A reduction mechanism of U(VI) on Shewanella oneidensis by spectral analysis

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Key words: *Shewanella oneidensis*; U(VI); mechanism; infrared spectroscopy; scanning electron microscopy; energy dispersive spectrometer.

Abstract: In order to realize the process mechanism that *Shewanella oneidensis(S. oneidensis*) reduced uranium(VI), and improve its reduction effect, the reduction mechanisms of uranium with *S. oneidensis* were studied by FTIR, SEM, EDS. The results show that after the *S. oneidensis* reduction of U(VI), the cell surface morphology is changed, the gaps and caverns on *S. oneidensis* surface decrease and more uncrystalmini grains through electron appear, which are binding each other. Much of new crystal structures were generated on the surface of cell. K^+ and PO_4^{3+} were involved in the process of U(VI) reduction. FT IR spectra analysis indicated that the main groups of S. *oneidensis* were association of hydroxyl, phenolic hydroxyl, amidogent, halogenated hydrocarbon, carboxyl in the process of U (VI) reduction.

Introduction

Uranium has been responsible for extensive contamination of groundwater due to releases from mill tailings and other uranium processing waster. The treatment of uranium-contaminated groundwater is a key issue of nuclear environmental problems to be solved in the uranium mining and metallurgy field. Conventional treatment of uranium contamination based on chemical and physical methods have several constraints, such as high cost, chemical wastes and complicated subsequent treatment. Alternative approaches of uranium remediation utilizing microorganisms including biosorption, bioacummulation, and biomineralization are considered. In contrast to traditional treatment methods, bioremediation methods with low cost, high efficiency and pollution free have huge potential in the treatment of uranium-contaminated groundwater.

S.oneidensis is a facultative anaerobic organism which exists in anaerobic environment of groundwater or deposition with rich organic matter of uranium mining regions. The organism has received wide attention in the reductive bioremediation of uranium-contaminated groundwater for it can grow aerobically and anaerobically on a vast array of electron acceptors including U(VI), Pd, Pu, Mn(IV), Fe(III) and Cr(VI). At present, most researches are about biodegradation of U(VI)

by *Shewanella*. But studies on the mechanisms of reductive precipitation of U(VI) is less, and the studies on the mechanisms are of great importance to improve the effect of biological remediation. In this study, the reduction mechanisms of uranium with *S. oneidensis* were studied by FTIR, SEM, ED

Materials and methods

Strain and main reagents. *S.oneidensis* MR-1, Purchased from Marine Culture Collection of China. (MCCC, NO.1A01706). The main reagents were Uranosouranic oxide (U_3O_8, Analytical reagent), standard uranium solution prepared according to GBW04201; the other reagents were analytically pure.

Experiment apparatus. Infrared spectroscopy (NICOLET6700) purchased from Themo Fisher company, scanning electron microscopy (FEI Quanta-200) and energy dispersive spectrometer(EDX-GENESIS60) were from America.

Experiment method.

(1) Scanning Electron Microscopy and Energy Dispersive Spectrometer（SEM-EDS）. The bacteria dried by the vacuum freeze drier and reduction of U(VI) was prepared into SEM samples after spray gold, Then observe the sample's morphology, Using EDS analyze the sample surface element.

(2) The analysis of Fourier Transform Infrared spectroscopy（FTIR）. Takes a certain amount of bacteria separately which before and after of reduction, making the bacteria mixed with KBr fully, Then put the bacteria into the sample chamber, Determining the infrared absorption spectra under the same scanning frequency.

Results and discussion

Analysis on functional groups of *S. oneidensis* with U(Ⅵ) by FTIR.

The infrared spectra of cell are shown before the test of reduction of U(Ⅵ) and after the test in the Figure 10. As shown in figure10, compared with the spectra of cell before the test, there are a few changes in position and amplitude of some peaks. The vibration peak around 3403.2 cm^{-1} moves 21.1 cm^{-1} toward high wave number and the peak narrows down, and it could be caused of the vibration of —OH from phenol, alcohol or carboxyl. This illustrates oxygen atoms are involved in the complexation of UO_2^{2+} in the reductive process of U(Ⅵ) , when the length of O—H bond strengthens. The peaks around 2376.3cm^{-1} could be generated because of the vibration of triple bond or cumulative double bond, and the peak moves to low wave number for the complexation of the triple bond or cumulative double bond with U(VI). The peak of famide Ⅰ or C═O in carboxylate occurs around the position of 1653.0 cm^{-1}, the position of peak changes and its oscillation gets weaker. The peak at 1542.1 cm^{-1} is

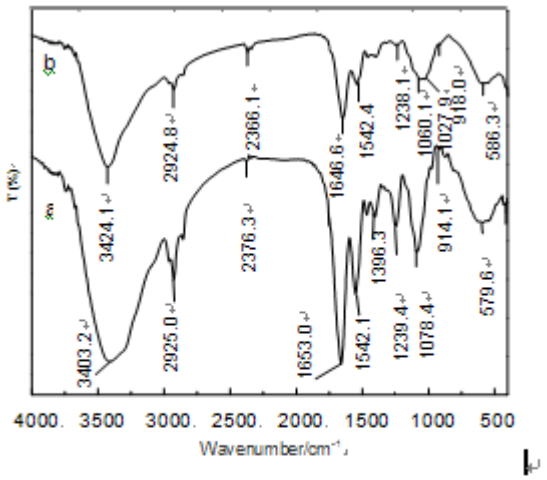

Fig.1 Infra-red spectra of cell before (a) and after U(Ⅵ) reduction (b)

formed for the flexural vibration of N—H and extendable of C—H in amide Ⅱ. The peak in the 1396.3 cm^{-1} could be formed in virtue of the vibration of C—H in —CH_3. The peak around 1239.4 cm^{-1} could be caused of the vibration of C—O, C═O in carboxyl or P═O in C—PO_3^{2-}. Infra - red absorption of symmetric and asymmetric—CH_2— at 2925 cm^{-1} gets weaker. So we could deduce that carboxy and phosphate from cell are involved in the reductive process of U(VI) from these changes of the peeks in the position and amplitude. The peak at 1078.4cm^{-1} could be generated for stretching vibration of C—O—C or the flexural vibration of —OH, and it occur two new peaks around 1060.1 cm^{-1} and 1027.9 cm^{-1}, respectively, in the experiment. It may be caused by the stretching vibration of O—H or P—O—C. FTIR spectra analysis indicates that the functional groups of hydroxyl, carboxyl, phosphate, amidogen, amide and other groups are involved in the reductive process of U(VI) by bacteria.

Scanning Electron Microscopy (SEM) analysis of S. oneidensis reduce U(VI). As can be seen from Fig.2(a). S. oneidensis bacteria surface texture is clearer and relatively smooth, the surface of the cell have a lot of voids. Some hollow appears between cell body, forming a deep cavity. There are less mutual adhesion among the cell, some buds can be seen between the part of the cell. AS shown from Fig.2(b), The results show that after the *S. oneidensis* reduction of U(VI), the cell surface morphology is changed, the gaps and caverns on *S. oneidensis* surface decrease and more uncrystalmini grains through electron appear, which are binding each other. Some cell death and the surface have a lot of uranium crystals. It indicate that there is interaction between cell and U(VI). There are many crystals in the surface and surrounding of S. oneidensis bacteria cell. It is probably the cell produce a substance which impact with U(VI) and generate a plenty of crystal crystallization. S. oneidensis cell's cytoderm is composed of carbohydrates and periplasmic protein. These biologic materials may provide a large number of organic groups, which are bound to each other as a ligand and free track U(VI) ,thus changing its morphology.

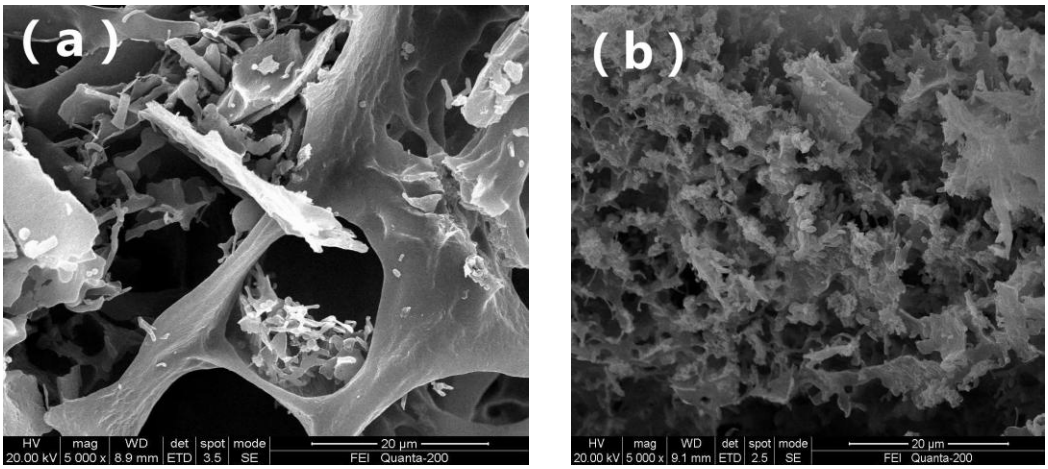

Fig.2 The picture of scanning electron microscope (SEM) on cell before (a) and after U(VI) reduction (b)

EDS analysis of *S. oneidensis* reduce U(VI). 72h spectroscopy analysis (Fig 3,4)shows that after reduction, S. oneidensis bacteria indicate the absorption peaks of U. Binding energy is 3~3.5keV, its content 59.11% of the cell mass fraction and 17.72% of atomic fraction. A high content of C , O which consistent with the sample and the bacteria that itself contains lots of C , O . The reducing of content of P, K element shows that K^+, PO_4^{3+} is involved in the process of S. oneidensis bacteria cells' reduction .The bacteria contain a strong peak after the reduction of U, It indicate that bacteria has a strong ability to reduce U. In the process of energy spectrum analysis, the sample will lead to Au element appear in before and after cell reduction after spraying gold.

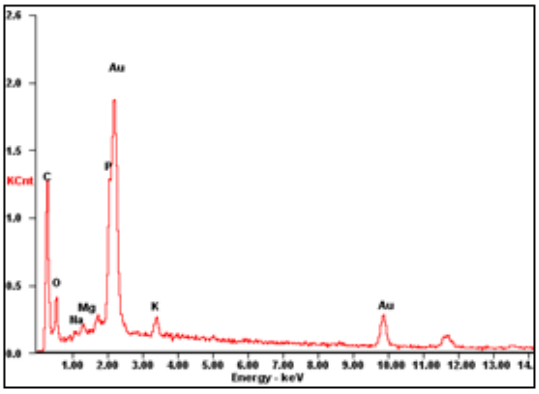

Fig.3 The spectra of cell before U(Ⅵ)reduction by EDS

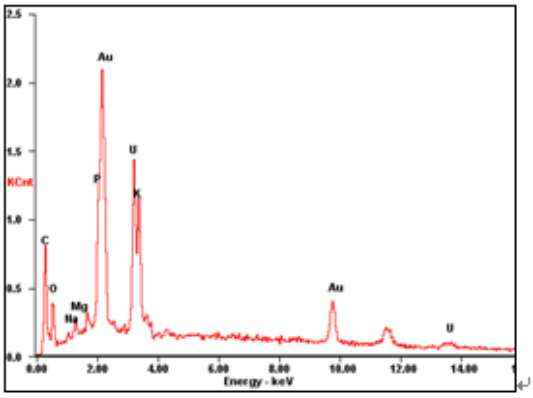

Fig.4 The spectra of cell after U(Ⅵ) reduction for72h by EDS

Summary

(1)The picture by SEM on cell before and after U(Ⅵ) reduction showed the cell surface morphology is changed after *S. oneidensis* reduction of U(VI), the gaps and caverns on *S. oneidensis* surface decrease and more uncrystalmini grains through electron appear, which are binding each other. Much of new crystal structures were formated on the surface of cell.

(2) EDS spectra analysis indicated that K^+ and PO_4^{3+} were involved in the the reduction process of U(VI), and *S. oneidensis* has the very strong compatible ability to reduce.

(3) FTIR spectra analysis indicated that the main groups of *S. oneidensis* were association of hydroxyl, phenolic hydroxyl, amidogent, halogenated hydrocarbon, carboxyl in the process of U (Ⅵ) reduction.

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