Study on microbial remediation of heavy metal contaminated soil

Qin Jiaming\textsuperscript{1,a}, Jiang Biguang\textsuperscript{2,b}, Nan Xiaolong\textsuperscript{2,c}, Xu Rui\textsuperscript{1,d}, He Youyu\textsuperscript{2,e}, Jiang Guoqing\textsuperscript{2,f}, Qin Jinning\textsuperscript{2,g}, Yang Yongbin\textsuperscript{2,h}, Li Qian\textsuperscript{1,i}\textsuperscript{*}

\textsuperscript{1}School of Minerals Processing and Bioengineering, Central South University, Changsha, Hunan, China
\textsuperscript{2}306 Brigade of Hunan Nuclear Geology, Hengyang, Hunan, China
\textsuperscript{a}email: qinjiaming@csu.edu.cn, \textsuperscript{b}email: 173865019@qq.com, \textsuperscript{c}email: 317526209@qq.com, \textsuperscript{d}email: ruixu1923@csu.edu.cn, \textsuperscript{e}email: 364325708@qq.com, \textsuperscript{f}email: jgq13974775943@126.com, \textsuperscript{g}email: hn635566qin@126.com, \textsuperscript{h}email: 1835633289@qq.com, \textsuperscript{i}email: csuliqian@126.com

Abstract: This paper studied the leaching effect of various strains on the heavy metal contaminated soil around the mining area of Dong’an County, Hunan Province. By measuring the form of heavy metals of antimony and arsenic in the soil and the concentration of heavy metals in the solution, the remediation effect of microorganisms in different pH conditions and different soil environments on the heavy metal contaminated soil was explored. The results showed that \textit{Acidithiobacillus thiooxidans} (\textit{A.c}) and \textit{Acidithiobacillus thiooxidans} (\textit{A.t}) had better leaching effect. Under the initial conditions of pH equal to 1, 10% bacteria content and 10 g/L S, the active metal components in the soil could be dissolved. The results showed that microorganism could remediate the soil contaminated with heavy metals and make the available metal components in the soil reach the safety standard.

1. Introduction
The pollution of heavy metals in soil caused by non-ferrous metal mining and smelting activities has become more and more prominent for a long time. Antimony and arsenic slowly migrate, diffuse, dilute, accumulate and have strong regional characteristics in soil\textsuperscript{[1]}. The antimony content in the soil of Yunnan, Guizhou, Yunnan and Guangxi provinces is as high as 5045 mg/kg. Some crops (such as rice, corn, vegetables,) have high arsenic content.

At present, the level of heavy metal pollution has exceeded the scope of natural remediation, and it is urgent to treat heavy metal pollution. Physical methods include mixing of soil, soil replacement and deep tillage methods. However, this kind of technical engineering is relatively large, such methods are rarely used in the treatment of heavy metal pollution. Chemical remediation mainly includes soil leaching technology and chemical fixation technology, which can promote the dissolution or migration of heavy metals in the soil. Sun\textsuperscript{[2]} used tartaric acid, malic acid and artificial chelating agent EDTA As eluents to leach Sb and As from the heavily polluted soil in the north bank of the waste reservoir of Qinglong Antimony Smelting Plant in Guizhou Province. The results showed that the three chelating agents had good effects on Sb and As due to their dissolution, desorption and chelation. The advantages are rapid and thorough, but the cost is high and may produce secondary pollution. Sanders et al\textsuperscript{[3]} found that Escherichia coli with glycerol facilitator can facilitating the leaching Sb from soil. Wu\textsuperscript{[4]} found that...
cyanobacteria have adsorption capacity for Sb(V), and its adsorption effect is greatly affected by the amount of adsorbent dosage, pH value and organic solvent. Lee et al\[5\] found 10 strains of Sb oxidizing bacteria from mining areas sediments, and found that the IMH strain can only reduce pentavalent arsenic As(V) when As and Sb coexist in the soil.

In summary, microorganisms have shown great development potential for antimony and arsenic pollution remediation. Sulfur oxidizing bacteria can utilize elemental sulfur to produce sulfuric acid, which is commonly used for metal leaching in industry. Thus, sulfur oxidizing bacteria could be used a tool for extracting metals from the contaminated soil. At present, there have been studies on arsenic leaching from contaminated soil by sulfur oxidizing bacteria, but the leaching of metal antimony are few, and the key role of bacteria in leaching is also less. Based on this, this article selects a variety of microbial strains with heavy metal leaching function to treat heavy metal contaminated soil, and studies the effect of heavy metal leaching under different conditions, in order to provide a reference for the remediation of heavy metal contaminated soil.[6].

2. Materials and Methods

2.1. Test soil and bacteria

2.1.1. Test soil
The experimental soil was taken from the soil around the mining area in Dong'an County, Hunan. Five representative soils were Fujiatian (S-1), Qianjiangchong (S-2), Jinjiang Reservoir (S-3), Tailings Mine (S-4) and Vegetable soil in mining area (S-5). The heavy metal form content in the soil was obtained by the BCR stepwise extraction method.

2.1.2. Test strain
Acidithiobacillus thiooxidans (A.t), Acidithiobacillus caldus(A.c), Acidithiobacillus ferrooxidans(A.f), Streptococcus thermophilus(S.t) and Leptospira ferrophila(L.f) provided by Central South University.

2.2. experimental method

2.2.1. Strain culture
In this experiment, 9K medium was used for the culture of bacteria, and the ingredients were: (NH4)2SO4 3 g/L, KCl 0.1 g/L, K2HPO4 0.5 g/L, MgSO4•7H2O 0.5 g/L, Ca(NO3)2 0.01 g/L, FeSO4/S 44.7 g/L/10 g/L.

2.2.2. Speciation determination of heavy metals
The content of heavy metals in the soil is extracted using the BCR stepwise extraction method. The forms of heavy metals include water-soluble, exchangeable, iron-manganese, organically bound and residual states, as well as the total amount of metals in the soil.

2.2.3. Leaching experiment
Set the liquid-solid ratio to 1%, the bacterial inoculation amount to 10% (v/v), add 44.7 g/L of FeSO4•7H2O for bacteria A.f, S.t, L.f as energy substance, and add 10 g/L of S for bacteria A.c and A.t as energy substance, and a prepared 100mL solution is added to a 250mL Erlenmeyer flask. S.t and L.f are subjected to the leaching test under constant conditions of 40 degrees Celsius, and the leaching test of A.c, A.t, A.f is performed under the conditions of 30 degrees Celsius, and the speed is 160r/min.

2.2.4. Filter residue analysis and toxicity detection
The "Solid Waste Leaching Toxicity Leaching Method Sulfuric Acid and Nitric Acid Method" (HJ/T299-2007) is used for testing, and a mixture of concentrated sulfuric acid and concentrated nitric acid with a mass ratio of 2:1 is added to water to form an extractant, so that the pH is 3.20± 0.05, oscillate at 23±2°C for 18±2h. The extractant is used to determine the leaching toxicity of heavy metals in
samples.

2.3. Detection method
The leaching experiment days were 1, 2, 3, 4, 6, 8, 10, 12, 14, and 16 days. Repeat sampling, use ICP analyzer to detect the ion concentration of Sb and As in the solution, and use pH meter Changes in pH and ORP during leaching.

3. Results and Discussion

3.1. Content and total amount of available heavy metals in soil
The effective metal content and total metal content are shown in Table 1.

| Samples | Effective state content/ Total(mg/kg) |
|---------|-------------------------------------|
|         | Sb                                  |
| S-1     | 854/ 3148                           |
| S-2     | 154/ 862                            |
| S-3     | 1101.4/ 2545.4                      |
| S-4     | 2418/ 5422                          |
| S-5     | 298.7/ 7984.7                       |
|         | As                                  |
|         | 41/ 261                             |
|         | 15/ 61                              |
|         | 455.6/ 579                          |
|         | 442.4/ 1995.4                       |
|         | 2.3/ 122.4                          |

3.2. The leaching effect of five strains on heavy metal soil
The pH, ORP and leaching rate of the biological leaching process are shown in Figure 1. The pH of the five strains gradually decreased over time. Among them, A. f bacteria, S.t bacteria and L.f bacteria became stable at around 1.6-1.8 on the 8th day, while A.c bacteria and A.t bacteria multiply to produce sulfuric acid, which makes the pH continue to drop. The oxidation-reduction potential (ORP) rises, and the A.c oxidation-reduction potential can reach up to 630mV. After leaching the soil, the leaching removal rate of each metal increases significantly. As the leaching days increase, the leaching removal rate of each heavy metal in the soil. The leaching removal rate of arsenic and antimony by bacteria A.f, S.t and L.f is low, and reaches a peak quickly, and the effect after the leaching cycle is far from reaching the soil heavy metal content standard; A.c bacteria and A.t bacteria have a good leaching effect on antimony and arsenic, and the best effect of A.t bacteria can reach 25% Sb and 56% As. The best leaching effect of A.c bacteria can reach 13% Sb and 30% As.
3.3. The effect of pH on the leaching of heavy metals in soil s-1

The change in the dissolution rate of heavy metals is shown in Figure 2. The effect is best under the condition of initial pH=1. The removal rate of antimony and arsenic leaching increases significantly. As the leaching days increase, in the 1-10d stage, heavy metals in the soil The removal rate of leaching is on the rise, and the leaching rate of heavy metals remains stable in the 10-16d stage. Among them, the effect of A.c on antimony and arsenic reaches 26% and 45%, and the effect of A.t on antimony and arsenic reaches 30% and 45% As.

3.4. The leaching effect of heavy metals in different soils

Figure 3 uses s-1 as a control to reflect the leaching effect of A.c and A.t under different soil conditions (s-2, 3, 4, 5). The effect of A.t is better than that of A.c. The leaching rates of antimony and arsenic of s-2 are 34.81% and 37.49%; the leaching rates of antimony and arsenic of s-3 are 40.79% and 95.19%; the leaching rate of antimony and arsenic of s-4 is 54.98% and 99%; the leaching rate of antimony and arsenic of s-5 is 7.36% and 51.79%.
This study shows that the pH continues to decrease and the leaching rate increases, indicating that the biological leaching causes elemental sulfur to generate sulfuric acid, and the reaction of antimony arsenic with sulfuric acid can generate substances such as $\text{Sb}_2(\text{SO}_4)_3$ and $\text{H}_3\text{AsO}_3$. Antimony sulfate can be dissolved in an acidic environment, and bacteria. Because of its eosinophilic mass reproduction, the species can survive in an acidic environment, thereby increasing the repair of antimony and arsenic in the soil.

3.5. Filter residue analysis and toxicity test

After the residue is determined by the BCR continuous extraction method, the content of antimony and arsenic in the filter residue in water-soluble, weak acid soluble, oxidizable, and reducible states are all zero. The antimony and arsenic in the filtered residue all exist in the residue form. In addition, after the filtered leachate passed the toxicity test of "Solid Waste Leaching Toxicity Leaching Method: Sulfuric Acid and Nitric Acid Method" (HJ/T299-2007), the solution contains almost no antimony and arsenic metal components, which can achieve the purpose of bioremediation of metallic soil.

4. Conclusions

a) Microbial strains A.c and A.t have the function of leaching heavy metals in the soil. Under the condition of initial pH=1, 10% (v/v) inoculation amount, the effective components of heavy metals antimony and arsenic can be completely dissolved.

b) When dealing with different heavy metal contaminated soils, the microbial strains A.c and A.t still have a good leaching effect on the available metals, and the microbial leaching of heavy metals is feasible.

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