



Uranium bioleaching from low-grade carbonaceous-siliceous-argillaceous type uranium ore using an indigenous *Acidithiobacillus ferrooxidans*

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Abstract

We evaluated the effectiveness of bioleaching uranium from a low-grade carbonaceous-siliceous-argillaceous type uranium ore using an indigenous iron-oxidizing bacteria, *Acidithiobacillus ferrooxidans*, isolated from local uranium ore. The effects of initial acidity, pulp density and ferrous ion concentration of the feed solution were investigated. The uranium (U₃O₈) content was 0.036% by weight. Using uranium ore acidified leachate as medium with initial ferrous ion concentrations of 3 g/L, pH 1.7 and pulp density of 20% as optimal conditions, the maximum rate of dissolved uranium recovery was 85.14%. This approach is thus, suitable for recovering uranium from low-grade CSA ore using bacterial leaching.

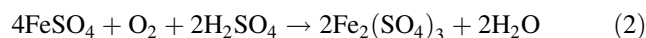
Keywords Uranium bioleaching · Low-grade · Carbonaceous-Siliceous-Argillaceous uranium ore · *Acidithiobacillus ferrooxidans*

Introduction

Carbonaceous-siliceous-argillaceous (CSA) type uranium deposit is one of the four types of uranium deposits in China and is an unconventional uranium resource. It has the properties of shale stability, large thickness and complex composition, with an abundance of organics, pyrite, clay, silica and phosphorus, a typical uranium content of 0.03–0.05% by weight [1, 2]. For CSA uranium ore leaching, the chemical acid extraction method is widely used in large operations. However, this process is accompanied with significant disadvantages, such as high

costs due to the addition of sulfuric acid and an oxidant (usually MnO₂, H₂O₂, or KClO₃), environmental non-compatibility and potential ecological risks [3–7]. Therefore, the development of a new strategy that focuses on an effective and environmentally-sound leaching process of low-grade CSA uranium ore is necessary.

The process of bioleaching has the potential to save costs, which is environment friendly and can recycle resources for the hydrometallurgy of metal ores [8, 9]. The mechanism of the uranium bioleaching process is that the bacteria oxidized pyrite (FeS₂) and soluble Fe²⁺ to Fe³⁺ as the leaching oxidants detailed in Eqs. (1–2), Fe³⁺ readily attacks minerals incorporating U⁴⁺ to oxidize it to U⁶⁺ according to Eq. (3).



The Fe²⁺ can be re-oxidised by microbes to Fe³⁺, which takes part in the oxidation process again. In an acidic solution Fe²⁺ is stable and the leaching mediated by Fe³⁺ would be slow. The bio-oxidation is about 10⁵–10⁶ times faster than the chemical oxidation thus enhancing the

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uranium dissolution rate. It is believed to be one of the most promising technologies because it consumes less acid, does not have a requirement of an oxidant additive, is a simple process with no need of sophisticated operator training and it can successfully treat certain mineral assemblages not amenable to treatment by conventional processes [10]. Moon-Sung Choi [11] reported the feasibility of bacterial recovery of uranium from low-grade black schists from the Okcheon District, Korea, using *A. ferrooxidans*. The uranium recovery reached 80%, while only 18% was leached without involving microbial activity. Over the past few decades, the bioleaching process has been applied for the extraction of copper [12–14], zinc [15, 16], gold [17], uranium [18–21], multiple metals [22, 23] and precious metals from printed circuit boards [24–26]. Therefore, this technique is proving to be a profitable alternative to the conventional processes.

With the rapid development and construction of China's nuclear power program, the demand for uranium resources has increased dramatically. Based on the view that CSA uranium ore and other unconventional uranium resources would make a positive contribution to the sustainable supply of uranium resources, officials have already begun to pay attention to the exploitation of uranium from CSA uranium deposits in China and many other countries and regions. In this study, the feasibility of uranium bioleaching from low-grade CSA uranium ore was explored. The effects of critical parameters was comprehensively studied in batch experiments.

Experimental

Preparation of uranium ore samples

The CSA uranium ore used in this investigation was collected from a local uranium mine in the Xiushui District, Jiangxi Province, China. For experimental use, the ore samples were prepared in a three-stage crushing operation. Initially, the uranium ore samples were manually cut into small pieces (diameter < 50 mm) using stainless steel blades. Next, the ores were crushed by a vibration jaw crusher. Finally, the uranium ores were sieved using a vibrator shifter through 16 mesh screen (diameter < 1 mm) dried to a constant weight in a drying oven at 105 °C and used for all the leaching experiments.

Microorganism isolation, culturing and domestication

The bacteria strain used in the experiments was *A. ferrooxidans*, which was isolated from the CSA uranium ore. The isolation was effected by using a dilution/enrichment

technique in ferrous sulphate medium followed by single-colony isolation on silica gel solid medium. 16S rDNA sequencing and phylogenetic analysis [27] identified the isolate as an indigenous acidophilic strain *A. ferrooxidans*, which belongs to the autotrophic bacteria. Specifically, it was an aerobic microorganism capable of oxidizing Fe^{2+} and S to obtain the energy required for growth.

The bacteria strain of *A. ferrooxidans* was cultured and domesticated in a modified 9 K medium composed of CSA uranium ore. The composition of the modified 9 K medium was as follows: 24.8 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ as an energy source, 3.0 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g of K_2HPO_4 , 0.1 g of KCl, 0.01 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02% (w/v) yeast extract and 1000 mL distilled water. *A. ferrooxidans* was inoculated at 20% (v/v) in a 250 mL Erlenmeyer flask containing 100 mL of 9 K medium, 20 g CSA uranium ore, an initial Fe^{2+} concentration of 5.0 g/L and the initial pH adjusted to 1.8 using 5.0 M sulfuric acid. The culture was incubated at 30 ± 1 °C on a constant-temperature rotatory shaker at 120 rpm. Bacteria inoculation would be repeated until the oxidation rate of Fe^{2+} reaches 90%. The bacteria stocks used in the leaching experiments were adapted to the CSA uranium ore through continuous subculturing in the way described above until a constant iron oxidation rate was achieved.

Bioleaching experiments

In order to investigate the influence of sulfuric acid content, pulp density and initial Fe^{2+} concentration, a series of flasks were arranged with sulfuric acid content at 1 g/L, 3 g/L, 5 g/L, 10 g/L, 20 g/L and 30 g/L; pulp density at 10% (w/v), 20%, 30%, 40% and 50%; initial Fe^{2+} concentrations of 0 g/L, 1 g/L, 3 g/L and 5 g/L. The bioleaching experiments were conducted using a 250 mL triangular flask and incubated at 30 °C with shaking at 120 rpm. At different elapsed times, samples were collected and filtered through 0.22 μm membranes. The samples were analyzed for pH, redox potential (Eh), uranyl-VI (U^{6+}), ferric ion (Fe^{3+}) and ferrous (Fe^{2+}) concentration.

Analytical methods

The chemical composition of uranium ore sample was analyzed using a wavelength-dispersive X-ray fluorescence spectrometer (WD-XRF, Tiger S8, Germany) described in our previous study [28]. The pH and Eh (redox potential) were measured using a pH meter (ST3100, Ohaus, USA). The concentration of ferrous and total iron concentration in solution were determined by titration with EDTA. Concentration of the ferric iron was calculated by subtracting the concentration of ferrous from the total iron. Uranyl ions in solution were assayed using titanium

reduction-ammonium vanadate titration [18]. The surface characteristics and morphology of the bioleaching residues were characterized by scanning electron microscopy (SEM, QuantaTM 250, FEI, USA). The X-ray diffraction (XRD, Bruker D8, Germany) characterizations were conducted for the phase composition detection.

Results and discussion

The mineral components of uranium ore

The chemical composition of raw uranium ore sample is listed in Table 1.

Analysis of the sample using XRD indicated that quartz (SiO_2), pyrite (FeS_2) and hematite (Fe_2O_3) were the dominant component present and that it contained small amounts of other impurities including illite ($\text{KAl}_2[(\text{Al},\text{Si})\text{Si}_3\text{O}_{10}](\text{OH})_2 \cdot n\text{H}_2\text{O}$), plagioclase ($\text{Na}(\text{AlSi}_3\text{O}_8)$, $\text{Ca}(\text{Al}_2\text{Si}_2\text{O}_8)$) and kaolinite ($\text{Al}_4(\text{Si}_4\text{O}_{10})(\text{OH})_8$).

The growth curve of *A. ferrooxidans*

Analysis of the ferrous oxidation rate of *A. ferrooxidans* in modified 9 K medium was conducted under conditions of pH 1.8, an initial Fe^{2+} concentration of 5 g/L and a pulp density of 20%. The growth curve of *A. ferrooxidans* is shown in Fig. 1.

As it depicted in Fig. 1, the ferrous oxidation rate of the bacteria was very slow from 0 to 50 h, bacteria grew in the lag phase and adapted to the new leaching environmental conditions. The bacteria steadily grew from 50 to 105 h and the oxidation rate of Fe^{2+} reached approximately 2.5 g/(L·d) in 105 h. Bacteria with high oxidation activity

Table 1 Chemical composition of raw low-grade CSA uranium ore sample

Component	Contents (wt. %)
SiO_2	60.54
Al_2O_3	7.16
Fe_2O_3	4.00
FeO	0.236
CaO	0.123
MgO	5.02
K_2O	1.77
Na_2O	0.037
TiO_2	0.504
MnO	0.066
P_2O_5	0.2
CO_2	0.18
$\text{C}_{\text{organic}}$	13.01
U	0.036

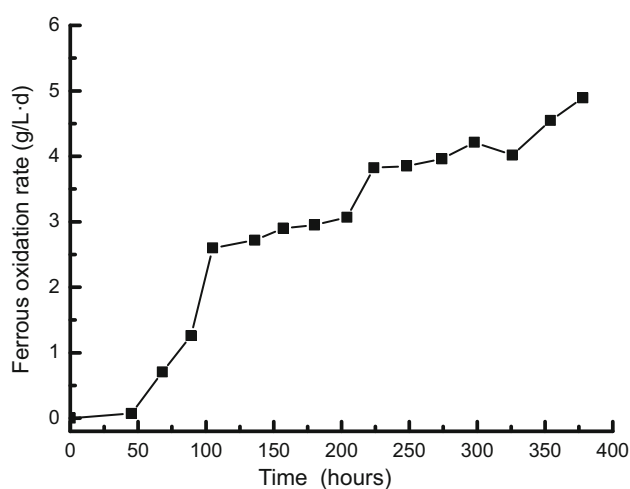


Fig. 1 Ferrous oxidation rate of bacteria in modified 9 K medium with time (conditions: 1.8 pH, 5 g/L Fe^{2+} , 20% PD, 30 °C, 120 rpm)

were obtained after 378 h of domestication and culturing. The oxidation rate of Fe^{2+} was greater than 4.3 g/(L·d), which indicated that *A. ferrooxidans* adapted remarkably well to the CSA uranium ore leaching solution following its acclimatizing culture. Good viability and ferrous oxidation capacity was gradually discovered during the subsequent bioleaching process.

Effect of sulfuric acid concentration

Acidified sulfuric acid concentration (or pH) is a crucial parameter for bacterial leaching of metals. In this study, the additive content of sulfuric acid was controlled at 1 g/L, 3 g/L, 5 g/L, 10 g/L, 20 g/L and 30 g/L. The pulp density was 40% (w/v) and the initial Fe^{2+} concentration was 5 g/L. The uranium leaching rate and pH change with respect to different initial sulfuric acid concentrations are depicted in Fig. 2.

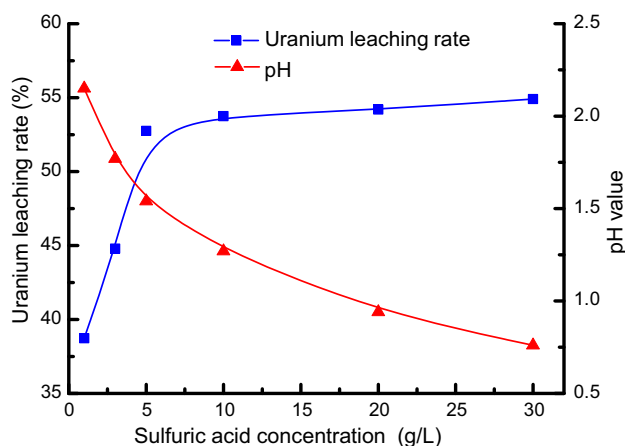


Fig. 2 Uranium leaching rate and pH with respect to different initial sulfuric acid concentration (other conditions: 5 g/L Fe^{2+} , 40% PD, 30 °C, 120 rpm)

As shown in Fig. 2, an increase in sulfuric acid content lowered the pH of the system. An opposite trend for the uranium leaching rate vs. the initial acid content was observed. When the acid content ranged from 3 g/L to 5 g/L, the uranium leaching rate improved significantly and the corresponding pH was 1.77 to 1.54. Under conditions of relatively low pH (< 2.0), the formation of jarosite through iron precipitation is inhibited [27]. The solid state of the uranium component should be efficiently oxidized to the dissolved form UO_2^{2+} by the ferric iron formed during microbial metabolism. As the acid content increased from 5 g/L to 30 g/L, the uranium leaching rate slowed. Any positive effect of acid consumption on the uranium leaching rate was negligible. The pH decreased from 1.54 to 0.76. The results indicated that higher concentrations of sulfuric acid could inhibit microbial growth and hinder the oxidation of U^{4+} to U^{6+} , which resulting in the increase of uranium leaching rate is slow and other problems associated with the dissolution of impurities. Furthermore, the increase in acid consumption would increase the investment costs.

A suitable acidity would reduce the extra mineral dissolution from the surrounding rocks, thus decrease the content of sulfuric acid and maintained the pH conditions for the microorganism metabolism. It has been documented [20] that *A. ferrooxidans* displays the highest uranium extraction capacity when the pH is less than 2.0. On the contrary, when the pH is greater than 2.0, a side reaction of the formation of jarosite occurs. This results in the ore surface being covered by the generated jarosite particulates and the uranium extraction is inhibited, leading to a decrease in the uranium extraction efficiency of the entire system.

Effect of pulp density

Experiments at different pulp densities of 10% (w/v), 20%, 30%, 40% and 50% were conducted under operating conditions of pH 1.7 and an initial Fe^{2+} concentration of 5 g/L (Fig. 3). The inoculated bacteria were in a 100 mL solution with a Fe^{2+} oxidation efficiency of 95%.

As shown in Fig. 3, Fe^{3+} and U^{6+} increased in relation with the increase in pulp density, while the uranium leaching rate underwent an opposite response. The reason for this phenomenon may be at low pulp densities, uranium minerals were easily extracted to the aqueous solution, which contributed to the high uranium leaching rate. However, because of the limitation of total uranium content in the added ore, U^{6+} concentration remained at a low level. The Eh value of the leaching solution was greater than 400 mV at all the pulp densities (Fig. 3b), which indicated that pulp density had no effect on the Eh of the leaching solution. A lower pulp density would be favorable

for a high uranium leaching efficiency. However, the amount of supplied water required would be increased. Also, along with the disadvantage of the lack of bacterial concentration and the intense acid consumption, the low pulp density also led to a low uranium concentration in the leaching liquid, which increased the difficulty of concentrating the leached uranium. Therefore, the dominant pulp density was determined according to a comprehensive consideration of the uranium leaching rate, the consumption needs of water and the amount of inoculated bacterial.

As shown in Fig. 3c, when the pulp density was 10% and 20%, the concentration of Fe^{3+} 5 g/L, the leaching efficiency was 88.33% and 84.17%, respectively. The uranium concentration in the leaching liquid was about 31 mg/L and 60 mg/L, respectively. This indicated that a pulp density ranging from 10% to 20% was most beneficial for uranium bioleaching. When the pulp density was greater than 30%, the concentration of Fe^{3+} in the system reached 7.17 g/L and the uranium leaching efficiency decreased to 70% (Fig. 4c). The high pulp density had a negative effect on the uranium bioleaching process. This may be ascribed to the improved shear force generated from the high pulp density, which was detrimental to the immobilization of the bacteria on the uranium ore surface. On the other hand, at a high pulp density the dissolved Fe^{3+} concentration increased dramatically and the components that increased the utilization of acid increased, the pH to easily exceed 2.0. This situation caused the precipitation of Fe^{3+} on the uranium ore surface, which further inhibited the dissolving of the uranium. On the other hand, *A. ferrooxidans* is noted to be a microorganism that uses carbon dioxide as carbon source and oxygen as an electron acceptor; so, under the high pulp density conditions, aggregated fine ore particles would be formed and the mass transportation of carbon dioxide and oxygen would be inhibited [11, 29]. Therefore, in subsequent experiments, a 20% pulp density was maintained.

Effect of ferrous ion concentration

Ferrous ion was the energy source for the uranium bioleaching process. The suitable Fe^{2+} concentration favored the growth of the microorganism and ensured an improved microorganism concentration and oxidation capacity. The cultured *A. ferrooxidans* was put in three triangle bottles each containing 20 g of uranium ore. The pulp density was 20% and the pH was 1.7. To investigate the influence of ferrous ion concentration on the uranium bioleaching efficiency, the initial ferrous ion concentration was controlled at 0 g/L, 1.0 g/L, 3.0 g/L and 5.0 g/L.

As shown in Fig. 4a, the oxidation rate of ferrous ion was increased with the higher concentrations of the ferrous ion. After leaching for 245 h, the ferrous ion concentration

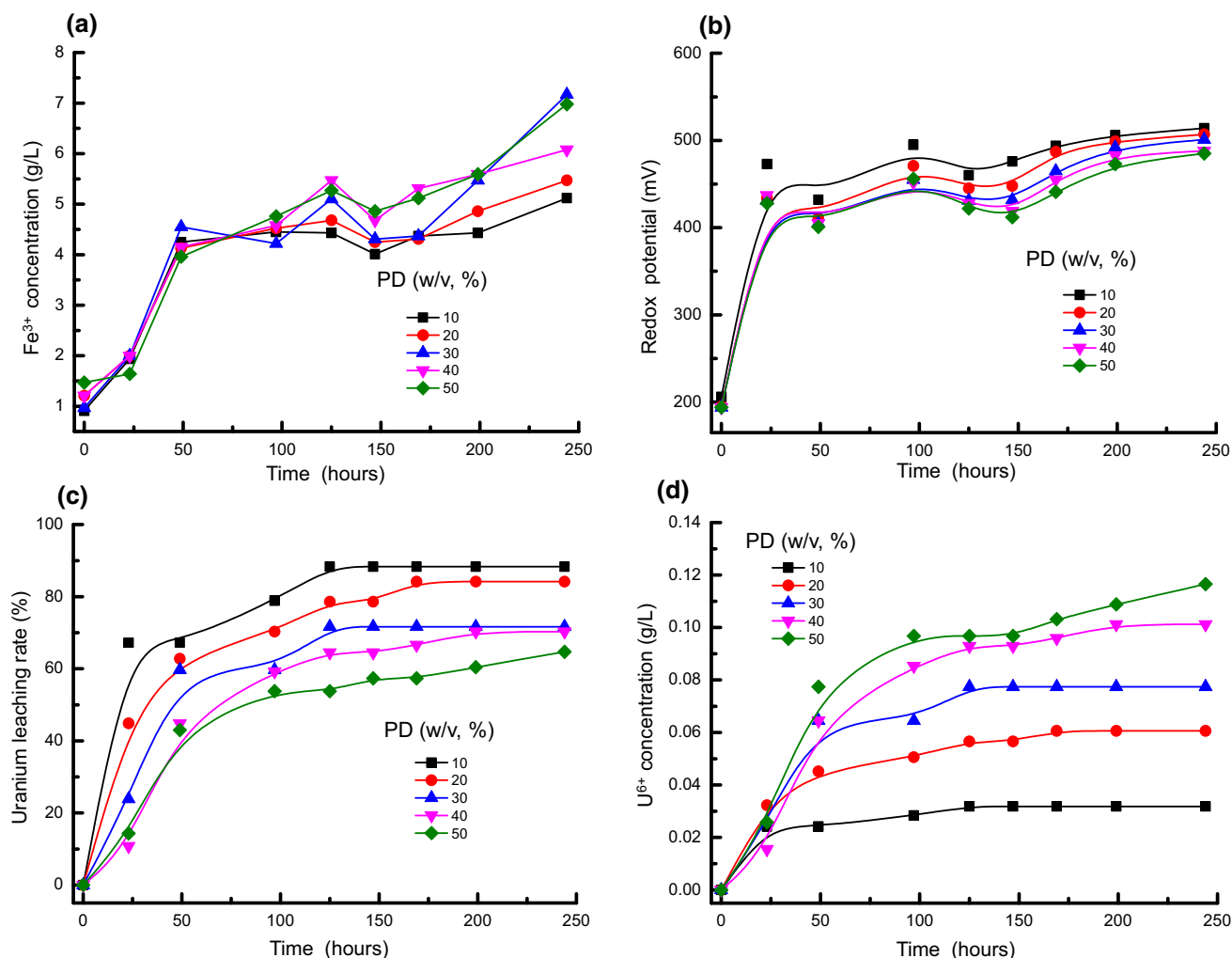


Fig. 3 Ferric iron concentration (a), redox potential (b), uranium leaching rate (c) and uranium concentration (d) under different pulp density with respect to time (other conditions: 1.7 pH, 5 g/L Fe^{2+} , 30 °C, 120 rpm)

decreased to 0.12 mg/L due to its consumption by the microorganisms (Fig. 4a). When the initial ferrous ion concentration was 0 g/L, the Eh was 460 mV (Fig. 4c) and the corresponding uranium bioleaching efficiency was only 68.89% (Fig. 4d). This indicated that a low ferrous ion concentration would result in a lower oxidation capacity and therefore to a lower uranium bioleaching efficiency.

The concentration of ferric iron and the Eh values were increased with the higher initial ferrous ion concentrations (Fig. 4b, c). When the ferrous ion concentration was 3 g/L and 5 g/L, the Eh value was maintained at 500 mV, the ferric iron concentration was 6.68 g/L and 8.20 g/L, respectively. This indicated that the ferric iron concentration had less of an effect on the Eh value and the leaching efficiency when the ferric iron concentration was greater than 6 g/L.

The highest uranium leaching efficiency was obtained when the initial ferrous ion concentration was 3 g/L

(Fig. 4d). This may be ascribed to the strongest bacterial activity and the highest capacity for the biological metabolism of carbon dioxide at the 3 g/L Fe^{2+} concentration. When Fe^{2+} concentration was 5 g/L, Fe^{3+} concentration of leaching solution reached 8.2 g/L, a decrease in the uranium leaching efficiency was observed (Fig. 4d). This indicated that at high Fe^{2+} concentrations, Fe^{2+} tended to be oxidized to Fe^{3+} by the microorganisms and precipitated onto the ore surface [27, 29]. It is clear that the formed precipitates would inhibit further dissolving of the uranium ores. Similar studies [30, 31] have established that only small quantities of ferrous ion and ferric iron are needed to maximize uranium solubilization.

SEM and XRD analyses of the leached residues

Representative results from SEM images of the leached residues from the different bioleaching conditions are

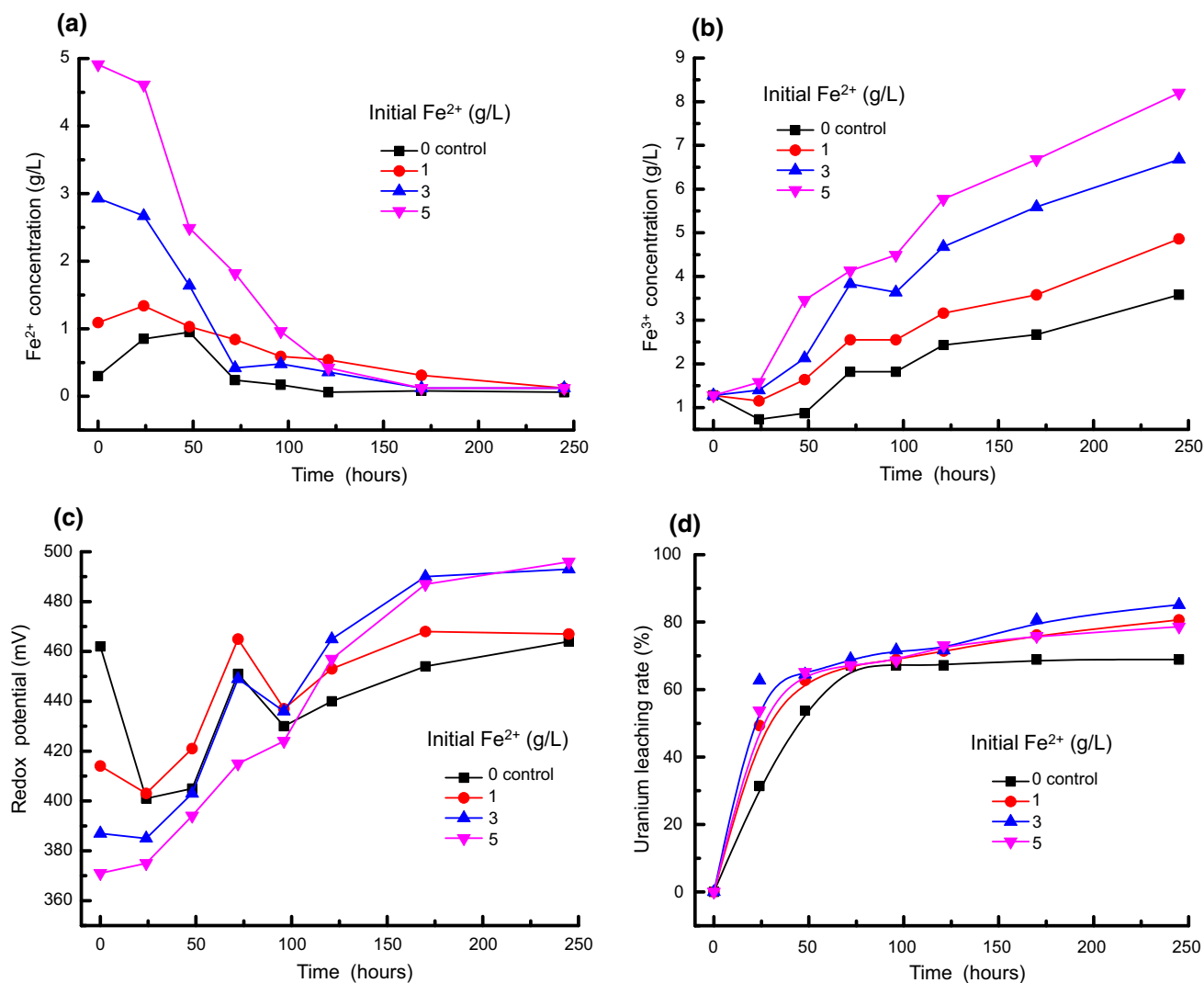


Fig. 4 Ferrous iron concentration (a), ferric iron concentration (b), redox potential (c) and uranium leaching rate (d) under different initial concentration of Fe^{2+} with respect to time (other conditions: 1.7 pH, 20% PD, 30 °C, 120 rpm)

shown in Fig. 5. It can be seen that the surfaces of the leached residues were wrapped by a layer of reaction product, which inhibited the movement and diffusion of the mineral surface bacteria and leaching agents in mineral fissures [21, 32, 33]. The layer on the residue obtained from the bioleaching with 5.0 g/L of additional external Fe^{2+} ions was dense surface and less porous than that with 3.0 g/L of additional external Fe^{2+} ions. The addition of Fe^{2+} ions accelerated the uranium bioleaching, while the concentration of additional Fe^{2+} over 3.0 g/L weakened this positive effect and even hindered the dissolution of the uranium.

The XRD data of the leached residues after bioleaching, at the different leaching conditions is presented in Fig. 6. It can be seen that the XRD of the leached residues after 245 h show that jarosite ($\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$) formed during the bioleaching process. The lower uranium leaching rate

may have been related to the passivation layer formed on the surface of mineral particles, which may have hindered the uranium bioleaching. It can also be seen that with additional 5.0 g/L Fe^{2+} ions, the intensity of peaks corresponding to jarosite were stronger than that observed with additional 3.0 g/L Fe^{2+} ions. This suggested that there was more jarosite produced on the surface of particle, which then inhibited the dissolution of the uranium. The results of the mineralogical analysis by XRD were also confirmed above SEM observations.

Conclusion

The native culture of the mesophilic bacteria *A. ferrooxidans* isolated from a source of mine water showed good potential for the bioleaching of uranium from the low-

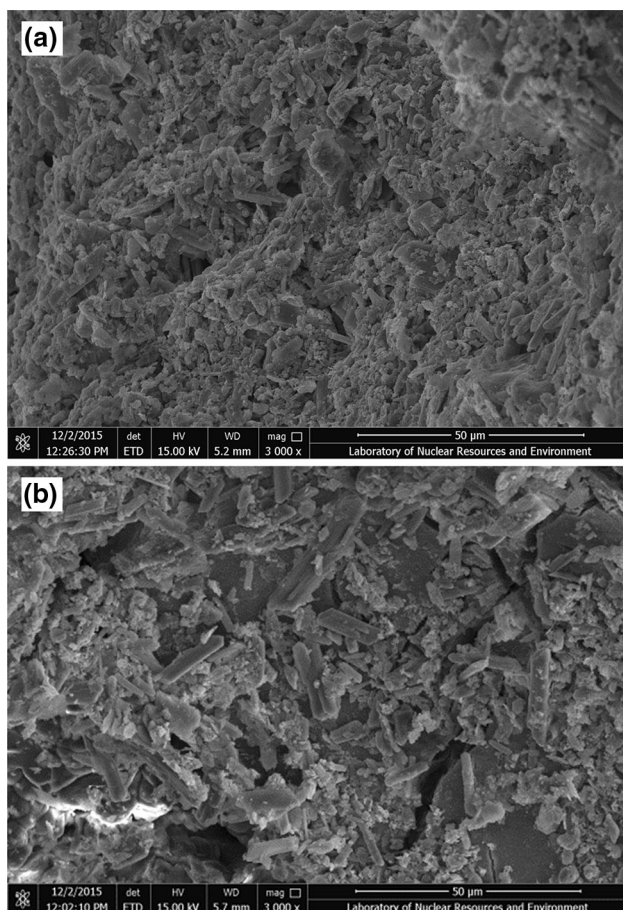


Fig. 5 SEM images of the bioleaching residues: (a) 3.0 g/L and (b) 5.0 g/L of Fe^{2+} ions

grade CAS uranium ore. The surrounding rock of the uranium ores used in this work was acidic, which contains less acid consuming gangue minerals. When the addition of sulfuric acid concentration was 5 g/L, the leaching solution pH was below 2.0, iron precipitation could be avoided. With the pulp density ranges from 10 to 20%, relatively high uranium bioleaching efficiency was obtained and the pulp density had almost no effect on bacterial growth or the final uranium leaching efficiency. Ferrous ion is the energy source for *A. ferrooxidans*. The additional external Fe^{2+} ions over 3 g/L resulted in the uranium leaching efficiency to decrease. SEM and XRD analyses of the leaching residues revealed that a product layer composed of jarosite was formed on the mineral surface when the concentration of additional Fe^{2+} was over 3 g/L, which resulted in a low uranium leaching rate during the later phase. A bio-recovery of 85.4% uranium in the leaching liquid was achieved in 10 days with the ore-adapted culture under the optimal conditions of pH 1.7, 20% pulp density, 30 °C, 120 rpm and with 3 g/L of additional Fe^{2+} ions. Furthermore, these experiments showed that the bioleaching

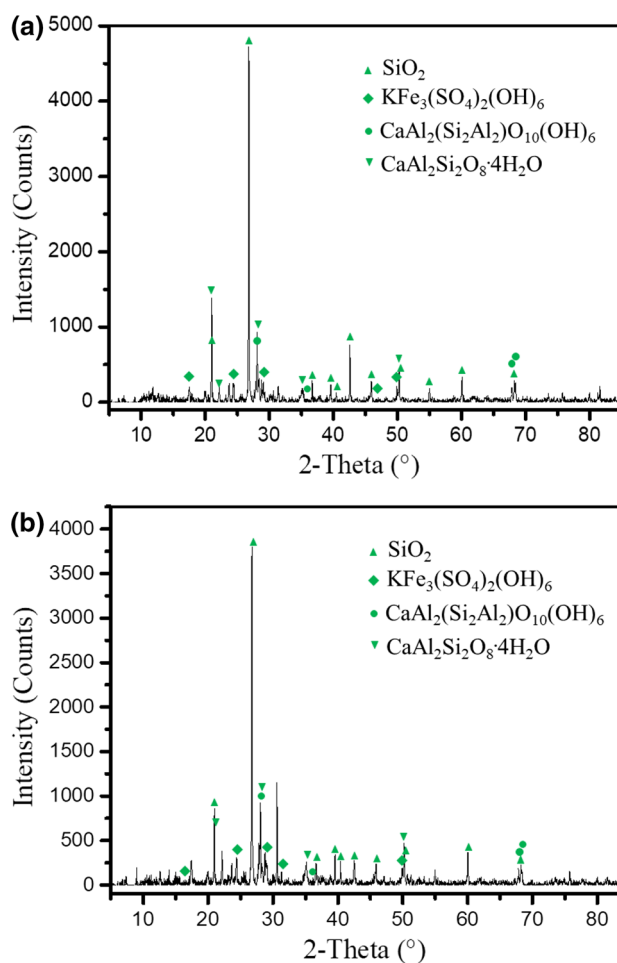


Fig. 6 XRD patterns of the bioleaching residues: (a) 3.0 g/L and (b) 5.0 g/L of Fe^{2+} ions

process can be more effective and economical for uranium extraction from low-grade CSA type uranium ores than other processes that are currently available.

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