Solubilization of Pu-239 in low-level radioactive contaminated soil by the addition of microbial leaching solution of *Acidithiobacillus ferrooxidans*

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Abstract

Researches on microbial solubilization of traces of radionuclide plutonium in contaminated soil with *Acidithiobacillus ferrooxidans* were conducted. When *A. ferrooxidans* was cultured in medium containing sulfur and iron, 92.93\% of the Pu-239 in solution was removed during bioleaching. In addition, the changes of pH value, concentrations of Fe\textsuperscript{2+}, Pu-239, organic matter, nitrogen and phosphorus were periodically analyzed. The results showed that bioleaching affected the combination forms of Pu-239 existing in the soil. The percentage of Pu-239 exits in exchangeable state increased significantly because the carbonate state, iron and manganese oxidation state and residual state transformed into exchangeable state during bioleaching. At the same time, the percentage of organic state kept steadily. The changes of form into exchangeable state which can improve the leaching rate effectively. The results showed that the bioleaching process can be used to remove Pu-239 from contaminated soil.

Keywords:

1. Introduction

Since July 1945, the United States conducted the first nuclear test in New Mexico desert, a total of eight countries around the world had conducted more than 2,000 times of nuclear weapons tests (including atmospheric tests). At the same time, about 30 nuclear explosion test sites have been established. Nuclear legacy pollutants have become a

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serious threat to ecosystem [1-4]. The United States, Russia and other nuclear-armed countries have investigated and studied the effect of plutonium to environment in nuclear test sites [5-8].

There are 20 different plutonium isotopes, while 239Pu need to be specially considered because 239Pu has unique properties [9-10]: i. Easy to decay; ii. Half-life is 24,100 a, and it can decay into 235U whose half-life is 7 billion years. At the same time, 239Pu has the characteristics of radiation toxicity, high activity and high heat energy [11]. And it will generate internal irradiation, causing serious consequences when the radioactive nuclide came into our body. Broadly, the technologies used to remedy soils contaminated by 239Pu can be classified into two categories: physical and chemical technologies. The physical technology includes high gradient magnetic separation (HGMS), electrodynamics decontamination and in situ verification, while chemical method mainly includes washing decontamination and heap leaching [12]. In comparison to the traditional physical and chemical treatment methods, using in situ stabilization techniques cannot only lower the cost and the secondary pollution, but also provide a practical way for the treatment of radioactive soil, and a lot of experiments have been carried out on this subject [13].

*A. ferooxidans* was firstly separated by Hinkle and Colmer from acid mine drainage in 1947 [14]. Since then Temple conducted further researches on the strain [15]. The short rod bacterium, belonging to acidophilus chemoautotroph strain, obligate aerobic, suitable growth pH value of 2.0-2.5, suitable growth temperature for 28-35°C. Because it can obtain the energy through oxidation of Fe^2+*, sulphur, and metal sulfides, and it can take oxygen as the final electron acceptor to make a strong acid environment [13], and it was widely used in bioleaching of sulfide ore, coal biological desulphurization and environmental pollutions treatment fields [16-19]. But solubilization of radioactive plutonium with respect to soil has not been reported so far. Also most of the earlier studies only concentrated on the distribution of plutonium in soil. Hence the present study was designed based on these facts to assess the applicability of *A. ferooxidans* for the removal of 239Pu from low-level radioactive contaminated soil.

2. Materials and methods

2.1. Contaminated soil

Soil contaminated with 239Pu was collected from Lop Nur. Firstly, the roots of plants and rubble need to be removed, and all through the sieve of 2.0 mm. Then, in order to provide for a single cumulative sample, all the soil samples need to be mixed completely. Finally, the soil were dried for 48 h at 110°C in thermoelectric thermostat drying box, then stored in jars [20-22]. Before the bioleaching, in order to reduce its volume, the soil samples need through grille screen, dual plate sieve and spiral separator. According to our experiments, particle size had an influence on the soil sample specific activity and when the particle size is larger than 0.25 mm the activity is far lower than the standard of China’s provisional regulation. So soil samples used to mix with the *A. ferooxidans* only when particle size less than 0.25 mm [23-25].

Representative physic-chemical properties of soil samples were determined, including particle size of soil, changes of pH, changes of content in organic matter, nitrogen and phosphorus and the activity of 239Pu and the changes of the combination forms of 239Pu is also determined.

2.2. Culturing of *A. ferooxidans*

*A. ferooxidans* was separated from acidic soil independently in this experiment, which has been preserved in China Center for Type Culture Collection for long-term (Accession number: CCTCC M208130, *Acidithiobacillus ferooxidans* HT-3). *A. ferooxidans* was pre-cultivated in a modified 9K liquid medium contains the following compositions in 1.0 l distilled water: 2.50 g of (NH4)2SO4, 2.50 g of KH2PO4, 0.50 g of MgSO4·7H2O, 0.25 g of CaCl2·2H2O and 40.40 g of FeSO4·7H2O. Then added *A. ferooxidans* culture medium 150 ml to a conical flask; added 20 ml liquid of *A. ferooxidans* active bacteria to culture medium, culture at 28°C in thermostat oscillator.

2.3. Preparation of culture medium

The culture medium was rich in iron and sulfur and contained the following compositions in 1.0 l distilled water: 3.45 g powdered sulfur, 3.85 g of pyritic ash, 2.50 g of (NH4)2SO4, 2.50 g of KH2PO4, 0.50 g of MgSO4·7H2O, 0.25 g of CaCl2·2H2O and 40.40 g of FeSO4·7H2O. Then added *A. ferooxidans* culture medium 150 ml to a conical flask; added 20 ml liquid of *A. ferooxidans* active bacteria to culture medium, culture at 28°C in thermostat oscillator.
MgSO₄·7H₂O, 0.25 g of CaCl₂·2H₂O and 6.80 g of FeSO₄·7H₂O.

2.4. Preparation of Auxiliary reagent

In order to promote the dissolution of $^{239}$Pu in the soil more effectively, auxiliary reagent requires in the experiments, which contains the following compositions in a 1.0-l distilled water: 21.90 g of Na₃S₂O₅·5H₂O, 21.20 g of DTPA, 12.50 ml of formic acid, 11.00 g of citric acid, 0.40 g of ammonium phosphate, 13.50 g of EDTA-Na₂, 24.20 g of organic fertilizers.

2.5. Bioleaching experiments

Mixed auxiliary reagent with active bacteria liquid in 1.8:1.0 (ratio of volume), then added it to the soil samples at the ratio of 9.0 ml/100g and culture medium 1.8 ml/100 g proportionally. Finally, aerated and agitated 7 days at 28°C. And the contrast experiment with an equal volume of deionized water and leaching soil samples under the same culture conditions.

2.6. Determination methods of organic matter, nitrogen and phosphorus

The total content of organic matter, nitrogen and phosphorus in soil samples was measured according to the national standard of P. R. China of GB 9834-1988, GB 7137-1987 and GB 9837-1988, respectively.

2.7. Measure activity of $^{239}$Pu during bioleaching

The technology of activity measurement of $^{239}$Pu has been researched. Extraction chromatography was applied to determining the radioactivity of $^{239}$Pu [26]. Then measure radioactivity of $^{239}$Pu with low background alpha measuring instrument (FD-3005, detection limit $2.0 \times 10^{-5}$ Bq·g⁻¹, relative deviation <30%) [27-28].

3. Results

3.1. Changes of pH and Fe²⁺ in culture liquid

In the experiment, the high accuracy pH meter and analyzer of ion concentration was applied to measure the change of pH and concentration of Fe²⁺ in the culture liquid. As is shown in Fig. 1, in the early culture time the pH increase rapidly and reach its maximum in third day. However, in the next 5 days, the pH was decline gradually; even at last, the pH is smaller than the initial value. At the same time, the concentration of Fe²⁺ in the liquid is in a gradual decline all the time shown in Fig. 2. In the first 5 days, the trend of decline kept steadily. However, after 5 days, an inflection point occurs on the concentration curve.
Surprisingly, the color of the culture liquid also changed. In the early time, the color is white, and then changed to light yellow, finally became brown yellow. At the same time, we find some precipitation on the bottom. Precipitation was analyzed by the technology of scanning electron microscope (SEM, Zeiss EVO-18, made in Germany) shown in Fig. 3. The SEM shows that \textit{A. ferooxidans} adsorbed on the precipitation in a large number.

### 3.2. Specific activity with different particle size

Soil samples were divided into four groups according to the quality. And then each group was divided into three groups again according to the particle size, the specific activity of 239Pu different particle size shown in Fig. 4.

The experiment results show that when the particle size is larger than 2.0 mm or between 0.25-2.0 mm the specific activity is far below 0.25 mm group. And the maximum value is 2.56 Bq·g$^{-1}$, the minimum value is 2.0×10$^{-2}$ Bq·g$^{-1}$. And during bioleaching, soil treatment required when their particle size less than 0.25 mm [24].

### 3.3. Changes in contents of soil during bioleaching

The content of organic matter, nitrogen and phosphorus in soil sample after \textit{A. ferooxidans}'s and deionized water’s treatment is presented in Fig. 5.

The solubilization of \textit{A. ferooxidans} group was obvious after 7 days, organic matter, nitrogen and phosphorus were significantly reduced, and removal rates are 8.89%, 5.04% and 3.94% respectively, while the deionized water almost had no obvious change, the removal rate is 0.74%, 1.44% and 0.097% which is far below \textit{A. ferooxidans}. 
3.4. Dissolution of $^{239}$Pu during bioleaching

Under the influence of various environmental conditions, heavy metal ions in soil can exist in many forms; such as exchangeable state, carbonate state, iron and manganese oxidation state, residual state and organic state. The percentage of different fractions of $^{239}$Pu in soil before and after the experiment is presented in Table. 1.

| Culture time(days) | M(EX) | M(CS) | M(FM) | M(O) | M(RE) |
|-------------------|-------|-------|-------|------|-------|
| 0                 | 23.65 | 25.12 | 21.76 | 1.57 | 26.82 |
| 3                 | 48.73 | 17.46 | 14.65 | 1.44 | 16.34 |
| 7                 | 88.40 | 6.12  | 2.80  | 1.21 | 1.21  |

*Note:* EX- Exchangeable fraction; CS-Carbonate and specifically adsorbed fraction; FM- Fe-Mn oxides fraction; O-Organic matter fraction; RE- Residual fraction

The sequential extraction results shows that plutonium distribution in sample was not uniform, on the contrary plutonium exits in different forms. After 7 days bioleaching, the percentage of $^{239}$Pu in exchangeable state increases significantly, and carbonate state, residual state and iron and manganese oxidation state reduced obviously, while the percentage of organic state has no obvious change. The leaching of $^{239}$Pu in exchangeable state is more effective than in residual state and iron and manganese oxidation state, thus other states changes into exchangeable state encourages the process of bioleaching of $^{239}$Pu.

As well as, we also have conducted a contrast experiment. After 3 days and 7 days, changes of quality activity by *A. ferooxidans* shows in Fig. 6 compared with deionized water.

Contrast experiment results shows that after 7 days bioleaching, $^{239}$Pu in deionized water group the removal rate is only 1.01%, the content of $^{239}$Pu still remains in a high level, while the *A. ferooxidans* removal rate is 92.93% much higher than the deionized water group. When *A. ferooxidans* grows in sulfur and iron as substrate and $^{239}$Pu as final acceptor in the respiratory chain, 92.93% of the $^{239}$Pu in solution was consumed during bioleaching. No considerable increase in $^{239}$Pu in solution is detected when culture have finished.
4. Discussion

The culture liquid consist of Na₂S₂O₃ shows weak alkaline, so traces of OH⁻ dissociate from H₂O. But the OH⁻ is not stable in the acid environment and disproportionate to sulfite and sulfur easily. As is shown in Fig. 1, in initial stage of culture OH⁻ can neutralize with H⁺ produced by biochemical reaction, thus pH increased was observed. And when A. ferooxidans came into logarithmic growth phase, acid production increased rapidly. So after 3 days, the pH began to fall soon when reached its maximum [30].

FeSO₄ is easier consumed by A. ferooxidans shows in the experiments. Bacteria growth can be seen from Fig. 2 according to the consumption of Fe²⁺. After 2 days lag phase, A. ferooxidans enters the logarithmic growth phase, Fe²⁺ is consumed entirely. Substrate and product in ionic state can get through the cell wall and diffusion more easily. In 9K culture medium Fe²⁺ changed to Fe³⁺ under the catalysis of Fe-Cytc oxidoreductase. Number of Fe³⁺ exists in the form of precipitation of Fe(OH)₃ in later period of culture, because Fe³⁺ exceeds its solubility. At the same time a little precipitation of Fe(OH)₃ turns into yellow or yellow-brown precipitation such as KFe₃(SO₄)₂(OH)₆ or NH₄Fe₃(SO₄)₂(OH)₆ etc. And the overall reaction procedure is as follows:

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\begin{align*}
4\text{Fe}^{2+} + \text{O}_2 + 10\text{H}_2\text{O} & \rightarrow 4\text{Fe(OH)}_3\downarrow + 8\text{H}^+ \quad (1) \\
3\text{Fe}^{3+} + 2\text{HSO}_4^- + 6\text{H}_2\text{O} + \text{NH}_4^+ & \rightarrow \text{NH}_4\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6\downarrow + 8\text{H}^+ \quad (2) \\
3\text{Fe}^{3+} + 2\text{HSO}_4^- + 6\text{H}_2\text{O} + \text{K}^+ & \rightarrow \text{KFe}_3(\text{SO}_4)_2(\text{OH})_6\downarrow + 8\text{H}^+ \quad (3)
\end{align*}
\]

And a common perception is that Pu is not very mobile under typical subsurface conditions. This perception is based on the fact that Pu is generally highly insoluble in the soil [31]. And a particle with a smaller diameter has larger surface for sorption than a larger one relative to its mass. So the same amount of finer particle is assumed to have higher radionuclide content.

Organic matter refers to a variety of carbon and nitrogen-containing compounds, including the animal and plant residues, micro-organisms and their products in different decomposition stages. Though organic matter in a low content, which has a great influence on the properties of physical, chemical and biological of soil. Mass fraction and supply condition of nitrogen and phosphorus depends on accumulation of organic and its decomposition rate. In the experiment, the dissolution of soil organic matter, nitrogen and phosphorus can provide energy sources for A. ferooxidans growth, promote the growth of bacteria. So the dissolution of elements can improve the leaching of ²³⁹Pu to a degree.

Radionuclide adsorbed non-exclusively in the surface of soil by diffusion and outer complexation. And in this condition the ²³⁹Pu exits in exchangeable state. The chemical form of radionuclides is sensitive to changes in environmental conditions and susceptible to rain leaching. So we observed the percentage of exchangeable state of ²³⁹Pu increased gradually during bioleaching. The ²³⁹Pu also can combine with carbonate in soil, exits in precipitate. So the carbonate state can transform into exchangeable state under acidic conditions. The iron and manganese oxides can also exits in soil. Specificity adsorption mechanism make nuclide is easily bound to the surface of iron and manganese. Natural organic matter (such as humic acid) have an ability of complexation, and the main component of humus is humic acid, which has a sponge-like structure, having a large surface area under alkaline
conditions, thus the humic acid can absorb large amounts of radionuclides [32], while the vast majority of organic components is macromolecular compound, such as humus, and extremely difficult to be degraded. So the percentage of $^{239}$Pu exits in organic state has no obvious change during bioleaching. In addition, the nuclides can get into mineral crystal lattice, and in this condition forms residual state.

During bioleaching, \textit{A. feroxoixdans} can change other forms of $^{239}$Pu into exchangeable state which can improve the leaching rate. As for the mechanism of bioleaching, there are three mechanisms now: indirect effect, direct effect and indirect-direct effect. In 1964, Melvin and Henry put forward direct effect during bioleaching for the first time, which is the decomposition of minerals through the secretion of enzymes of bacteria [33]. With the development of bioleaching, the limits of direct effect were found. Then the theory of indirect effect emerged. In this theory, Fe$^{2+}$ and sulfur generated during chemical reaction process, and those elements will be biological oxidized to Fe$^{3+}$ and sulfate, and the bacteria did not contact to the surface of minerals [34]. And another theory is indirect-direct effect, both indirect effect and direct effect plays a critical role during bioleaching [35].

In a word, the present study reports bioleaching of $^{239}$Pu from low-level radioactive contaminated soil using \textit{A. feroxoixdans}. Bioleaching was found efficient for removal of $^{239}$Pu from soil. After bioleaching carbonate state, iron and manganese oxidation state and residual state were basically removed from soil. After bioleaching it was mainly present in exchangeable fraction. The results indicate that $^{239}$Pu pre-existing in soil in various forms that can be easily transformed into removable forms initially during bioleaching. The experimental results provide sufficient grounds for a field application of the newly suggested biological treatment technology with inoculation of \textit{A. feroxoixdans}, which proved to be effective in the remediation of soils contaminated by $^{239}$Pu.

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