SRB enriched anaerobic consortium as inocula and nitrogen as gas headspace to impose anaerobic condition. In the early stage of the reaction, medium was not supplemented during the culture process. After 35 days, the same volume of medium solution was added after each sampling. Control treatments without addition of SRB were also set up for comparison. All the bottles were anaerobic incubation at 25-30°C in static conditions. Each experiment was carried out in triplicate. At regular intervals, liquid supernatant samples (5.0 mL) were filtered through a sterile syringe to analysis of aqueous Sb and As. The redox potential (Eh) and pH were also monitored at each sample internal.

2.4 Analytical Methods

Soil samples were heated at 105°C for 2 h to determine water content. The pH of soil samples was measured at solid/water ratio of 1:2.5 with a pH meter (Thermo Scientific Orion 3-Star, Germany). To determine the leaching toxicity of heavy metals in soil samples, sulfuric acid and nitric acid method were applied.

The pH and Eh of water samples were immediately measured after sample collection with

pH meter and redox potential meter (pH/ORP controller PC-350, China), respectively. Total dissolved Sb and As content were quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Agilent 725, USA).

3 RESULTS AND DISCUSSION

3.1 Characterization of The Soil

The antimony mining area soil sampled at Qinglong County were reddish brown in appearance. The contaminated soil was almost neutral, and the average pH values are 7-7.8. The soil moisture content was 6.8%. The main element of the sample as conducted by XRF analysis were summarized in Table 1. Totally, Sb contents were several times greater than that of As, and the concentration of Sb and As was 17550 and 3231 mg/kg, respectively. Besides, the soil also contained high concentrations of Fe (18.74%), Ca (6.85%), S (6570 mg/kg), Ti (4720 mg/kg). According to the Chinese national standards for groundwater quality, the leaching concentration of Sb and As exceeds the groundwater IV standard, in which As exceeds 1.256 times and Sb exceeds 728 times.

Table 1: The XRF results of the antimony mining area soil.

Elements	Con. (mg/kg)	Elements	Con. (mg/kg)	Elements	Con. (mg/kg)
Fe	187400	Ti	4720	Sr	330
Ca	68500	As	3231	Ba	273
Sb	17550	Cl	1070	Cr	215
K	10610	Zn	861	Cu	115
S	6570	Mn	498	V	114

3.2 Variation of Physicochemical Properties in the Soluble Medium

After 3 days of reaction, the solution in the remediation group began to turn black and hydrogen sulfide was produced (Figure 1), while there was no significant change in the control group, indicating that SRB in the remediation group could quickly adapted to the high concentration of antimony and arsenic pollution environment and played a biological reduction function in this system.

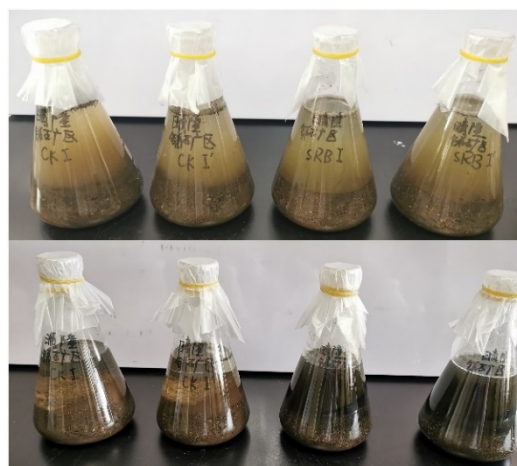


Figure 1: Solution color changes during remediation.

ORP and pH are fundamental properties which can reveal the activity of microorganism and the process of mineral transformation (Gao, et al., 2021). Changes of Eh and pH during the 50d reaction period was shown in Figure 2. The pH in biotic systems increased from the initial value of 7.2 to 8.3 within 28 d, which may be attributed to the alkalinity (HCO_3^-) generation and biogenic H_2S accumulation due to the sulfate reduction by SRB (Fan, et al., 2018). Subsequently, pH decrease was observed in the treatment may be relative to the death of SRB in the system. Thus, when liquid medium was supplemented to the system, the pH of solution began to increase again. With the initiation of the remediation treatment by SRB, the Eh was decreased in the first 4 days, then increased gradually and reached a peak to approximately 50 mV (Figure 3), reflecting the decrease of SRB activity. After the addition of medium in 35d, the Eh decreased markedly from 40 mV to -220 mV, indicated that SRB was growing well and resulted in a strong reducing condition in the culture. In the abiotic control treatments, the pH presented an upward trend first and then falling, and the Eh nearly remain unchanged at ~100 mV throughout the entire incubation period.

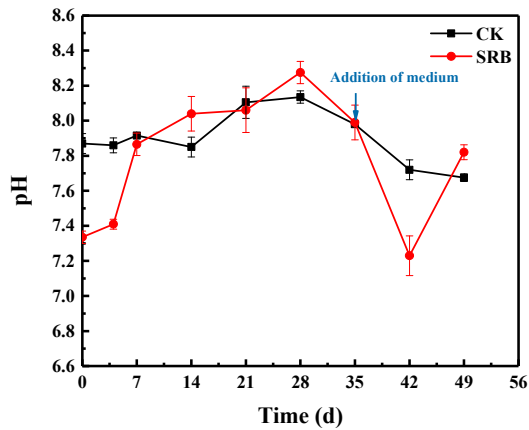


Figure 2: pH changes during remediation.

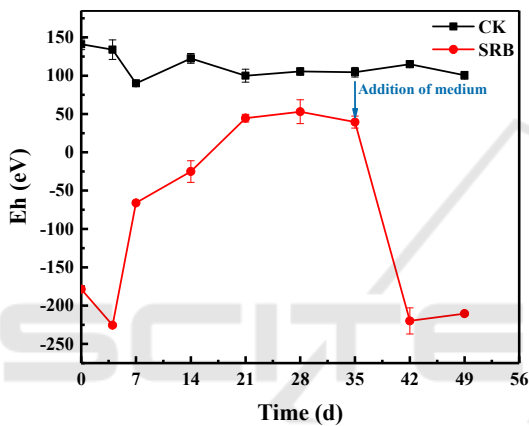


Figure 3: ORP changes during remediation.

3.2 Concentration Changes of Sb and As in Solution

The Sb concentration in the CK group increased from 3.62 mg/L to 10.37 mg/L (Figure 4). While in the SRB bioremediation group, the Sb concentration decreased rapidly from 4.45 mg/L to 0.91 mg/L within 7 days, after that, there was a slightly increase, and then began to increase sharply, reaching 7.80 mg/L on the 35 days. After supplemented with the medium, Sb concentration quickly decreased to 0.36 mg/L, and maintained a low concentration during the whole experiment. The change of As concentration was shown in Figure 5. The As concentration in the CK group increased from 0.37 mg/L to 1.30 mg/L from 0-7d, and then remained relatively stable. The As concentration in the SRB bioremediation group decreased from 1.05 mg/L to 13.97 mg/L from 0-7d, and it began to decrease significantly on the 14 days, and the concentration decreased to 7.64 mg/L on the 35

days. After supplemented with medium, As concentration started to increase rapidly at the end of this experiments.

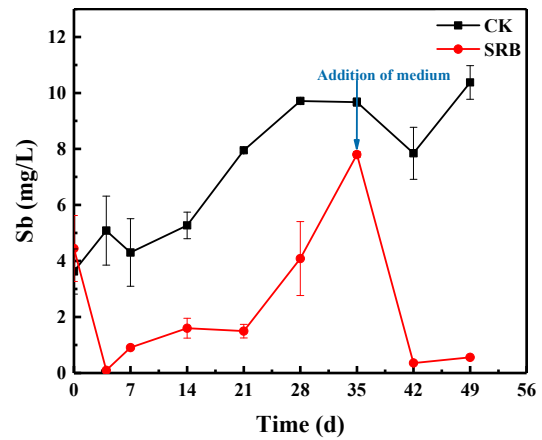


Figure 4: Sb concentration changes during remediation.

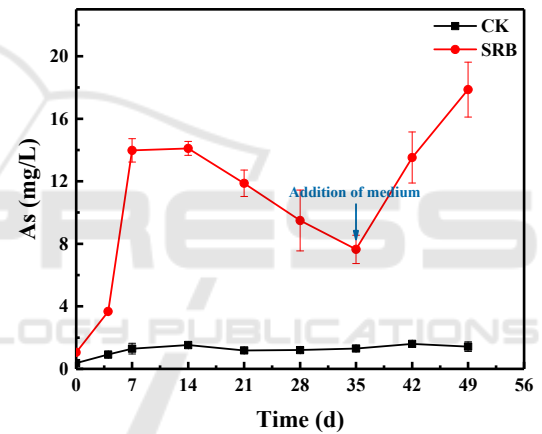


Figure 5: As concentration changes during remediation.

SRB mediated sulfate reduction to produce H_2S , and H_2S reacted with Sb to produce insoluble precipitate Sb_2S_3 , which may be the reason for the decrease in Sb concentration in the initial stage. With the consumption of the substrate, we observed that the ORP gradually increased, the activity of SRB decreased and the production of H_2S decreased, and inability to precipitate the dissolved Sb completely, resulting in an increase in the concentration of Sb in the solution. In addition, the medium was supplemented in the later stage, and ORP and the concentration of Sb decreased rapidly, confirming that the highly active SRB can precipitate Sb by mediating sulfate reduction to produce H_2S . However, the change of As concentration in solution was opposite to Sb, which indicated that SRB may lead to the release of arsenic. This may be because As (V) was reduced to

As (III) in the presence of SRB, while As (III) is more unstable and easily released into the environment. Although H₂S can react with As to form insoluble As₂S₃, this precipitate is not stable (Matos et al., 2018). In the presence of high concentrations of sulfide and at near-neutral pH, the transformation of insoluble As₂S₃ into soluble As (OH)S₂²⁻ could be observed in the system (Sun et al., 2019). Therefore, it is difficult to remediation of As by SRB alone, which requires strict reaction conditions.

4 CONCLUSIONS

During the reductive process of contaminated soil from antimony mining area by SRB, the changing trend of Sb and As were different. The change of aqueous Sb concentration showed two stages, firstly, when the activity of SRB decreased, the Sb immobilization by SRB would be gradually released in the early stage of the reaction. next, the released Sb quickly removed from the solution when the OPR value decreased to negative. However, SRB caused a negative effect on As in this study. This process verified a cost-effective biological process to remove Sb from Sb-As contaminated soil, while the remediation of As through SRB needs further study.

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