

Study on Occurrence Speciation and Activity of Uranium Tailing Contaminants in the Presence of *S.oneidensis* MR-1 under Anaerobic Conditions

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Abstract. Taken a uranium tailings pond in Hunan province as the research object, three groups of reaction systems including min (mineral group), min-dbio (mineral-dead bacteria) and min-bio (mineral-viable bacteria) were set up for the sequential batch experiments. The metal elements of U, Fe, Cd, and Pb in the uranium tailings which have reacted with *S. oneidensis* MR-1 for 2d, 4d, 10d, and 20d were studied by the sequential chemical extraction methods, to analyzed the effect of species of contaminants in Uranium Tailings by *S. oneidensis* MR-1. The results showed that the residual state is the main occurrence mode of each metal element. The release of contaminants from tailings is mainly attributed to the dissolution of exchangeable state. The complexation or adsorption of the dead bacteria and metal elements leads to the increase of the organic matter. The effect of *S.oneidensis* MR-1 promoted the release of each metal elements in the exchangeable state, carbonate binding state, organic matter binding state and the amorphous iron manganese oxide binding state of tailings sample, and the reduction of *S. oneidensis* MR-1 caused U^{6+} to produce U^{4+} , resulting in an increase in the content of residuals, while Fe^{3+} was reduced to Fe^{2+} , resulted in an increase in the binding state of crystalline iron-manganese oxides.

1. Introduction

Nuclear power, as a safe, clean, low-carbon and reliable energy source, has been accepted and adopted by more and more countries in recent years [1]. According to the forecast of IAEA 2014, the world's total installed nuclear capacity will reach 401 to 699 gigawatts by 2030[2]. Along with it came the development of uranium mining industry and the production of uranium tailings. As of December 2014, there were 467 and 154 radioactive waste storage and disposal facilities worldwide, with storage and disposal volumes of $1.2 \times 10^8 \text{ m}^3$ and $7.6 \times 10^7 \text{ m}^3$, respectively[3]. These waste rocks and tailings sands contain a large amount of radionuclides and heavy metal elements, which will occupy a large amount of land. Under long-term exposure, harmful metal elements released from them may also migrate along with groundwater and surface water, thus causing various pollutions. Exposure to U can cause both



radiological and chemical toxicity [4]. Among the main chemical effects related to exposure to U and its decay compounds are kidney disease and lung cancer [5,6].

As the research progressed, the researchers found that the mobility and bioavailability of toxic pollutants in tailings not only depended on the size of the total amount, but also were influenced by their existing forms. Many studies have confirmed that different forms of heavy metals determine the size and bioavailability (i.e. bioavailability) of their migratory capacity, thus exhibiting different biological activities and toxicity [7,8]. Fe(III) reduction phenomenon mediated by iron-reduction microorganisms occurs at any time under anaerobic environment. Microbial alienation of Fe(III) may be one of the earliest forms of respiration on the planet [9]. It can affect the distribution of iron elements and the morphology of minerals, and the reduction process of iron has great influence on the speciation changes of heavy metals or toxic elements (such as chromium, arsenic, selenium, uranium, etc.) [10-12] in anaerobic environment. Uranium tailings contains large amounts of iron oxides. Therefore, the effect of typical dissimilatory Fe(III)-reducing bacteria *S.oneidensis* MR-1 on the occurrence mode changes of toxic metal elements in uranium tailings under anaerobic conditions was studied, which is of great significance to understand the toxicity and control the pollution of uranium tailings.

2. Materials and Methods

2.1. Reagent and instrument

Reagent: MgCl_2 , CH_3COONa , CH_3COOH , 30% H_2O_2 , $\text{CH}_3\text{COONH}_4$, $\text{C}_2\text{H}_2\text{O}_4$, $\text{C}_2\text{H}_8\text{N}_2\text{O}_4$, $\text{Na}_2\text{S}_2\text{O}_4$, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, NaHCO_3 , HNO_3 , HCl , HF , H_3PO_4 , $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, TiCl_3 , NaNO_2 , $\text{CO}(\text{NH}_2)_2$, $\text{C}_{12}\text{H}_{10}\text{NNaO}_3\text{S}$, NH_4VO_3 , *et al.* The reagents used in the experiment are analytical pure, and the water used in the experiment is pure water ($\text{pH} = 7$).

Instrument: AA-6300 atomic absorption spectrophotometer; 721 visible spectrophotometers; Z36HK bench-top high-speed refrigerated centrifuge; SHA-B thermostatic water bath shaker.

2.2. Experimental Method

2.2.1. Treatment of uranium tailings. 5 samples of tailings were randomly collected from a uranium tailings pool in Hunan Province of China. The samples were dried naturally at room temperature, then crushed, mixed uniformly, and milled to 200 mesh by ball mill. Finally, the samples were dried at 105°C for 2 hours until constant weight.

2.2.2. Experimental strains and culture. *S.oneidensis* MR-1 was selected as the bacterial strain. The bacteria were activated, enriched and cultured in the sterilized LB liquid medium, then centrifuged for 10 min under the condition of 3000 g to collect the bacteria. The bacterial suspension with concentration of 1g/L was prepared and set aside. Another small amount of broth was placed in a constant temperature water bath at 80°C for 20 minutes to obtain a suspension of dead bacteria [13].

2.2.3. Experimental design. The experiment was divided into 3 groups (Table 1). The min group was the tailings group without bacterial, the min-bio group was the group of added bacteria and tailings, and the min-dbio group is the control group for investigate the effect of bacterial activity on contaminant release in tailings, which was added the killed bacteria liquid and tailings.

Table 1. Experimental Design

Groups	Bacterial inoculation quantity (mL)	mineral dust(g)	culture medium(mL)	Parallel group number
Min	0	2.0	100	4
Min-bio	2	2.0	98	4
Min-dbio	dead bacteria liquids 2	2.0	98	4

The experimental containers were 150 mL erlenmeyer flask. According to the experimental design, the experimental sample was added into the sterilized erlenmeyer flask and sealed with butyl rubber plug, then washed into the mixed gas (high purity nitrogen: carbon dioxide=4: 1) for 15 min. After the gas was inflated, all rubber tubes were sealed and placed in a biochemical incubator at a constant temperature of 30°C. During the experiment, samples were carried out at specific time and analyzed the occurrence mode. Each sample were fed with mixed gases to maintain the anaerobic environment.

2.2.4. Extraction of Metal Forms

The classic Tessier five-step extraction method was improved [14]. Samples from three groups were taken after 0d, 2d, 4d, 10d, and 20d respectively for refrigerated centrifugation. After centrifugation, the solid precipitate was freeze-dried and placed at 150 mL erlenmeyer flask for the extraction of the occurrence mode, which includes exchange state, carbonate state, the organic combination state, the amorphous iron manganese oxide combined with state, as well as the combination of crystalline iron manganese oxide state and residue.

2.3. Test Methods

Determination of uranium: the supernatant solution of 5 mL of each step extract liquid was determined in 150ml conical bottle by TiCl_3 reduction- NH_4VO_3 oxidation titration; Other heavy metals were determined by flame atomic absorption spectrophotometer (AAS).

3. Result analysis

3.1. Analysis of Metal Elements Content in Uranium Tailings

The total amount of uranium in uranium tailings samples was determined by TiCl_3 reduction -- NH_4VO_3 oxidation titration (one-step total solution method), and the total amount of uranium contained was 188.4 mg/Kg. After digested by used the electric heating plate method, the total contents of other major metal elements such as Fe, Mn, Pb, Zn, and Cd in the uranium tailings sample measured by AAS were 28726 mg/Kg, 35.9 mg/Kg, 122.6 mg/Kg, 17.6 mg/Kg, and 30 mg/Kg, respectively. The contents of U, Fe, Cd, and Pb are far beyond the background values of these elements in the soil of Hunan [16]. Therefore, they were selected as the research objects to study the change of the state of existence in the presence of *S. oneidensis* MR-1 under anaerobic conditions.

3.2. Analysis of Occurrence Forms of Heavy Metals in Raw Uranium Tailings

A series of extraction of raw uranium tailings was carried out, and the results of heavy metal speciation analysis were shown in Fig.1.

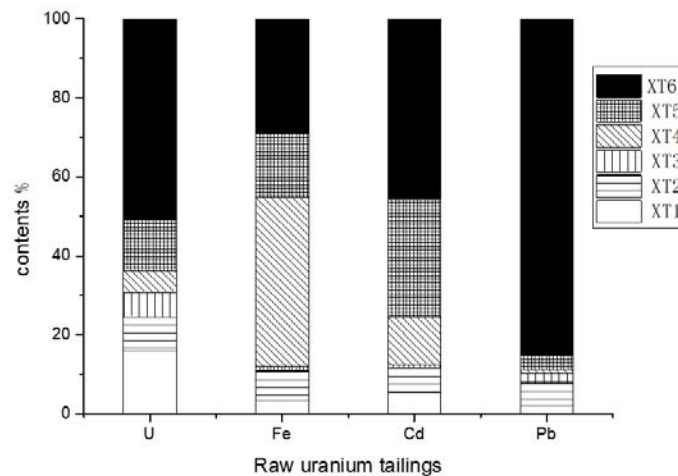


Figure 1. The occurrence forms of elements in the original uranium tailings

(XT1—exchangeable state; XT2—carbonate bound state; XT3—organic matter bound state; XT4—amorphous iron-manganese oxide bound state; XT5—crystalline iron-manganese bound state; XT6—residue state)

As can be seen from Fig.1, U in the primary uranium tailings is mainly exists in exchangeable state (15.9%), crystalline ferromanganese oxide state(12.84%) and residue state(50.87%); Fe mainly exists in the combination state(42.92%), crystalline ferro-manganese oxides state(16.15%) and residue state(29.00%); and Cd is mainly composed of crystalline ferromanganese state(30.18%) and residue state(45.49%); Pb is dominated by residual state, accounting for 85.19%.

3.3. The morphological analysis of metal elements in the solid phase of different reaction time

The analysis results of heavy metals in uranium tailings in different reaction time of min reaction system are shown in Fig.2.

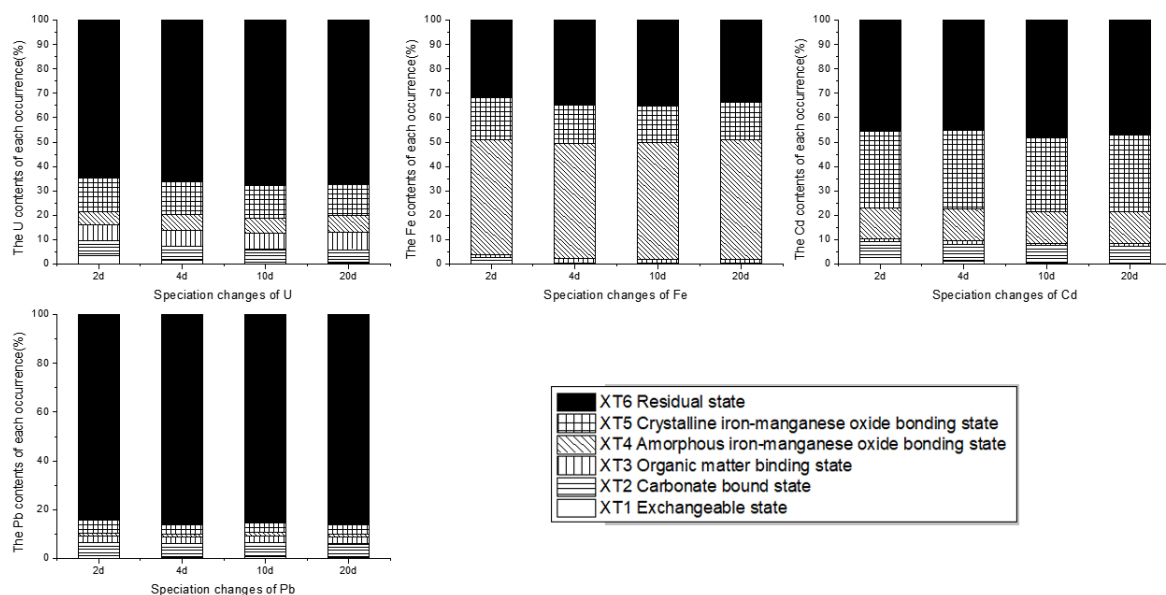


Figure 2. Occurrence forms of elements in uranium tailings at different reaction times in min groups

According to Fig.2, as the reaction progressed, the XT1 content of U in the min groups gradually decreased from 3.2% for 2d to 0.19% for 20d, and The XT4 content of U was increased from 6.55% of the reaction 2d to 6.98% of the reaction 20d, while the contents of XT2, XT3, XT5 and XT6 are basically unchanged. The contents of XT1 and XT2 of Fe decreased from 1.32% and 1.23% of the reaction 2d to almost 0 at the 4d of the reaction, while the content of XT4 increased slightly from 46.89% on the 2d to 48.96% on the 20d of the reaction, and the other existing forms remained unchanged. However, the changes of Cd and Pb occurrence pattern are similar to that of U, as the content of XT1 decreases gradually, and the content of XT4 increases slightly, while other forms of occurrence are basically unchanged.

The speciation analysis of heavy metals in uranium tailings at different reaction times in min-dbio reaction groups are shown in Fig.3.

As shown in Fig.3, in the min-dbio groups, the changes of the occurrence forms of the metal elements are similar to those in the min groups. As the reaction proceeds, the content of XT1 in each element gradually decreases. The XT1 contents of U, Fe, Cd and Pb was reduced by 4.3 %, 1.49 %, 1.98 % and 1.16 % of reaction 2d to 0.12 %, 0.09 %, 0.16 % and 0.31 % of reaction 20d, respectively; but the XT2 of Fe decreased from 0.98% of 2d to be not visible of 4d. At the same time, the XT4 content of each element was slightly increased, as U, Fe, Cd, and Pb increased from 5.79%, 47.53%, 13.12%, and 1.87% at the 2d to 5.97%, 48.12%, 13.74%, and 2.63% at 20d, respectively. However, unlike the min groups, the XT3 contents of the metal elements in the min-dbio reaction groups also slightly increased, as U, Fe, Cd, and Pb increased from 6.87%, 1.43%, 1.32%, and 2.93% for 2d to 8.83%, 3.63%, 2.78%, and 3.78% for 20d, respectively. While the contents of XT5 and XT6 in the min-dbio groups were almost unchanged.

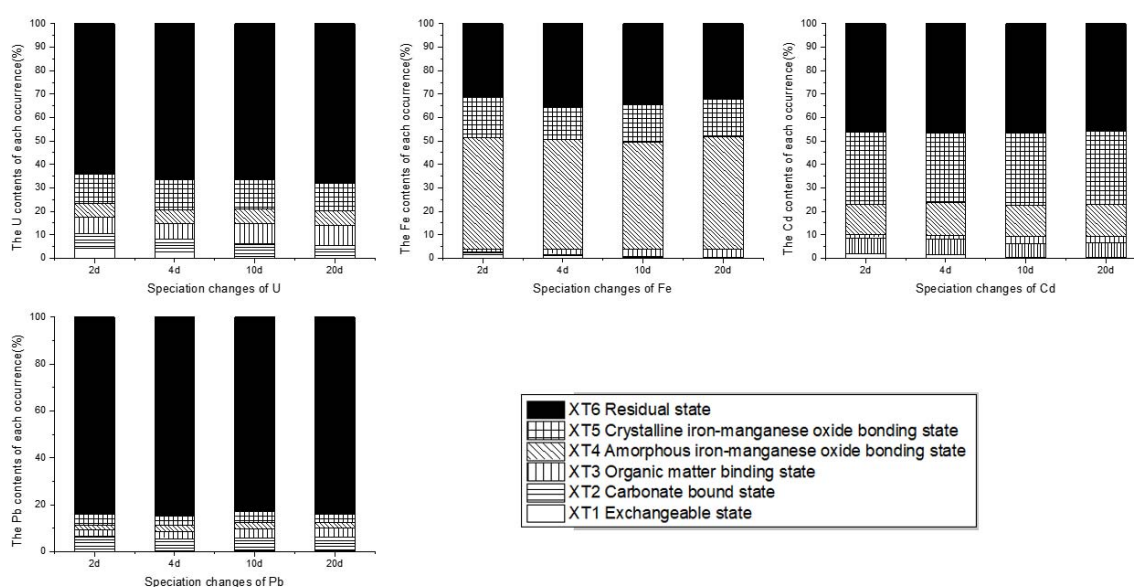


Figure 3. Occurrence forms of elements in uranium tailings at different reaction times in min-dbio groups

The analysis results of heavy metals in uranium tailings in different reaction time of min-bio reaction groups are shown in Fig.4.

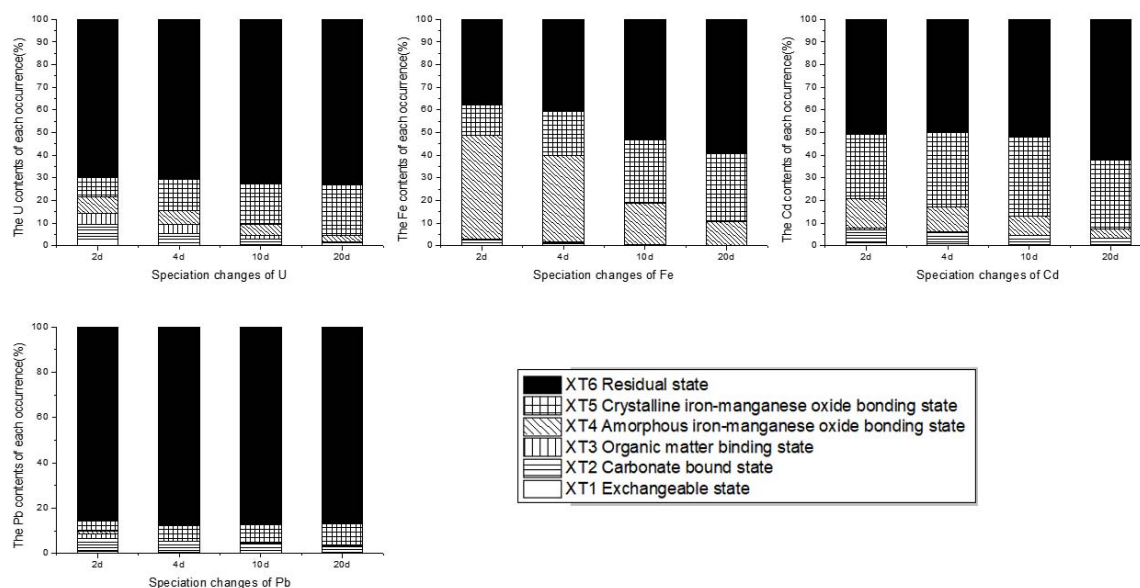


Figure 4. Occurrence forms of elements in uranium tailings at different reaction times in min-bio groups

The results in Fig.4 show that as the reaction proceeds, the XT1, XT2, XT3, and XT4 contents of the four metal elements in the min-bio reaction groups gradually decrease. The contents of XT1, XT2, XT3 and XT4 of U and Fe decreased gradually from 3.1%, 6.45%, 4.61%, 7.26% and 1.12%, 1.23 %, 0.49%, 45.76% of 2d to 0.03%, 1.16%, 0.19%, 2.79%, and 0.05%, 0.03%, 0.07%, 10.32% of 20d, respectively; and the XT5 contents of U and Fe increased from 8.74% and 13.76% for 2d to 22.49% and 30.19% for 20d, while the XT6 contents of U increased from 69.84% for 2d to 73.8% for 20d. And the contents of XT1, XT2, XT3 and XT4 in Cd and Pb decreased from 1.34%, 5.78%, 0.74%, 12.98% and 0.78%, 5.71%, 2.11% 1.36% on the 2d of the reaction to 0.08%, 3.12%, 0.07%, 4.22% and 0.06%, 2.99%, 0.31% 0.16% of 20d, respectively, while the XT5 contents of Cd and Pb increased gradually from 28.49% and 4.21% on the reaction of 2d to 30.18% and 9.73% of 20d, respectively. The contents of XT6 of Cd and Pb remained basically unchanged.

4. Discussion

According to Fig.2 and the analysis results, the XT1 contents of the four heavy metal elements of U, Fe, Cd, and Pb in the min groups gradually decreased with the reaction, and the XT4 contents slightly increased, while the XT2, XT3, XT5, and the XT6 contents were basically unchanged. So the release of the four metal elements in the min groups are mainly attributed to the soluble dissolution of the exchangeable state; other forms in the aqueous solution is not easy to dissolve, so their contents are almost unchanged; the XT2 of Fe also contributes to the release of Fe, as the combination of CO_3^{2-} and Fe^{3+} are unstable and easily hydrolyzed. The hydrolysis equation is shown in equation (1):



Due to the $\text{Fe}(\text{OH})_3$ colloid was formed, the increase of XT4 contents of other metal ions in the solution can be attributed to the adsorption of ferric hydroxide colloid.

According to Fig.4 and its analysis results, the changes of the occurrence forms of metal elements in the min-dbio reaction groups were similar to those in the min groups, but the contents of XT3 of each metal element in the min-dbio groups also increased slightly, which is because of the presence of dead bacteria. The organic matter complexed with metal elements or the bacteria adsorbed metal elements

after the broken of the dead bacteria, which resulted in the decrease of the contents of Cd, Fe and U in the solution at the later of reaction.

According to Fig.5 and the analysis results, the changes of the occurrence forms of each metal elements in the min-bio groups differ greatly from the min and min-dbio groups. The XT1, XT2, XT3, and XT4 contents of the metal elements in min-bio decrease gradually, and the contents of XT5 increase gradually, while except the XT6 contents of U increased slightly, the XT6 contents of other elements remained unchanged. It is due to the strong bioavailability of exchangeable states, carbonate bound states, organic bound states and amorphous iron-manganese oxide binding states. Under the action of *S. oneidensis* MR-1, Most of metal elements in these four forms are released into solution. However, the occurrence forms changes of U and Fe is due to the reduction of U and Fe by *S. Oneidensis* MR-1 under anaerobic conditions, in which the U^{6+} was reduced to U^{4+} , leads to the increase of residual state, while Fe^{3+} was reduced to Fe^{2+} and leads to an increase of the crystalline iron and manganese oxides state.

The residual state contents of the four metal elements in the three groups were almost unchanged, which indicates the poor bioavailability of the residual state and it is difficult to release. Since the forms of Cd and Pb mainly consist of the crystalline iron-manganese oxide bound state and the residual state, the release promotion effect of *S. oneidensis* MR-1 is not obvious, which is mainly the release of non-residuals and less release of harmful substances. Therefore, heavy metals in the tailings are mainly in the form of residuals state, low in exchangeable state, relatively stable in chemical activity, weak in migration ability, and less harmful to the ecological environment.

5. Conclusion

(1) The total contents of U, Fe, Cd, and Pb in the uranium tailings are 188.4 mg/Kg, 28726 mg/Kg, 30 mg/Kg, and 122.6 mg/Kg, which far exceeds the background value of these elements in the soil of Hunan Province.

(2) The release of metal elements in mineral groups are mostly attributed to the dissolution of exchangeable state.

(3) The increase of organic matter bound state of metal elements in the mineral-dead bacteria groups were due to the complexation of organic matter with metal elements after the crushing of dead bacteria or the adsorption of metal elements by dead bacteria.

(4) Because of the reduction of *S. oneidensis* MR-1 under the anaerobic condition, the contents of exchangeable state, carbonate bound state, organic bound state, amorphous iron oxide bound state in the mineral -living bacteria groups were all reduced. And the reduction of U^{6+} into U^{4+} with weak mobility resulted in the increase of the residual state of U, while the reduction of Fe^{3+} to Fe^{2+} resulted in the increase of the crystalline ferromanganese oxide binding state of Fe.

(5) The metal elements in tailings are mainly residuals with low exchangeable state contents, poor bioavailability, relatively stable chemical activity and little direct harm to ecological environment.

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