



## U(VI) reduction by *Shewanella oneidensis* mediated by anthraquinone-2-sulfonate

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**Abstract:** Anthraquinone-2-sulfonate (AQS) was employed in humus substitutes to evaluate the effects and influencing factors of U(VI) reduction by *Shewanella oneidensis* MR-1 (*S. oneidensis* MR-1) under anaerobic condition. The removal rate of U(VI) at 30 °C reaches 99.0% after 96 h with the pH value of 7.0 and AQS concentration of 1.0 mmol/L. The effective concentrations of AQS as the accelerator for U(VI) bioreduction are approximately 0.5–1.0 mmol/L. The bioreduction of U(VI) is inhibited when the concentration of AQS exceeds 2.0 mmol/L. The coexistence of ions, such as  $\text{Cu}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Mn}^{2+}$ , shows a remarkable negative effect on the U(VI) reduction, and  $\text{Zn}^{2+}$  shows less influence on the process compared with other tested ions. The U(VI) reduction is remarkably inhibited when the concentration of nitrate ion exceeds 1.0 mmol/L. Otherwise, no difference is found when the nitrate ion concentration is less than 0.5 mmol/L. Sulfate ion (<5.0 mmol/L) slightly promotes the U(VI) reduction. Zero-valent iron (ZVI) promotes the U(VI) reduction by *S. oneidensis*, and the reduction rate improves with increasing the amount of ZVI in the range of 0–2.0 g/L. The XPS result indicates that uranium deposits on the cell surface are in U(VI) and U(IV) forms, and the majority of uranium in the solution is stable  $\text{UO}_2$ .

**Key words:** U(VI); anthraquinone-2-sulfonate (AQS); *Shewanella oneidensis*; XPS

## 1 Introduction

Uranium-contaminated groundwater is a key issue in uranium mining and metallurgy. This groundwater contains radioactive pollutants (uranium), heavy metal ions ( $\text{Cu}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ ), anions ( $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ), and toxic organics [1,2]. The treatment of uranium-contaminated wastewater using conventional processes based on chemical and physical methods is limited by high cost, chemical wastes, and complex subsequent treatment. Alternative approaches, such as biosorption, bioaccumulation, and biomineralization, have been considered for uranium remediation using microorganisms. Contrary to traditional processes, bioremediation methods are less expensive, having high removal efficiencies, and pollution-free, so this alternative process can be applied to treat uranium-

contaminated wastewater [3].

*Shewanella oneidensis* (*S. oneidensis*) is a facultative anaerobic organism that can survive at low environmental temperature of 4 °C and in anaerobic environment of groundwater or deposition with rich organic matter in uranium mining regions. This organism has attracted much attention in the reductive bioremediation of uranium-contaminated wastewater because it could grow aerobically or anaerobically on a vast array of electron acceptors, such as U(VI), Pd, Pu, Mn(IV), Fe(III), Cr(VI), nitrates, and organic pollutants [4–8]. Recent studies have indicated that *S. oneidensis* reduced aqueous U(VI) to insoluble U(IV) with the precipitation of immobile uraninite ( $\text{UO}_2$ ) in contaminated groundwater. LOVELY et al [9] found the vast existence of microbial humus respiration in soil, sediment, or other anaerobic environments. They also showed that humic acid acting as a medium for

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electronic transformation can effectively promote Fe(III) reduction under experimental conditions with the coexistence of humic reductive bacteria and humic acid. Humus can also promote the reductive precipitation of U(VI), Cr(VI), and other heavy metals [10–12]. This organic matter can also enhance the biodegradation of azo dyes [13] and other toxic organic compounds.

Most studies have focused on the biodegradation of toxic organic compounds, such as azo dyes, using *Shewanella* [14–16]. However, the studies on reductive precipitation of aqueous U(VI) are highly important for biological remediation of uranium-contaminated wastewater. In this work, *S. oneidensis* was employed as the model organism, and its effects and major factors for U(VI) reduction in the presence of anthraquinone-2-sulfonate (AQS) were examined.

## 2 Experimental

### 2.1 Organism and culture medium

*S. oneidensis* MR-1 was provided by Marine Culture Collection of China (No. 1A01706).

The basal media consisted of 2.5 g/L NaHCO<sub>3</sub>, 0.1 g/L KCl, 0.25 g/L NH<sub>4</sub>Cl, 0.1 g/L NaCl, 0.04 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.05 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 g/L MgCl<sub>2</sub>·6H<sub>2</sub>O, and 1.0 g/L yeast extract. A certain amount of sodium lactate was added into the above culture medium as the electron donor in the reduction experiments.

### 2.2 U(VI) reduction by *S. oneidensis* in presence of AQS

Figure 1 illustrates the test equipment. The experiments were conducted in 150 mL conical flasks, which contained 100 mL microbial medium that consisted of sterilized basal media, 10.0 mmol/L sodium lactate, 20.0 mg/L U(VI) and 1.0 mmol/L AQS. The pH value of the medium was subsequently adjusted to 7.0 by using NaOH or HCl. Pure nitrogen and carbon dioxide were bubbled through bacteria filter into the conical flasks of the culture media for 15 min to remove the oxygen inside the bottles. The bacterial suspension, of which the optical density value in the wavelength of 600 nm (OD<sub>600</sub>) was 0.81, with the volume ratio of 2% was inoculated into the above culture media under the protection of mixed gases. Finally, the bottle mouths were sealed with butyl rubber stoppers, and the bacterial suspensions were incubated at 30 °C on an orbital shaker at 120 r/min. All experiments were performed under the aforementioned experimental conditions. Additionally, subsequent controls were incubated along with the treatment groups. The samples with similar volumes were extracted with a syringe from the sampling tube at regular intervals, and the mixed gases were again flushed into bottles to maintain anaerobic conditions during the

experiment. The supernatant samples obtained through 0.45 μm filtration membrane after centrifugation at 8000 r/min for 10 min were analyzed.

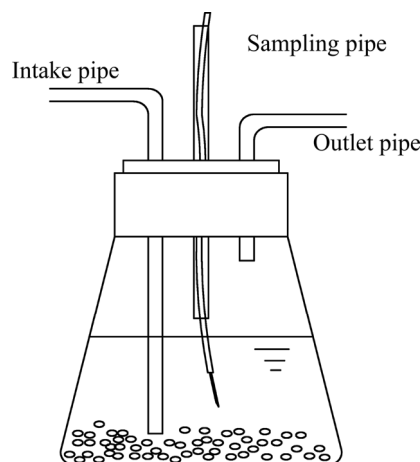


Fig. 1 Anaerobic culture device

The influencing factors of U(VI) reduction by *S. oneidensis* in the presence of AQS were investigated under the following conditions: pH values of 4.0, 5.0, 6.0, 7.0 and 8.0, coexistent metal ions of Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>6+</sup> and Mn<sup>2+</sup>, main anions of NO<sub>3</sub><sup>−</sup> (0.2, 0.5, 1.0 and 2.0 mmol/L) and SO<sub>4</sub><sup>2−</sup> (0.2, 1.0, 2.0 and 5.0 mmol/L), and zero-valent iron (ZVI) dosages of 0, 0.5, 1.0 and 2.0 g/L. Each experimental group set had three parallel experiments, and the average values from the tests were recorded as the experimental results. The same group of microorganism was used at each test to ensure experiment comparability.

### 2.3 Main reagents and analytical methods

The main reagents were uranosouranic oxide (U<sub>3</sub>O<sub>8</sub>, analytical grade), standard uranium solution prepared according to GB W04201, and AQS (analytic grade, Sigma Company). The iron powder was of analytical grade and obtained from Tianjin Chemical Reagent Factory, China. All other reagents were of analytical grade, and ultra-pure water was used in the experiment.

Spectrophotometric method according to national standard GB 6768–86 was used to determine trace uranium. The removal rate of U(VI) ( $R_{U(VI)}$ ) was obtained using the following equation:

$$R_{U(VI)} = (A_0 - A_1) / A_0 \times 100\% \quad (1)$$

where  $A_0$  and  $A_1$  are the concentrations of U(VI) in the solution before and after the reaction, respectively.

## 3 Results and discussion

### 3.1 Tolerance of *S. oneidensis* for U(VI)

The tolerance of the microorganism to uranium was investigated. The experiments were performed at U(VI)

concentrations of 0, 20, 50, 80, 120 and 160 mg/L. The results are shown in Fig. 2. In Fig. 2, the bacteria grow and reproduce in the basal media without uranium. The organisms grow slowly in the early growth phase because of the lack of nutrients from the media. Then, after this short period of stagnation, the bacteria quickly grow into logarithmic period, and the OD<sub>600</sub> values of the bacteria are 0.42 after 24 h and 0.97 after 36 h. The bacterial growth is significantly inhibited, and the bacteria need an extended period to adapt to the environment when the uranium concentration is 20 mg/L, in which OD<sub>600</sub> is only 0.05 after 24 h and 0.73 after 60 h. When the uranium concentration increases to 50 mg/L, the uranium causes a toxic effect on the organisms, so that the organisms grow very slowly with OD<sub>600</sub> of only 0.39 after 60 h. When U(VI) concentration is more than 80 mg/L, the bacterial structure is damaged, and the bacteria even die [17]. The effects of U(VI) concentration on *S. oneidensis* are distinct, and *S. oneidensis* tolerate toxicity at uranium concentrations below 50.0 mg/L.

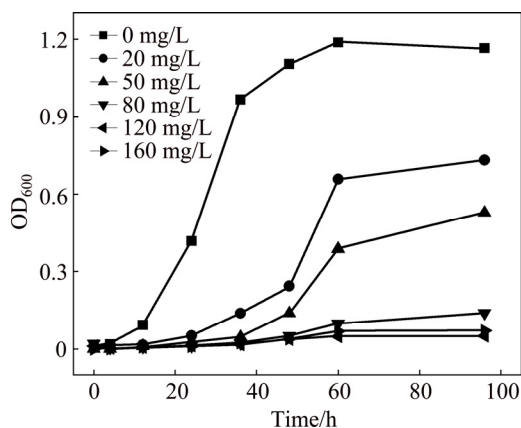


Fig. 2 Resistance of *S. oneidensis* on uranium concentration

### 3.2 U(VI) reduction in different redox systems

Control groups were prepared to accurately verify the contributions of AQS and microorganisms to U(VI) reduction. The control groups consisted of bottles with sole 1.0 mmol/L AQS or bacteria. The effects of different AQS concentrations on U(VI) reduction were evaluated. The experiments with both bacteria and AQS were performed with AQS concentrations of 0.5, 1.0 and 2.0 mmol/L. The results are shown in Fig. 3. AQS or bacteria each could reduce U(VI) in the solution. In the control group with only bacteria, approximately 40% of U(VI) is reduced after 4 d. In the group with AQS only, the reduction rate of U(VI) is stable at approximately 30%. Compared with the control groups, the reduction rate of U(VI) of the experimental group with both AQS and bacteria is relatively higher. The reduction removal rate of U(VI) reaches 99.0% with AQS of 1.0 mmol/L after 4 d. This rate is more than the superposition of that

of the control groups with either sole AQS or bacteria. AQS can be used as electron shuttle vector between U(VI) and electron donor to accelerate U(VI) reduction.

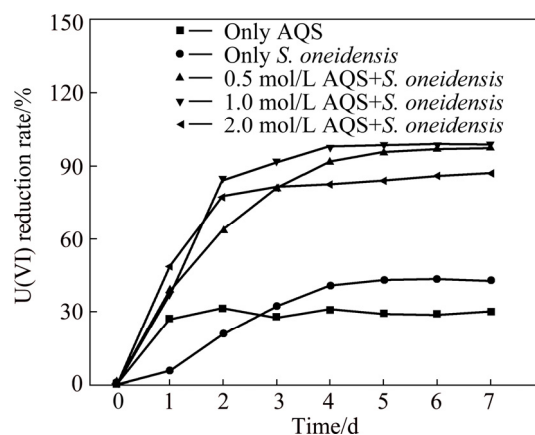


Fig. 3 U(VI) reduction rates of different redox systems

The addition of AQS in the experiments stimulates the U(VI) reduction to varying degrees. However, AQS concentration slightly influences the U(VI) reduction by *S. oneidensis*. The removal rates of U(VI) under AQS concentrations of 0.5, 1.0 and 2.0 mmol/L are 63.51%, 84.23% and 77.62%, respectively, after reduction for 2 d by microorganisms. Compared with the control group with bacteria only, the removal rate of U(VI) with addition of AQS increases by 43.01%, 63.73% and 57.12%, respectively. The stimulative role of AQS in the reduction of U(VI) by bacteria is positively correlated with the concentration of AQS in the range of 0.5–1.0 mmol/L. The catalytic role of AQS on the reduction of U(VI) weakens when the AQS concentration exceeds 1.0 mmol/L. The results are consistent with that of the reduction of azo dyes by *Shewanella* [18]. For most synthetic quinones, certain toxic effects are exerted on biological cells. *S. oneidensis* has limited tolerance to 2.0 mmol/L quinones with continuously increasing the AQS concentration. The electronic competition between AQS and U(VI) weakens the accelerative effects on U(VI) reduction.

### 3.3 Effect of pH value on U(VI) reduction

The pH value plays an important role in bacterial growth, and the biomass of bacteria greatly affects the U(VI) reduction. The effect of initial pH value on U(VI) reduction is plotted in Fig. 4. The pH value from 6.0 to 8.0 is more suitable for the growth of *S. oneidensis*. Under this condition, the reduction rate of U(VI) of each experimental group is more than 90% after 5 d, and the best removal efficiency is obtained at pH value of 7.0 with the removal rate of 95.21% after 5 d. However, 52.38% U(VI) is reduced at pH value of 4.0, and 76.42% U(VI) is reduced at pH value of 5.0 after 5 d.

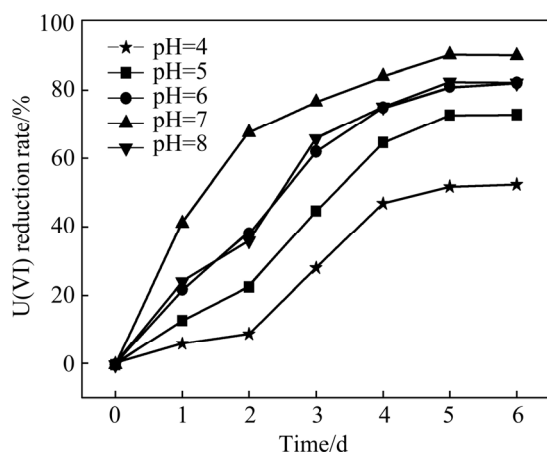


Fig. 4 Effect of pH value on U(VI) reduction

The results indicate that neutral environment is suitable for the growth of *S. oneidensis*, and the acidic environment of pH value less than 5.0 can inhibit the growth of microorganisms. However, *S. oneidensis* still induce certain reductive effects on U(VI) at pH values of 4.0–5.0. Consequently, the organism has high tolerance under acidic conditions.

### 3.4 Effects of ZVI on U(VI) reduction

In the past few years, the study on ZVI in pollution control and remediation of uranium-contaminated wastewater has become a hot spot. ZVI has several advantages such as rapid reaction rate, high oxidation–reduction potential, low price and readily available resources. One pair of control groups were prepared. One group was only added with ZVI, and the other group was only added with bacteria. The effects of ZVI dosages on U(VI) reduction were verified. Tests with both bacteria and ZVI were performed with ZVI dosages of 0.5, 1.0 and 2.0 g/L. In the groups mentioned above, 1.0 mmol/L AQS and 20.0 mg/L U(VI) were added into the cell culture medium.

The removal rates of U(VI) at different ZVI dosages are presented in Fig. 5. With 1 g/L ZVI, the reductive removal rate of U(VI) of the group with both ZVI and bacteria reaches 89.59% after 18 h, and the removal rate increases nearly 6 times that of the group with bacteria only. The removal rate of U(VI) reaches 95.01% after 24 h, whereas the reduction rate of U(VI) of the group with either sole ZVI or bacteria is less than 20% after 24 h. Additionally, 120 h is needed for the group with bacteria only to reduce about 95.01% U(VI). The reduction rates after 48 h in the redox system with both AQS and *S. oneidensis* are more than those of the controls with only ZVI or bacteria. This phenomenon illustrates that ZVI plays a distinctly stimulative role on reducing U(VI) by bacteria. Three reasons can be cited for the accelerative role of ZVI on U(VI) reduction.

Firstly, under anaerobic condition, the hydrogen production via electrochemical corrosion of ZVI may assist the microbial growth. Secondly, the dissolved oxygen in the solution can be depleted with ZVI electrochemical reaction, maintaining anaerobic condition, which is beneficial for U(VI) reduction by *S. oneidensis*. Finally, the toxicity of U(VI) to the organism is weakened for the U(VI) reduction by ZVI.

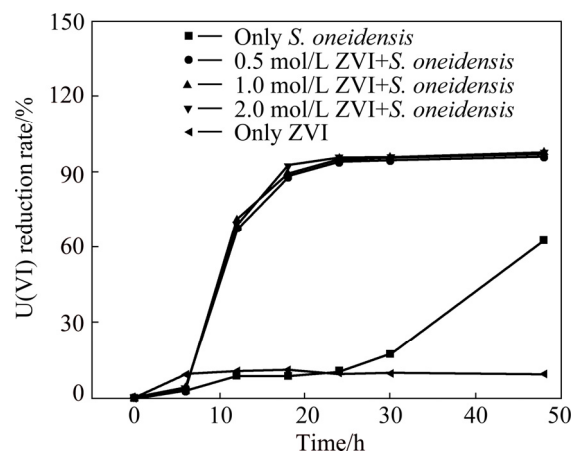


Fig. 5 Effect of ZVI on U(VI) reduction

The accelerative role of ZVI on the U(VI) reduction by bacteria is closely related to the amount of ZVI. The reductive removal rates of U(VI) at ZVI dosages of 0.5, 1.0 and 2.0 g/L reach 89.03%, 95.02% and 95.58%, respectively, after 24 h. Contrary to the group with 1.0 g/L ZVI, the removal rate of the group with 0.5 g/L ZVI decreases by 5.99%, and the removal rate of the group with 2.0 g/L ZVI increases by 0.56%. Consequently, when ZVI dosage ranges from 0.5 to 2.0 g/L, the higher the ZVI dosage, the greater the reduction rate of U(VI). In addition, when ZVI dosage ranges from 1.0 to 2.0 g/L, the reductive rate of U(VI) almost does not increase with increasing the ZVI dosage. This phenomenon may be due to the reaction of U(VI) reduction on the ZVI surface. Thus, the higher the ZVI dosage, the larger the surface of ZVI, which results in increased reductive rate.

### 3.5 Effects of coexisting metal ions of $\text{Cu}^{2+}$ , $\text{Cr}^{6+}$ , $\text{Mn}^{2+}$ and $\text{Zn}^{2+}$ on U(VI) reduction

The coexisting metal ions, such as  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , influence the reduction efficiency of U(VI). In the experiments, 2.0 mmol/L  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cr}^{6+}$  was separately added into the redox systems of *S. oneidensis* and AQS. The effects of the above ions on the U(VI) reduction are shown in Fig. 6. The removal rates of U(VI) in the experimental groups with  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cr}^{6+}$  are 5.69%, 40.52%, 92.82% and 47.25% after 72 h, respectively, and that of the control group without the above metal ions reaches 91.72% at the same time.

The results indicate that  $\text{Zn}^{2+}$  does not significantly affect the U(VI) reduction, but other metal ions of  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Cr}^{6+}$  inhibit the reduction. The inhibition of  $\text{Cu}^{2+}$  is the most prominent, followed by  $\text{Mn}^{2+}$ . TANG et al [19] found that  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$  also inhibited the reduction of Cr(VI) in the synergic removal of Cr(VI) in water by iron filings with microorganisms. The inhibition of  $\text{Cu}^{2+}$  may be due to the reductase activity of the protein on the cell membrane of *S. oneidensis* losing its ability of oxidation electron donor. This phenomenon is caused by the additional  $\text{Cu}^{2+}$  with the active center of the dehydrogenase protein from the initial respiratory chain, ultimately leading to the decrease of reductive rate of U(VI) [20,21]. The inhibition role of  $\text{Cr}^{6+}$  may be due to the strong oxidation ability of  $\text{Cr}^{6+}$ , leading to the damage of the cell structure.

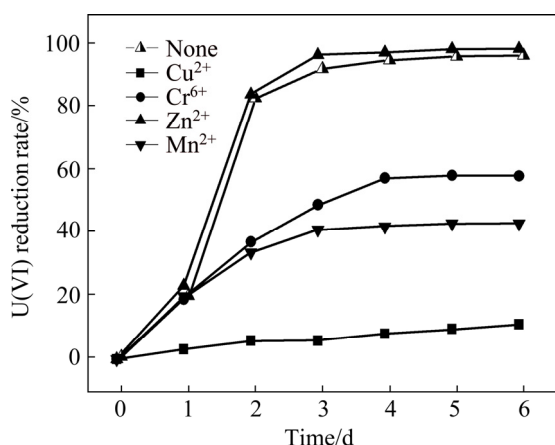


Fig. 6 Effect of different metal ions on U(VI) reduction

$\text{Mn}^{2+}$  has also been reported to hinder the U(VI) reduction by microorganisms [22].  $\text{Mn}^{2+}$  may be toxic to the organism and inhibit the bacterial growth. The removal effect of U(VI) is positively correlated with the biomass of bacteria, so the lower the biomass of the bacteria, the lower the removal rate of U(VI). In addition, the bacteria exhibit competitive selection of electron acceptor between  $\text{Mn}^{2+}$  and  $\text{Cr}^{6+}$ , resulting in reduced reductive rate of U(VI).

### 3.6 Effects of coexisting anions on reduction of U(VI)

#### 3.6.1 Effects of $\text{SO}_4^{2-}$ on U(VI) reduction

The reduction rate of U(VI) at different  $\text{SO}_4^{2-}$  concentrations is presented in Fig. 7. The reduction rates of U(VI) of the groups with  $\text{SO}_4^{2-}$  increase compared with that of the control groups without  $\text{SO}_4^{2-}$ . The reduction rates of U(VI) reach 88.88%, 89.73%, 93.82% and 95.23% at  $\text{SO}_4^{2-}$  concentrations of 0.2, 1.0, 2.0 and 5.0 mmol/L, respectively, after 3 d. The removal rates of the aforementioned groups remain stable at approximately 96% after 4 d. The reduction rates of the group without  $\text{SO}_4^{2-}$  are 73.77% after 3 d and only

81.14% after 4 d.

The results indicate that  $\text{SO}_4^{2-}$  can weakly promote the U(VI) reduction by *S. oneidensis*, and the reductive rate of U(VI) is positively correlated with  $\text{SO}_4^{2-}$  concentrations varying from 0 to 5.0 mmol/L. *S. oneidensis* can utilize sulfate as the electron acceptor for energy metabolism, and  $\text{SO}_4^{2-}$  anion can promote the activity of reductase in the oxidation–reduction process. Consequently, the reduction rates increase with increasing the addition of  $\text{SO}_4^{2-}$ .

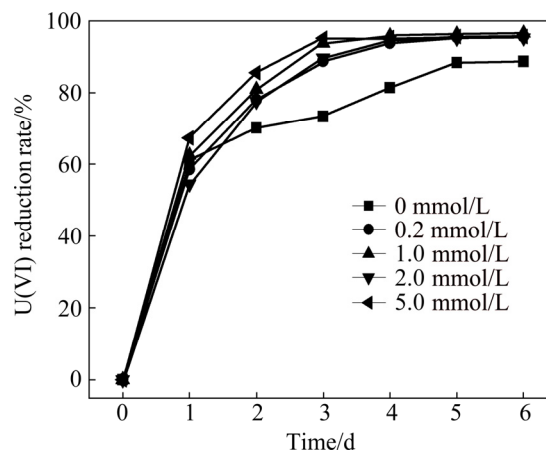


Fig. 7 Effect of  $\text{SO}_4^{2-}$  on U(VI) reduction

#### 3.6.2 Effects of $\text{NO}_3^-$ on U(VI) reduction

The experimental and control groups were set to evaluate the effects of  $\text{NO}_3^-$  concentrations on the U(VI) reduction. In the experimental groups with  $\text{NO}_3^-$ ,  $\text{NH}_4\text{Cl}$  in the culture medium was replaced with  $\text{NaNO}_3$ . However,  $\text{NH}_4\text{Cl}$  was not replaced in the control group without  $\text{NO}_3^-$ . The reduction rates of U(VI) at different  $\text{NO}_3^-$  concentrations are presented in Fig. 8. When the  $\text{NO}_3^-$  concentration is 0.5 mmol/L, 96.92% U(VI) is reduced by *S. oneidensis* after 6 d. When the concentration of  $\text{NO}_3^-$  increases from 0.5 to 1.0 and 2.0 mmol/L, the reduction rate of U(VI) decreases from 96.92% to 10.07% and 7.11% after 6 d, respectively.

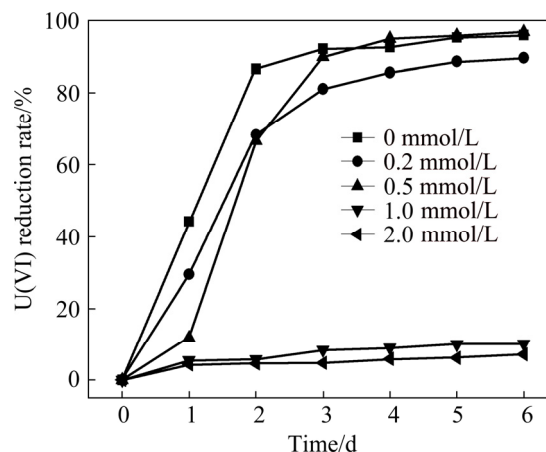


Fig. 8 Effect of  $\text{NO}_3^-$  on U(VI) reduction



However, the reduction rate of U(VI) of the control group without  $\text{NO}_3^-$  ion reaches 96.07% after 6 d.

The results show that  $\text{NO}_3^-$  has no obvious inhibitive influence on the reduction of U(VI) in the concentration range of 0–0.5 mmol/L. However,  $\text{NO}_3^-$  inhibits the reductive process when the concentration of  $\text{NO}_3^-$  is more than 1.0 mmol/L. This phenomenon is due to the fact that  $\text{N}^{5+}$  in the oxidation state from  $\text{NO}_3^-$  has powerful oxidative character. Moreover,  $\text{NO}_3^-$  (>1.0 mmol/L) probably competes for the free electron with U(VI) during the reduction, resulting in adverse reductive effects. However,  $\text{NO}_3^-$  (<0.5 mmol/L) can be used by the cell through nitrification to promote its growth and reproduction.

#### 4 Morphological analyses on reductive products of U(VI)

XPS was performed to analyze the valence state of uranium to the cell surface and characterize the reductive products of U(VI). Figure 9 shows two obvious peaks. One peak is in the orbit of U  $4f_{7/2}$  with binding energy of 380–382 eV, and the other peak is in the orbit of U  $4f_{5/2}$  in the 392–393 eV region. According to the Analytical Handbook of XPS [23], the peak at  $4f_{7/2}$  can be attributed to  $\text{UO}_2$  [U(IV)] with binding energy of  $380.3 \pm 0.4$  eV and  $\text{UO}_3$  [U(VI)] with binding energy of  $381.6 \pm 0.3$  eV, and the ratio of  $\text{UO}_2$  to  $\text{UO}_3$  is around 8:3. The other peak at  $4f_{5/2}$  can be attributed to  $\text{U}_3\text{O}_8$  in the 392 eV region and  $\text{UO}_3$  [U(VI)] in the  $392.65 \pm 0.15$  eV region, and the ratio of  $\text{U}_3\text{O}_8$  to  $\text{UO}_3$  is around 5:1. These results demonstrate that uranium is on the cell surface with U(IV) and U(VI) forms. The majority of U(VI) is induced to stable U(IV) by bacteria, and U(VI) on the cell is probably due to the biosorption of bacteria.

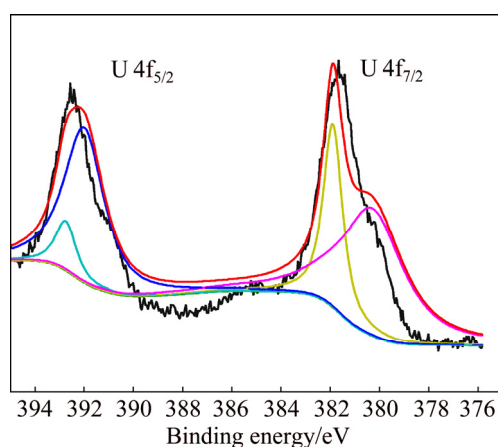


Fig. 9 Characteristics of uranium on cell surface

#### 5 Conclusions

1) In the presence of AQS, U(VI) can be reduced

effectively in anaerobic environment by *S. oneidensis*. The reduction rate of U(VI) reaches 99.0% at 30 °C after 96 h with the pH value of 7.0 and 1.0 mmol/L AQS. The effective concentration of AQS as the accelerator for U(VI) bioreduction is 0.5–1.0 mmol/L.

2) ZVI promotes the reduction of U(VI) in the system of AQS and *S. oneidensis*, and the reduction rate improves with increasing the ZVI dosage in the range of 0–2.0 g/L.

3) The coexistence of 2.0 mmol/L ions, such as  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Cr}^{6+}$ , inhibits the U(VI) reduction. Moreover, 2.0 mmol/L  $\text{Zn}^{2+}$  and less than 0.5 mmol/L  $\text{NO}_3^-$  exhibit no significant effect on the U(VI) reduction. Additionally, less than 5.0 mmol/L  $\text{SO}_4^{2-}$  slightly promotes the U(VI) reduction.

4) Uranium on the surface of bacteria is deposited in the U(IV) or U(VI) form. The majority of U(VI) in the solution forms stable  $\text{UO}_2$ .

#### References

- [1] RODGHER S, AZEVEDO D H, FERRARI C R, ROQUE C V, RONQUI L B. Evaluation of surface water quality in aquatic bodies under the influence of uranium mining (MG, Brazil) [J]. Environmental Monitoring and Assessment, 2013, 185(3): 2395–2406.
- [2] CHEN Hua-bai, XIE Shui-bo, LIU Jin-xiang, XIAO Shi-hong, ZENG Tao-tao, LING Hui, WANG Jin-song. Characteristics and mechanism of uranium(VI) absorbed by anaerobic granular sludge [J]. The Chinese Journal of Nonferrous Metals, 2014, 24(9): 2418–2425. (in Chinese)
- [3] FREDRICKSON J K, ROMINE M F, BELIAEV A S, AUCTIONG J M. Towards environmental systems biology of *Shewanella* [J]. Nature Reviews Microbiology, 2008, 6(8): 592–603.
- [4] LIU C, ZACHARA J M, ZHONG L, HEALD S M, WANG Z, JEON B H, FREDRICKSON J K. Microbial of reduction intragrain U(VI) in contaminated sediment [J]. Environmental Science and Technology, 2009, 43(13): 4928–4933.
- [5] LUAN F, BURGOS W D. Bioreduction of nitrobenzene, natural organic matter, and hematite by *Shewanella putrefaciens* CN32 [J]. Environmental Science and Technology, 2010, 44(12): 184–190.
- [6] ICOPINI G A, LACK J G, HERSMAN L E, NEU M P, BOUKHALFA H. Plutonium(VI) reduction by the metal-reducing bacteria *Geobacter metallireducens* GS-15 and *Shewanella oneidensis* MR-1 [J]. Applied and Environmental Microbiology, 2009, 75(11): 3641–3647.
- [7] DOLLHOPF M E, NEALSON K H, SIMON D M, LUTHER G W. Kinetics of Fe (III) and Mn (IV) reduction by the black sea strain of *Shewanella putrefaciens* using in situ solid state voltammetric Au/Hg electrodes [J]. Marine Chemistry, 2000, 70(1–3): 171–180.
- [8] CROSBY H, RODEN E. The mechanisms of iron isotope fractionation produced during dissimilatory Fe(III) reduction by *Shewanella putrefaciens* and *Geobacter sulfurreducens* [J]. Geobiology, 2007, 5(2): 169–189.
- [9] LOVLEY D R, COATES J D, BLUNTARRIS E L. Humic substances as electron acceptors for microbial respiration [J]. Nature, 1996, 382(6590): 445–448.
- [10] XIE Shui-bo, ZHANG Ya-ping, LIU Jin-xiang, LIU Ying-jiu, LI Shi-you, WANG Jin-song, LIU Hai-yan. Characteristics of reducing U(VI) by *Shewanella putrefaciens* in presence of anthraquinone-2-

- sulfonate (AQS) [J]. The Chinese Journal of Nonferrous Metals, 2012, 22(11): 3285–3291. (in Chinese)
- [11] ZHANG F, WU W M, PARKER J C, MEHLHORN T, KELLY S D, KEMNER K M, ZHANG G, SCHADT C, BROOKS S C, CRIDDLE C S, WATSON D B, JARDINE P M. Kinetic analysis and modeling of oleate and ethanol stimulated uranium(VI) bio-reduction in contaminated sediments under sulfate reduction conditions [J]. Journal of Hazardous Materials, 2010, 183(1–3): 482–489.
- [12] GUO J B, LIAN J, XU Z F, XI Z H, YANG J L, JEFFERSON W, LIU C, LI Z X, YUE L. Reduction of Cr(VI) by *Escherichia coli* BL21 in the presence of redox mediators [J]. Bioresource Technology, 2012, 123(3): 713–716.
- [13] XU Zhi-cheng, HONG Yi-guo, LUO We, XU Mei-ying, SUN Guo-ping. The effects of the humic substances on azo reduction by *Shewanella spp* [J]. Acta Microbiologica Sinica, 2006, 46(4): 591–597. (in Chinese)
- [14] CAI P J, XIAO X, HE Y R, LI W W, CHU J, WU C, HE M X, ZHANG Z, SHENG G P, LAM M H, XU F, YU H Q. Anaerobic biodecolorization mechanism of methyl orange by *Shewanella oneidensis* MR-1 [J]. Applied Microbiology and Biotechnology, 2012, 93(4): 1769–1776.
- [15] XIAO X, XU C C, WU Y M, CAI P J, LI W W, DU D L, YU H Q. Biodecolorization of naphthol green B dye by *Shewanella oneidensis* MR-1 under anaerobic conditions [J]. Bioresource Technology, 2012, 110(4): 86–90.
- [16] GRALNICK J A, VALI H, LIES D P, NEWMAN D K. Extracellular respiration of dimethyl sulfoxide by *Shewanella oneidensis* strain MR-1 [J]. Proceedings of the National Academy of Sciences of the United States of America, 2006, 103(12): 4669–4674.
- [17] TAPIA-RODRÍGUEZ A, LUNA-VELASCO A, FIELD J A, SIERRA-ALVAREZ R. Toxicity of uranium to microbial communities in anaerobic biofilms [J]. Water, Air and Soil Pollution, 2012, 223(7): 3859–3868.
- [18] XU Zhi-cheng, HONG Yi-guo, LUO We, CHEN Xing-juan, SUN Guo-ping. Anaerobic humus respiration by *Shewanella cinica* D14<sup>T</sup> [J]. Acta Microbiologica Sinica, 2006, 46(6): 973–978. (in Chinese)
- [19] TANG Jie, WANG Zhuo-xing, XU Xin-hua. Removal of Cr(VI) by iron filings with microorganisms to recover iron reactivity [J]. Environmental Science, 2013, 34(7): 2650–2657. (in Chinese)
- [20] BELIAEV A S, SAFFARINI D A, MCLAUGHLIN J L, HUNNICUTT D. MtrC, an outer membrane decahaem *c* cytochrome required for metal reduction in *Shewanella putrefaciens* MR-1 [J]. Molecular Microbiology, 2001, 39(3): 722–730.
- [21] FERNANDEZ V M, RUA M L, REYES P, CAMMACK R, HATCHIKIAN E C. Inhibition of desulfovibrio gigas hydrogenase with copper salts and other metal ions [J]. European Journal of Biochemistry, 1989, 185(2): 449–454.
- [22] VERRAMANI H, SCHOFIELD E J, SHARP J O, SUVOROVA E I, ULRICH K U, MEHTA A, GIAMMAR D E, BARGAR J R, BERNIER-LATMANI R. Effect of Mn(II) on the structure and reactivity of biogenic uraninite [J]. Environmental Science and Technology, 2009, 43(17): 6541–6547.
- [23] WAGNER C D, RIGGS W M, DAVIS L E, MOULDER J F. Handbook of X-ray photoelectron spectroscopy [M]. Minnesota: Perkin-Elmer Corp, 1992: 42–77.

## 蒽醌-2-磺酸钠介导下奥奈达希瓦氏菌还原 U(VI)

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**摘要:** 在厌氧环境下, 以蒽醌-2-磺酸钠(AQS)作为腐殖质模式物, 对奥奈达希瓦氏菌(*Shewanella oneidensis*, *S. oneidensis*)还原 U(VI)的效果与主要影响因素进行研究。结果表明: 在 30 °C, 当 pH 值为 7.0, AQS 为 1.0 mmol/L, 反应 96 h 后, *S. oneidensis* 对 U(VI)的去除率达到 99.0%。AQS 浓度为 0.5~1.0 mmol/L 时, 其能有效促进 U(VI)的还原, 当 AQS 浓度超过 2.0 mmol/L 时, 其对 U(VI)的还原促进作用明显减小。Cu<sup>2+</sup>、Cr<sup>6+</sup>和 Mn<sup>2+</sup>对 U(VI)的还原均表现出明显的抑制作用, 而 Zn<sup>2+</sup>对 U(VI) 的还原影响较小。NO<sub>3</sub><sup>-</sup>离子浓度低于 0.5 mmol/L 时, 其对 U(VI)的还原基本没有影响, 而当 NO<sub>3</sub><sup>-</sup>离子浓度高于 1.0 mmol/L 时, 其对 U(VI)的还原存在明显的抑制作用。当 SO<sub>4</sub><sup>2-</sup>离子浓度低于 5.0 mmol/L 时, 其对 U(VI)的还原有微弱的促进作用。在 AQS+S. *oneidensis*+ U(VI)还原体系中投加零价铁(ZVI), 能快速促进 U(VI)的还原, 且 U(VI)的还原率与 ZVI 的投加量(0~2.0 mg/L)呈正相关。XPS 分析结果表明: 菌体表面沉积 U(VI)和 U(IV)两种价态的 U 元素, 反应后的铀主要形成稳定的 UO<sub>2</sub>。

**关键词:** U(VI); 蒽醌-2-磺酸钠(AQS); 奥奈达希瓦氏菌; XPS

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