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Bioleaching of heavy metals from contaminated alkaline sediment by auto- and heterotrophic bacteria in stirred tank reactor

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Abstract: Bioleaching Xiangjiang River alkaline sediment contaminated by multiple heavy metals was investigated. Multiple metals in alkaline sediment possess significant toxicity to aquatic organisms or humans and will greatly inhibit bioleaching. The bioleaching method using autotrophic bacteria mixed with heterotrophic bacteria can solve this problem successfully. The experiment results showed that bioleaching efficiencies of Zn, Mn, Cu, and Cd were 95.2 %, 94.2 %, 90.1 %, and 84.4 %, respectively. Moreover, the changes of heavy metal concentrations in different fractions in contaminated sediment before and after bioleaching were analyzed by selective sequential extraction, and it was discovered that the main fractions of Zn, Mn, Cu and Cd after bioleaching are Fe−Mn oxide, organic associated form and a residual form. Its biotoxicity decreased greatly. The bioleaching heavy metals from sediment using autotrophic bacteria combined with heterotrophic bacteria can effectively improve the bioleaching efficiency and reduce toxicity.

Key words: bioleaching; alkaline sediment; heavy metals; sequential extraction；auto- and heterotrophic bacteria

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1 Introduction

With the development of metal processing, tanneries and electroplating industries [1], heavy metal contamination becomes a worldwide environmental problem. It is persistent when releasing into environment and can pose significant risks to public health and ecosystems through the contamination of food chains, breaking the normal metabolism activity of organisms [2,3]. Therefore, it is a global environmental concern to clean up the metal contaminated sites. The toxicity and mobility of metals depend not only on total concentration, but also on their specific chemical forms, so the knowledge on metal speciation may be as important as the total metal concentrations for hazard assessment studies [4]. Metals deposited in the sediment can be present in a number of chemical forms, such as exchangeable, carbonate associated, Fe−Mn oxide-associated, organic associated, and residual forms [4,5].

Bioremediation has been developed as an environmentally friendly and cost-effective technology for the removal of metals from sediments. Among the biological technologies, microbiological leaching of heavy metals from sediment is far more popular. Bioleaching is defined as “the solubilization of metals from solid substrates either directly by the metabolism of leaching bacteria or indirectly by the products of metabolism” [6]. Bioleaching process using acidophilic microorganisms (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) and neutrophilic microorganisms (*Aspergillus niger*) have been applied successfully to metal removal from sediment, municipal solid, and sludge [7−11]. Whereas these materials are commonly complex mixtures consisting mainly of organic compounds, such as organic acids, simple sugars and amino acids, which can intensively inhibit chemolithoautotrophic microbes, thus decreasing the efficiency of metal dissolution from solid substrates [9,12,13]. Heterotrophic microbe has gained

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an increasing attention since it can use the organic matter as energy to eliminate these toxic organic compounds to chemolithoautotrophic bacteria.

Xiangjiang River is a major tributary of the Yangtze River in Hunan province. A large number of non-ferrous metal and chemical enterprises, whose pollutants entering this river located in this valley, have contaminated the Xiangjiang River [14], and emissions of arsenic, chromium, cadmium, lead, and mercury in Hunan have ranked the first in China [15,16]. The basin suffered severely heavy metal pollution over a period of around 50 years. Simultaneity, the river organic pollution is an increasingly serious problem in recent years [17,18]. But little has been done about the removal of heavy metals from the highly contaminated alkaline sediments.

This work used an artificial consortium constructed by auto- and heterotrophic bacteria to improve the efficiency of removal of heavy metals from highly contaminated dredged alkaline sediments of Xiangjiang River in a stirred tank reactor, and the process was based on: firstly, inocula of heterotrophic bacteria mixture (HBM) able to oxidize organic compounds such as organic acids, simple sugars and amino acids, secondly inocula of *Pseudomonas aeruginosa* ZH able to assist the growth of sulfur-oxidizing microorganism by consuming microbial metabolites and oxidizing S^0 produced acid which is benefit for the sulfur-oxidizing microorganism growth and *Acidithiobacillus thiooxidans* DMC able to oxidize sulphur and sulfide.

2 Experimental

2.1 Contaminated sediment

The sediment for the present study was collected from the Xiawan port of Xiangjiang River located at Zhuzhou city, in September, 2010, which is an industrial town in Hunan Province. Sediment (0−10 cm in depth) was sampled and mixed completely and temporarily stored at 4 °C. Some sample was air-dried and divided into two parts, one was for bioleaching, and the other was processed with 2 mm sieve pore size for physiochemical analysis.

2.2 Sediment analysis

The selected physicochemical properties of sediment were analyzed according to the routine methods: pH of soil was measured on 1:2.5 (soil to distilled water) soil slurry. Total organic carbon (TOC) content was determined by loss-on-ignition, combusting at 450 °C for 4 h in a muffle furnace and at 105 °C for 7 h. The total concentrations of metals in sediment were analyzed by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500 series, USA). Fractionation of heavy metals present in sediment before and after bioleaching was carried out by selective sequential extraction following TESSIER et al [5] and then determined by ICP-MS.

2.3 Microorganism

This study used a procedure mixture of microorganisms, including autotrophic *A. thiooxidans* DMC, heterotrophic *P. aeruginosa* ZH, and heterotrophic bacteria mixture (HBM), which were isolated and preserved by the Key Laboratory of Biometallurgy of Ministry of Education. We used fresh sediment as the seed sediment to enrich and culture the indigenous heterotrophic bacteria added to LB medium. Until mixture resistant abilities to Zn^{2+} and Cd^{2+} were up to 1.5 g/L respectively, the inocula for the bioleaching experiments were obtained. The *P. aeruginosa* ZH and *A. thiooxidans* DMC were routinely cultivated in 9K medium with S^0 (1%, w/v), and S^0 and glucose as the energy source, respectively [19].

2.4 Bioleaching experiment

Bioleaching tests were performed in a 3 L glass cylindrical with 15 cm in inner diameter and 17 cm in height equipped with a pH controller, a temperature controller, and a stirrer. As shown in Fig. 1, the reactor was placed in a thermostatic bath to keep the temperature constant at 30 °C for 48 d. Working volume of the reactor was 1 L containing 90% (v/v) of distilled water, 5% (w/v) of contaminated sediment, and 1% (w/v) of S^0, 10% (v/v) of (100 mL) mixed proportional culture of HBM, *P. aeruginosa* ZH and *A. thiooxidans* DMC as inoculum. At first, the bacteria mud of HBM was inoculated into the tank reactor, in which the cell was approximately 1×10^6 mL^{-1}. Two days later the 100 mL equal proportional culture mixture (2×10^6 mL^{-1}) of *P. aeruginosa* ZH and *A. thiooxidans* DMC was

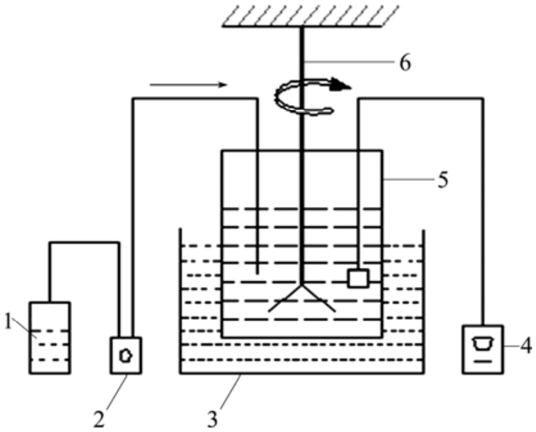

Fig. 1 Layout of bioleaching experimental apparatus (1—Distilled water; 2—Peristaltic pump; 3—Thermostat water bath; 4—pH meter; 5—Glass cylindrical reactor; 6—Stirrer)

inoculated into the reactor. Two sets of controls were used during the experiment. Chemical control was maintained using autoclaved uninoculated sulfur amended sediment and other conditions were the same as those used in experiments. In biological control, the sediment was inoculated and maintained without addition of S^0. We also analyzed the fraction variation of heavy metal before and after bioleaching by selective sequential extraction following TESSIER et al [5]. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to analyze the microbial community after bioleaching using a protocol described by ZHU et al [20].

The reaction process was monitored by periodic sampling and analysis of the sediment suspension for pH, oxidation–reduction potential (ORP), SO_4^{2-} and soluble heavy metals during the leaching period (48 d). pH, ORP and SO_4^{2-} were measured once 2 d and soluble heavy metals once 4 d. pH and ORP were measured using pH S-3C acid meter and platinum electrode with an Ag/AgCl reference electrode, respectively. Aliquot samples of 10 mL were drawn from the reactor centrifuged at 10000 r/min for 20 min. Then supernatant was filtered through a 0.45 μm filter membrane and analyzed for the sulfate and heavy metal. Sulfate was measured by turbidimetric method according to the procedure in standard methods [21]. Heavy metal concentrations in the samples were measured using ICP-MS. And SPSS 18.0 software was also used to analyze the relation between metal solubilization and sulfate concentration. The loss of water content in the reactor due to evaporation and sampling was replenished with distilled water by mass difference method.

3 Results and discussion

3.1 Physico-chemical characteristics of sediment

The partial physicochemical characteristics of the sediment are presented in Table 1. Sediment was found to be slightly alkaline in nature and rich in TOC. The organic compounds maybe present in the sediment in association with metals can inhibit chemolithoautotrophic bioleaching bacteria. The contents of Zn, Mn and Pb are the highest, followed by As, Hg and Cd. Fractions extracted by the selective sequential extraction procedure before and after bioleaching were used, in which metals are divided into exchangeable (F1), bound to carbonates (F2), bound to Fe−Mn oxides (F3), bound to organic matter (F4), residual (F5) [5]. The metal partitioning of sediment, as determined by the sequential chemical extraction, is shown in section 3.6.

Table 1 Physico-chemical characteristics of sediment

Parameter	Value
pH	7.7
TOC(w/w)/%	8.62
ρ(Cd)/(mg·kg^{-1})	74.8
ρ(Zn)/(mg·kg^{-1})	2273.1
ρ(Pb)/(mg·kg^{-1})	777.8
ρ(Hg)/(mg·kg^{-1})	79.3
ρ(As)/(mg·kg^{-1})	492.0
ρ(Mn)/(mg·kg^{-1})	1295.0

3.2 pH and ORP change during bioleaching

In bioleaching, elemental or reduced sulfur compounds are oxidized to sulfuric acid by the sulfur-oxidizing leaching bacteria, resulting in acidification of the sediment and promoting the leaching bacteria growth synchronously. Solubilization of heavy metals in bioleaching is highly pH-dependent [22], pH is the most important factor that influences metal solubilization during metal bioleaching. However, this leads to a final disposal needed to adjust pH of treated sediments to natural environment levels. The pH changes during bioleaching in the bioreactors are shown in Fig. 2. The results reveal that in the biological control without inoculation of bacteria and in chemical control without S^0, both two sets, the pH basically unchanged. In the first 2 d the sediment pH did not decrease and even slightly increased from 7.8 to 8.2, then decreased gradually to 6 in the first 14 d; the final pH was stabilized at about 1.0 in the first 44 d. This phenomenon of first increase and then decrease in pH may be due to a fact that heterotrophs consume organic matters, especially organic acids. The pH in bioleaching process of contaminated sediment is affected by the solids content of the sediments. The higher the sediment solid content, the higher the buffering capacity [23]. The pH in the bioleaching process is also related to the TOC in the sediment and TOC of this sediment is high, so the rate of

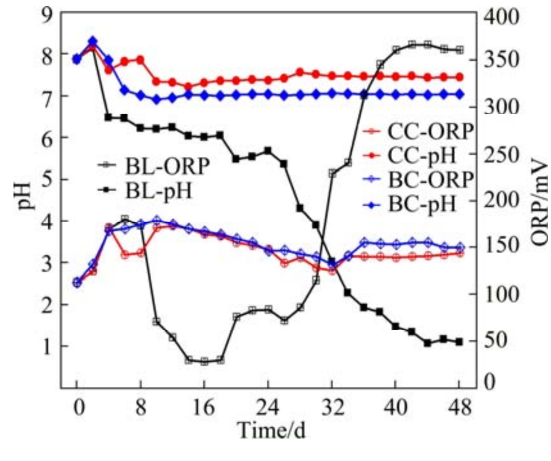

Fig. 2 Variation of pH and ORP in bioleaching process (BL—Bioleaching; BC—Biological control; CC—Chemical control)

decline of sediment pH of this work is less than that in the previous studies [24]. In the initial 6 d the heterotrophs consumed organic matter, and later during the growth of autotrophic bacteria, the pH declined. Then buffering capacity was slowly diminished by continuous production of acid from the oxidation of sulfur compounds by *A. thiooxidans*, so the pH rapidly declined 18 d later. Chemical control indicated that the endogenous bacteria do not play a role in bioleaching. And our previous research about the ecology of the sediment indicates that the microorganism community structure of the sediment has little sulfur-oxidizing bacteria [20]. Biological control indicated that addition S^0 as energy source of chemolithoautotrophic bacteria is a warrant for bioleaching process. So, to achieve sufficiently low pH, a good amount of S^0 and auto-and heterotrophic bacteria should be necessary.

Changes of oxidation–reduction potential (ORP) indicate the level of activity of sulfur-oxidizing bacteria. The variations in ORP during the bioleaching process are presented in Fig. 2. According to Fig. 2, the ORP of control sets stabilized at about 145 mV during the bioleaching period. In contrast, the ORP of the experimental group caused an increase by the oxidation of sulfur rapidly in 25 d. The ORP values increased to 365 mV when the final pH 1.0 was reached. Compared with the variation of pH in bioleaching, the variations of ORP were much related to those of pH. The increase of ORP had a similar trend with the decrease of pH.

3.3 Sulfate production during bioleaching

The sulfate concentration increased with the bioleaching time, resulting in the sulfur-oxidization. Sulfate production during bioleaching is the indicator for sulfur-oxidizing bacteria activity. Figure 3 shows the sulfate concentration produced in the bioleaching process. The sulfate concentration of chemical and biological controls basically unchanged. The sulfate production increased from 687.6 mg/L to 20324.1 mg/L in the bioleaching, showing that the S^0 is oxidized to sulfuric acid by sulfur-oxidizing organisms.

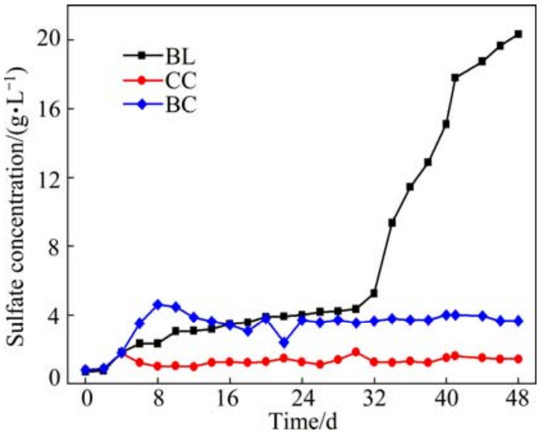

Fig. 3 Sulfate production during bioleaching process (BL—Bioleaching; BC—Biological control; CC—Chemical control)

3.4 Microbial life

At the end of bioleaching, analyses of the community structure of leachate and slag of the experiment group were carried out by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on 16S rDNA (Genbank accession numbers JF906976-JF906981). The results confirmed the former ecological data that the original sediment has a little sulfur-oxidizing bacteria [20]. And the mainly bacteria were *P. aeruginosa* and *A. thiooxidans*, whereas the heterotrophs cannot be detected at low pH. But the two kinds of bacteria distributions between leachate and slag were different; in the leachate the quantity ratio of *A. thiooxidans* to *P. aeruginosa* was 8:1 and in the slag the cell mainly was *A. thiooxidans*. Further research on the mechanism is required to better explain this phenomenon.

3.5 Heavy metal solubilization during bioleaching

The amounts of heavy metals (Zn, Cu, Cd, Mn, Pb, As, Hg) leached from the Xiangjiang River sediment during the bioleaching process are given in Fig. 4. Compared with the two controls, it is evident that the result of experimental group is satisfactory. Figure 4 reveals that the efficiencies of Zn, Mn, Cu and Cd were 95.2% 94.2% 90.1% 84.4%, respectively. These metals had good solubilization efficiency during the bioleaching process. By contrast, when pH reached a certain value, the efficiencies of As, Hg and Pb were 26.0%, 15.9%, 19.9%, respectively. The efficiency of metal solubilization from contaminated sediment is in the decreasing order: Zn>Mn>Cu>Cd>As>Pb>Hg, and the correlation between sulfate and Hg, Pb, Zn, Cd, Mn, Cu, As solubilization was significant with p=0.000 of all the metals and the Pearson coefficients were 0.981, 0.956, 0.896, 0.976, 0.968, 0.940, 0.980, respectively. Sulfate is a very important factor that affects the metals solution.

During the bioleaching period, Zn appeared first in the leachate and at the beginning a small amount of Zn^{2+} existed in the suspension, since Zn hydrolyzed at pH 7.0−7.5 [3]. Mn began to be solubilized after 4 d of bioleaching when pH declined to 6.5 and then slowly stabilized until the end of bioleaching. Solubilization in this test was lower than that in the previous research [25,26]. Until 28 d later only 1% As was solubilized and it was up to 26.0% at the end of bioleaching when pH was about 1. The efficiency of Pb solubilization was not high in the bioleaching process. The result showed the same response with previous literatures [24,27] that the solubilized Pb forms $PbSO_4$ with sulfate, which has low

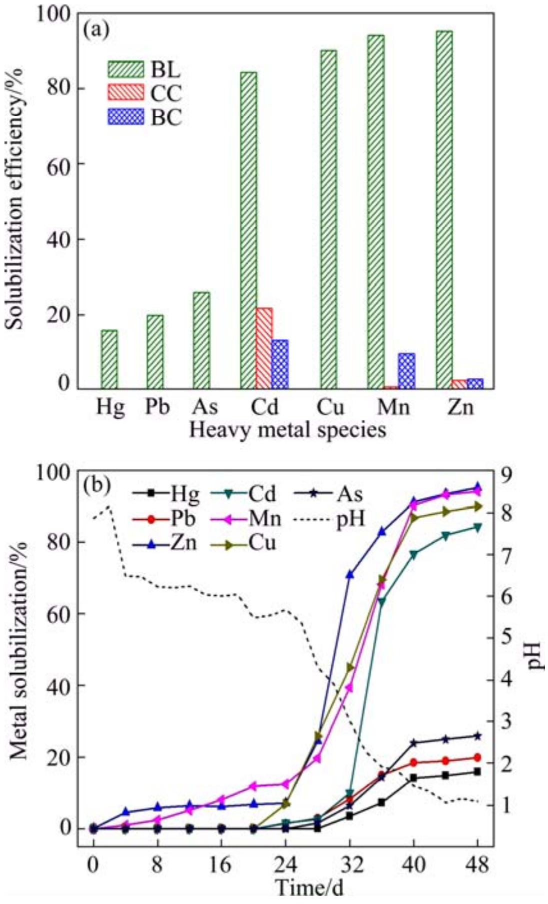

Fig. 4 Solubilization of heavy metals: (a) Final solubilization rate of heavy metals in different tests (BL—Bioleaching; BC—Biological control; CC—Chemical control); (b) Changes of heavy metals with time and pH during bioleaching

solubility (K_{SP}=1.62×10^{-8}), resulting in a decreased solubilization efficiency of Pb. After 32 d only 3.4% Hg was solubilized when the pH decreased to 2.5, but the bioleaching efficiency increased up to 15.9 % in 48 d.

Taken together, heavy metals such as Zn, Mn and Cu in the contaminated sediment were cleaned satisfactorily and their contents were below levels of environmental quality standard for soils (GB15618−1995). The remaining concentrations of Hg and As in sediment seem to be unsatisfactory.

3.6 Fraction variation of heavy metal during bioleaching

Figure 5 shows the chemical fractions of heavy metals as percent of their total concentrations in original sediment and the experiment group sediment after bioleaching for 48 d. Figure 5 reveals that the distribution of the metal fractions varied widely in the original sediment and had a significant change in partitioning of heavy metals after bioleaching. The original sediment As mainly bound to F1 and F5, Cd mainly bound to F2, Cu mainly bound to F4 and F5, Hg mainly bound to F1, F3, F4 and F5, Mn mainly bound to F2, F3 and F5, Pb mainly bound to F5, Zn bound to F2, F3 and F5. After bioleaching the distribution of the metals is mainly bound to F4 and F5, which are considered to be stable and non-bioavailable. Removing of residually bound fractions may be not necessary [28]. However, in this sediment bioleaching, the heavy metals in organic and residual forms can also be partially released in this study, which is consistent with result of TSAI et al [29]. The bioleaching treatment resulted in an overall decrease in heavy metal contents in sediment, except for Hg and As. Compared with the original sediment, the contents of metals in the sediment were lower than the permissible levels prescribed for unrestricted ones after bioleaching

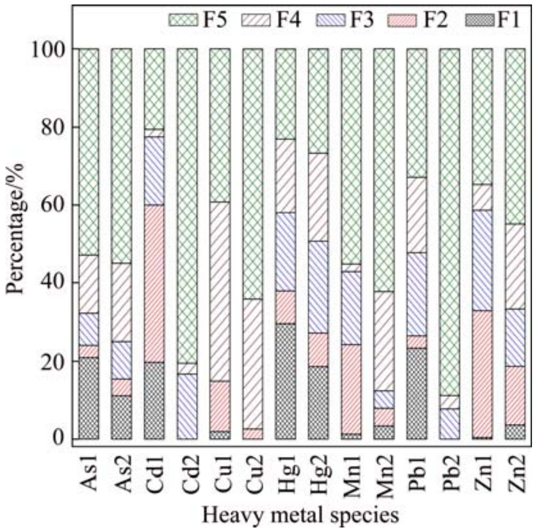

Fig. 5 Chemical fraction distribution of metals in sediment before (bar 1) and after (bar 2) bioleaching: F1—Exchangeable; F2—Carbonate associated; F3—Fe−Mn oxide-associated; F4—Organic associated; F5—Residual

4 Conclusions

1) The method of using consortium constructed by auto- and heterotrophic bacteria simultaneity and fed with S^0 as energy to remedy the contaminated alkaline sediment is workable.

2) Heterotrophic bacteria consume organic compounds as inhibitors to autotrophic microbes. S^0 is an essential energy substrate for the growth of sulfur-oxidizing and bacterially catalyzed metal solubilization in the bioleaching process. During bioleaching, the decrease of pH followed by the opposite trend with the increase of ORP and sulfate concentration.

3) Bioleaching was found to be efficient for removal of Zn, Cd, Cu and Mn from Xiangjiang River alkaline sediments. After bioleaching the metals residuals in the sediment are lower and are mainly bound to organic associated forms and residual forms.

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重金属污染碱性底泥的自养与异养细菌协同搅拌浸出

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摘 要：探讨了生物浸出法处理湘江河床多种重金属污染的碱性底泥。底泥中的多种重金属对水生生物与人类有较大的毒性以及显著抑制细菌的浸出，利用自养细菌与异养细菌协作的浸出方法可以解决这个问题。结果表明，底泥中锌、锰、铜和镉的生物浸出率分别达 95.2%、94.2%、90.1%和 84.4%。利用连续提取法分析了浸出前、后底泥中不同形态重金属的含量变化，发现浸出后底泥中残余重金属主要以铁锰氧化态、有机结合态和残渣态的形式存在，这些存在形态的生物毒性较低。本研究说明自养菌结合异养菌的浸出法可有效提高碱性底泥重金属的浸出率和降低生物毒性。

关键词：生物浸出；碱性底泥；重金属；连续提取；自养与异养细菌

(Edited by Hua YANG)