The Composite Interception Technology of Biochemistry (CITB) for Uranium Pollution Control at the Uranium Tailings

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Background

- Great deals of waste rocks, tailing sand and percolate effluent from tailing were produced in the process of uranium mining and metallurgy, which brought the radioactive pollution to groundwater and water bodies.
- The development of groundwater treatment technology of high efficiency and low consumption for uranium and other radioactive contamination, is a major task facing sustainable development of the nuclear industry and the environment protection.

Introduce of uranium mining & tailings in China

- There are more than 60 mines and 3 uranium mills in China, more than 20 units are underway or would be decommissioning.
- Naturally occurring radioactive material (NORM) due to unregulated activities and artificial radionuclides, mainly resulting from past practice.
- Most of processes of uranium mining are acid in situ leaching, heap leaching and in-situ blasting leaching.
- There are huge waste, high radiation dose and influence widely in uranium mining and metallurgy in China. The radiation dose accounts for 91.5% of entire nuclear fuel circulatory system, and including 80% of uranium tailings' radiation dose. The maximum effective dose of residents is up to 0.65 ~ 1.67 mSv/a by water vapor channels in uranium mining, and 17.5 Sv/a collective effective dose, beyond the criteria specific to existing situations of exposure.

Introduce of uranium mining & tailings in China

- **Both radioactive pollutants/contaminants and nonradioactive ones exist in situ. Uranium and other heavy metals, as copper, chromium, lead, cadmium, etc. Massive leaching agent, such as concentrated sulfuric acid, sulfate dissolved infusion**

Uranium and other radioactive or toxic substances: with high ability of migration, long half-life time, large quantity and complex.

No 272 Uranium Tailings

Those images come from the largest uranium tailings in Asia

BACK

Introduction of uranium mining & tailings in China

- Usually, control rainwater infiltration by surface covering, combined with shunting of cut-off ditch and drainage. The phenomenon of recurrent acid exists in some decommissioned facilities.
- There are many problems in decommissioning management or treatment in Uranium mining
- Due to radioactive material residues, such as uranium tailings, the ecological environment is or would be threatened by radionuclide in some regions.

Uranium contamination is one of the core environmental problems in China.

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2 Strategies ,Techniques and processes to control uranium migrate

- The forms of uranium in aqueous, mostly are U(Ⅳ) (tetravalent)or U(Ⅵ)(hexavalent), other valence state could be neglected. For U(Ⅳ), it is easy to form a stable complex with inorganic carbon, and it is difficult to migrate; For U(Ⅵ), form of uranyl ion（UO_2^{2+}）， it is soluble, easy to migrate.
- How to make U(Ⅵ) be immobiled and be isolated

  To reduce U(Ⅵ) to U(Ⅳ) precipitation state by biological, chemical oxidation and reduction
- Separate U(Ⅵ) directly by

  adsorption（biosorption) ion-exchange

  membrane separation
- Reduce (concentration)： evaporation

Techniques for control radioactive waste (water)

- **Comprehensive radioactive waste management**
- **Permanent segregation**

  Minimizing the loses to a considerable extent to isolate uranium from biosphere. The main method is disposition, including waste generation, treatment, disposal and predisposal etc.
- **To built tailings for near-surface disposal of low-intermediate level radioactive waste such as uranium tailings, waste rock of uranium mining and metallurgy. (storage or remediation)**
- **To treat radioactive polluted water :**

  Traditional Methods: Pump and treat, or physical chemical method etc.(ion exchange, evaporation-enrichment method, storage and diffusion; chemical precipitation)
- **Permeable reactive barriers (PRBs) and biological technology**

The limitation of currently available techniques

- Pump and treat remediation, physical chemical remediation method, are very expense and difficult to apply or promote in China.
- Disadvantages of PRBs: the accumulation and deposition of pollutants will shorten their life cycle while high load, and poor control etc.
- In PRBs systems, polluted by toxic heavy metals, how long the filler' s activity and effectiveness will last? and what will change the environmental conditions and could reactivate immobilized heavy metals?

Objective

- to seek the new process and technique with economic, environmental friendly on remediation of water contaminated by radioactive material residues, and control of radionuclide migration
- to develop high efficiency low consumption, long-term effective uranium pollution control technology, for low-grade uranium resource recovery.
- Aiming at solving the problem of the accumulation of pollutants in the course of restoration uranium and other radioactive, toxic heavy metals, improve the existing PRBs.

Theory & simulation and test methods

With biotechnology, environmental chemistry

Methods

The composite interception technology of biochemistry (CITB)

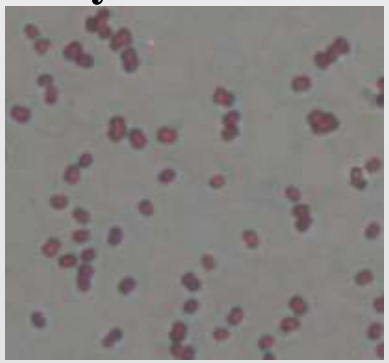
Study on biomaterial characteristics for processes of CITB

- The characteristics of sulfate reducting bacteria (SRB), *Citrobacter sp-* and *Bacillus subtilis*, as well as the characteristics of zero-valent iron reducing uranium from hexavalent to tetravalent in the chemical and biological conditions.

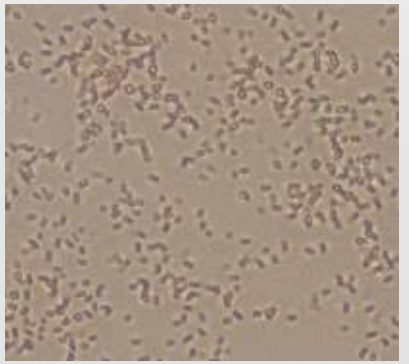
Advantages of biosorption

- There are a growing emphasis on biotechnology on treating uranium-containing wastewater and uranium mining and metallurgy.
- high efficiency, low operating costs, easy to regenerate biomass & recovery precious metals effectively; adapt to a wide range of pH & temperature; removal mental alternatively with dilute effluents

Bacillus subtilis



***Deinococcus* radiodurans**



R1

The adsorption capacity of microorganism to uranium

Biosorbents	Biomass	q / (mg·g $^{-1}$)
Bacteria	*Citrobacter sp-)*	9000
	Bacilllus subtilis	615
	Myxococcus xanthus	571
actinomycete	*Pseudomonas sp-*	541
	Zoogloea ramigeral	400 - 800
	Streptomyces sp-	440
	Penicillium chrysogenum	170
	S accharomyces cerevisiae	172
algae	*Sargassum fulitans*	560
	Anabaena azollae	1.6

The order of adsorption capacity of microorganism to uranium

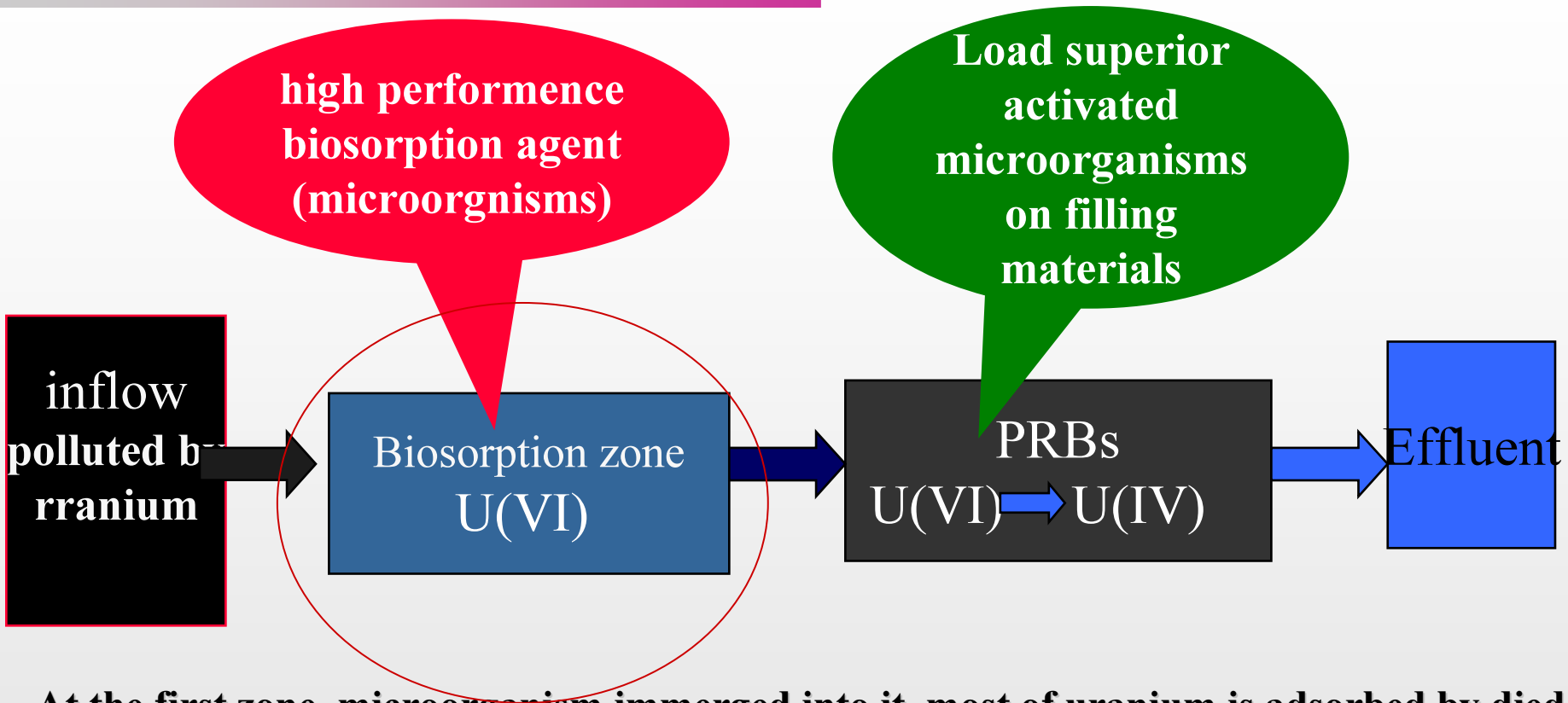
Bacteria ＞ actinomycete ＞fungi＞ algae

400-9 000

1.6-560

The uranium adsorption capacity of *Citrobacter sp-* was far lower than the former reports of 9000 mg·g^{-1} in our studies. Chinese researchers use *Rhizopus arrhizus* to adsorpt uranium, while in overseas using *bacillus* or *pseudomonas.*

3 The Characteristics of the CITB



At the first zone, microorganism immerged into it, most of uranium is adsorbed by died bacterial mass, the efficiency of adsorption to uranium would have been improved by pretreatment of bacterial mass; at the second zone (PRBs with engineering strain), the uranium is precipitated by biochemical reaction or adsorbed by microorganism. The metal which had been adsorbed could be desorbed for recovery. Through biological adsorption, and PRBs and SRB+ZVI coordination, the rest concentration of effluent is less than effluent-quality standard of China.

3 The Characteristics of the CITB

- **In front of PRBs, There is a biosorption zone with excellent sorption materials and suitable sorption environment; inducing SRB to PRB, optimizing fillings such as ZVI and fluor, developing the synergy of SRB and ZVI, the composite interception technology of biochemistry for uranium pollution control at the uranium tailings—efficient compound PRB was put forward.**
- **The uranium pollution control and the recovery of low-grade uranium resources would be achieved, taking full advantages of the synergy of inorganic materials and biomaterials, for which the uranium in effluent was efficiently absorbed in forepart, and then adjusting pH to 5. The experimental results proved that it is efficient to control uranium pollution.**

4 The Test for CITB

- Optimizing test, evaluate the functional biomaterials
- Biosorption agents for absorption zone: adsorption properties of superior microorganisms and its influencing factors（*Citrobacter sp-*, *Bacilllus subtilis*, *Saccharomyces cerevisiae*, ）
- Filling materials and strains for PRBs: SBR and others, reducing agent, the capability of ZVI, fluor, hematite etc.
- Mechanism of removing uranium coorperatively by *Sulfate reducing bacterial* &ZVI.
- Environmentally-friendly catalyst for biochemical reaction catalyst: *Deinococcus radiodurans*. SRB-1 is a kind of artificial breeding engineering stains
- Construction strategy and functional analysis of of *Deinococcus* radiodurans
- An plot experiment of uranium control in some solution mining uranium
- To investigate the effect factors condition for removal rates of U (VI), such as, pH, initial concentration of uranium, coexisting ions, reaction time by ZVI and hematite.
- The reaction kinetics and thermodynamics about the removal of uranium (VI) from wastewater by ZVI and hematite.

4 The results of test for the process of CITB

- The experiment indicated that the biosorption plays a primary role in the forepart of the efficient compound PRB, and SRB-1 catalyses and reduces uranium from hexavalent to tetravalent through ZVI after that.
- It is the key to control pH properly that prevent uranium and other metals remigration from causing secondary pollution. Also it is crucial for performance of engineering processing, through optimizing adsorption process, controlling pH and the concentration of heavy mental ions such as copper, and strictly controlling the effect of nitrate ion to the process.
- The steady and preponderant SRB-1, *Citrobacter sp-* and *Bacillus subtilis* were obtained through screening test. The mechanisms and immobilization of biomass were also studied and successfully applied in industrial process.

Removal of U(VI) from wastewater by ZVI and hematite

Iron is an active metal with reducibility, and was an efficiency filling material. Iron powder can react with heavy metals and some organics, and make them precipitate. It can reduce heavy metal ions or compounds with stronger oxidization and some organics 。In aqueous solution , iron and other elements could easily form primary battery:

anodic reaction：$Fe-2e \rightarrow Fe^{2+}$ $E_0(Fe^{2+}/Fe)=-0.440$ V

cathodic reaction：$2H+2e \rightarrow 2[H] \rightarrow H_2\uparrow$, $E_0(H^+/H_2)=0.00$ V

when oxygen dissolved in water:

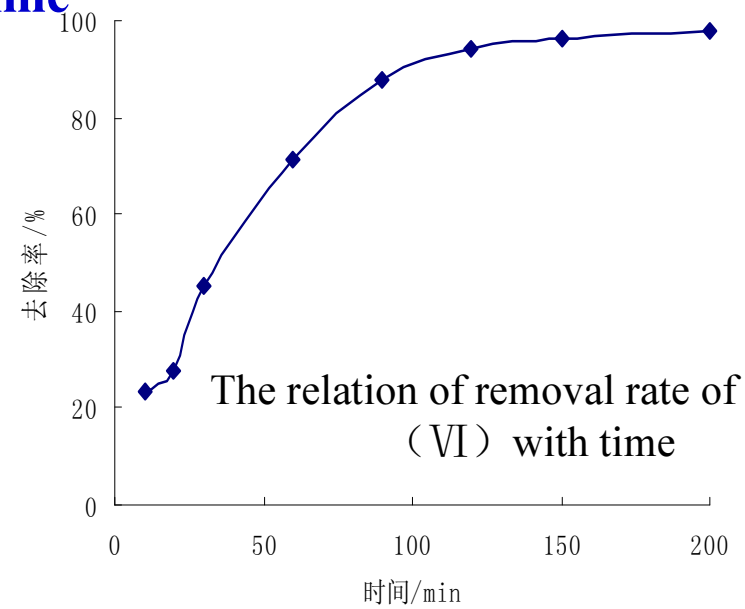
$$O_2+2H_2O+4e \rightarrow 4OH^- \quad E_0(O_2/OH)=0.40V$$

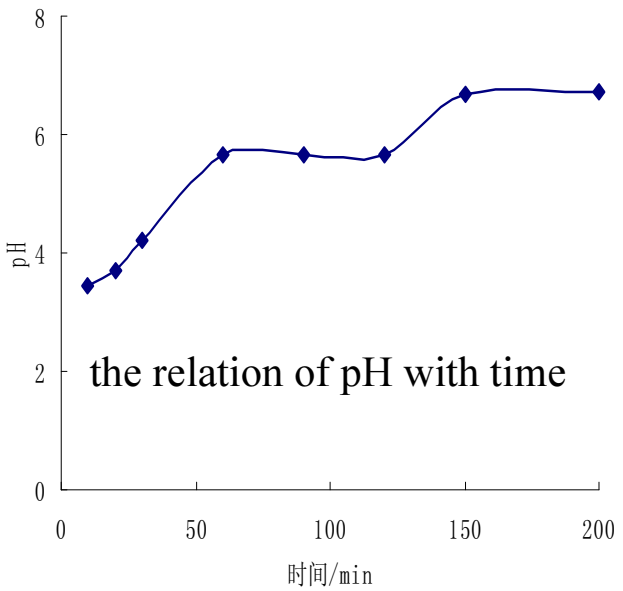
The oxides of iron, especially hematite and goethite are the common components of soil, sediments and water，there are large sp. surface area and strong adsorption.

ZVI 's good nature material for treating heavy metals in wastewater，it be selected to build large scales of PRBs for remediation of land and water contaminated by waste material residues.

Results and Analysis

Efficiency test of the removal of U(Ⅵ) in solution by ZVI with reaction time

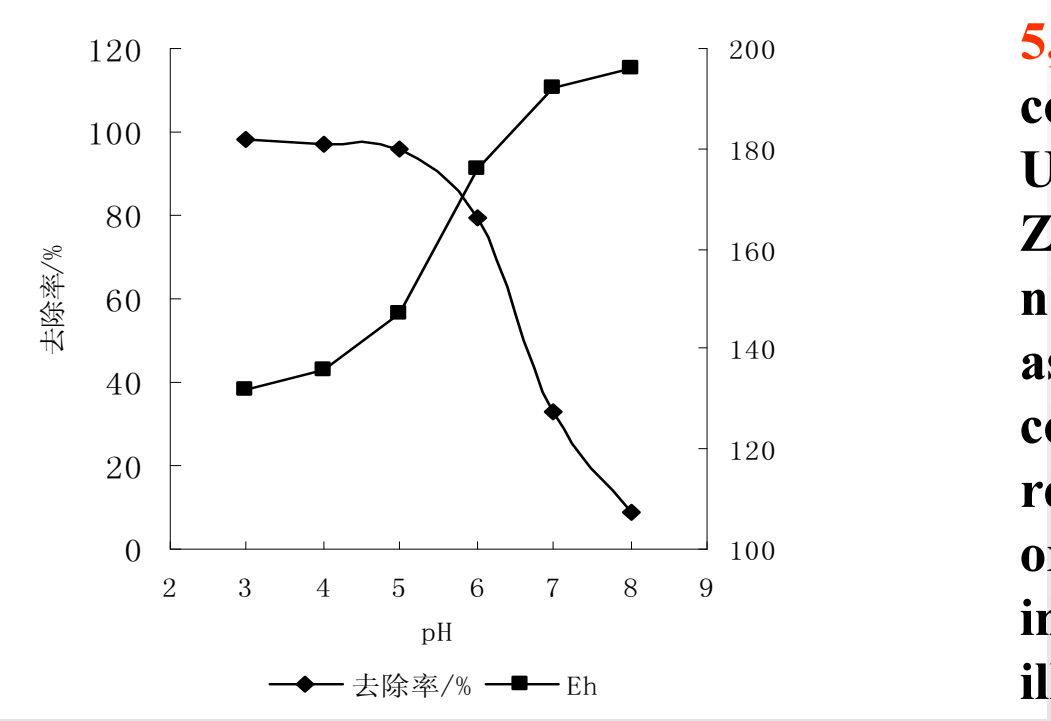




At the initial stage，the removal rate reached 88% when at 90min，when 200min，it reached 98.1%。Because of the higher concentration of U(Ⅵ)，the sediments adhered the iron surface were relatively less than to make the reaction of reduction precipitation of U(Ⅵ) quicker. With the reaction going on，pH increased，iron oxides, the corrosive products of ZVI would attach with the iron surface，holding back the reaction. In the reduction precipitation of U(Ⅵ) by ZVI，amounts of H^+ in the related electrochemical reaction were consumed，the concentration of H^+ decreased，pH increased.

Removal of U(VI) from wastewater by adsorption of ZVI

Effect of removal rate by initial pH



As the initial pH increased, the removal rate of U (VI) decreased, when pH was over 5,it decreased rapidly . At acid condition，uranium existed as UO_2^{2+} which was reduced by ZVI suitable，when near neutral and alkaline，it existed as other complexions，these complexions inhibited the reduction of uranium, oxidation-reduction potential increasing with pH increasing，illustrated that the decreased

Without organisms to catalyze，acidic（weakly acidic）conditions were helpful for reduction of uranium by ZVI.

Removal of U(VI) from wastewater by adsorption of ZVI

Effect of the removal rates of U（Ⅵ）by the amount of the iron powder

The removal rates of U(Ⅵ) were not improved significantly excessive iron powder

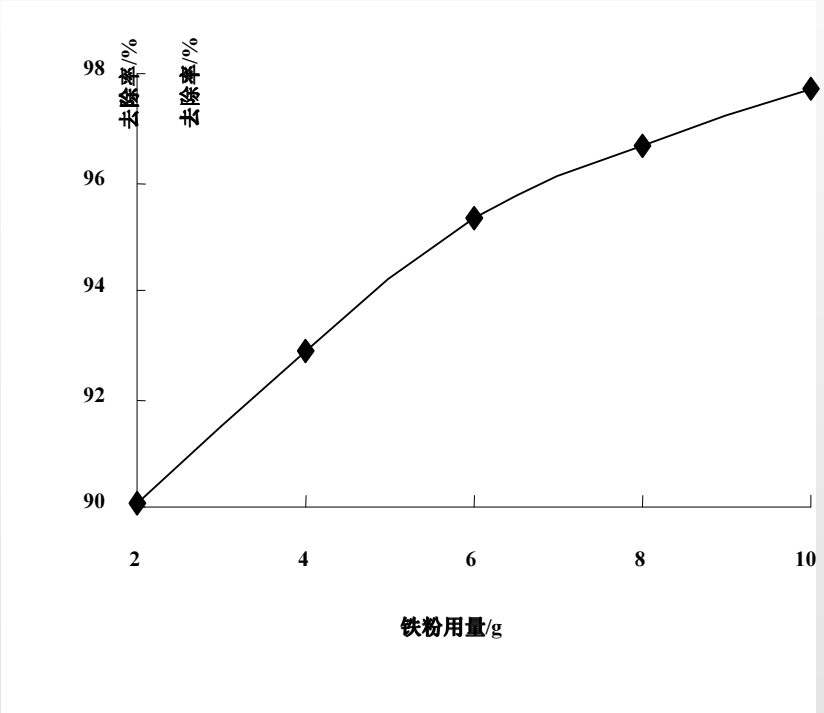


Fig.4 Effect of removal of U(VI) from aqueous solution by adsorption of ZVI

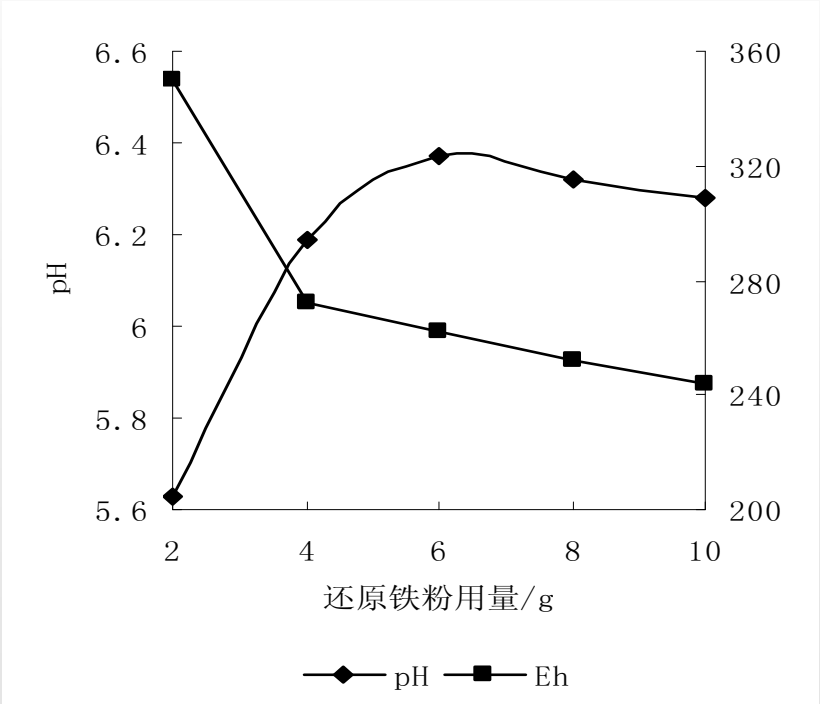
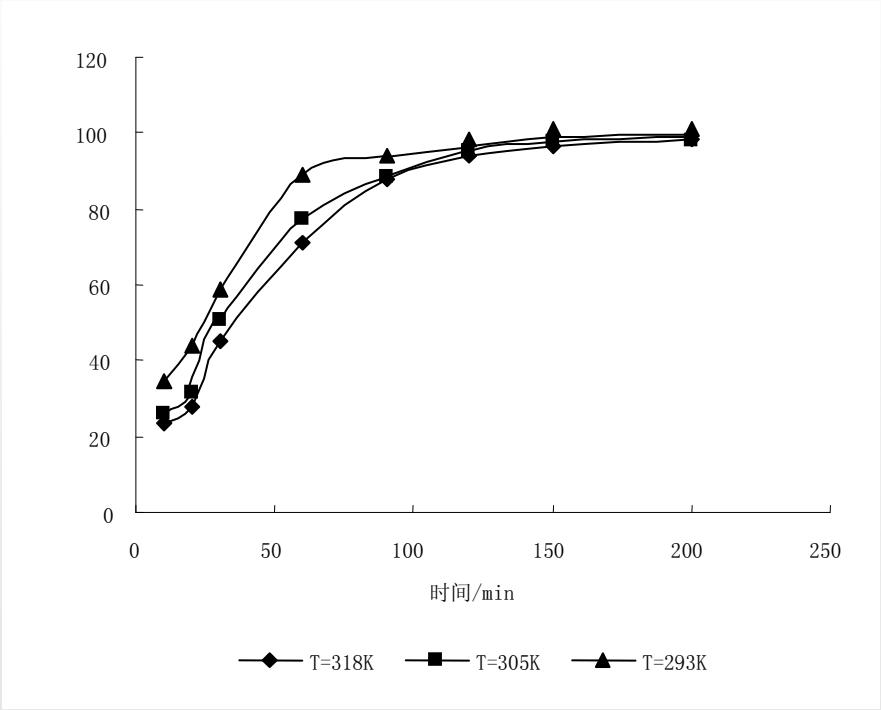


Fig.5 Relation of pH、Eh and the amount of reduced iron powder

The test of hematite and ZVI removing the U(VI) in wastewater

Relation of the U(VI) removal and time with the different temperature



Analysis of the reaction dynamics

The influence of temperature on reaction rate shows the degree of influence of the mass transfer on the reaction dynamics. For the typical mass transfer reaction dynamics, Diffusion is the limiting factor, activation energy of the reaction may be small; for the dynamics controlled by chemical reaction , the influence of temperature on reaction rate is not too big.

From the above graph, the degree of change is not so sharp within the different temperature, the reaction of the U(VI) and ZVI is controlled by the chemical reaction.

The table below is from the the result of the uranium concentration from the test along with the changing time and concentration calculation formula . And dynamics constant and correlation coefficent are calculated by the regression .

Table 1: C_t、 Ct ,－ln(Ct/C0) and －(1/C0－1/Ct) along with the changing time

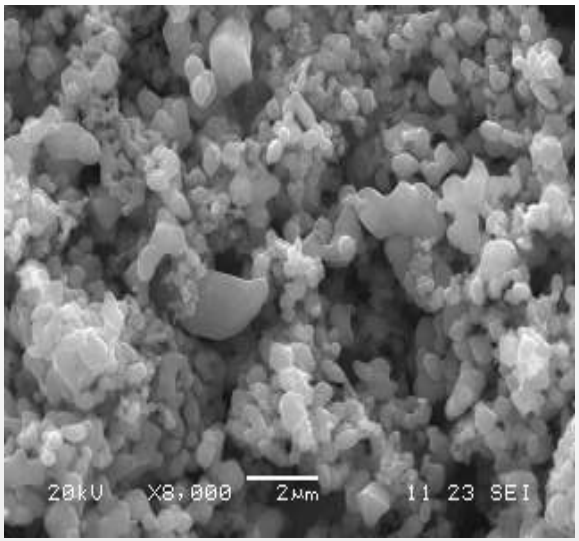
time/min	10	20	30	60	90	120	150	200
$-C_t$	－15.34	－14.49	－10.99	－5.74	－2.40	－1.23	－0.76	－0.38
$\ln(C_t/C_0)$	−2.73	−2.67	−2.4	−1.75	−0.68	−0.21	0.27	0.97
$-(1/C_0 -1/C_t)$								

In the scope of the initial concentration, the zero-valent iron to remove U (Ⅵ) in the water is the first reaction , whose correlation coefficient is 0.9863.

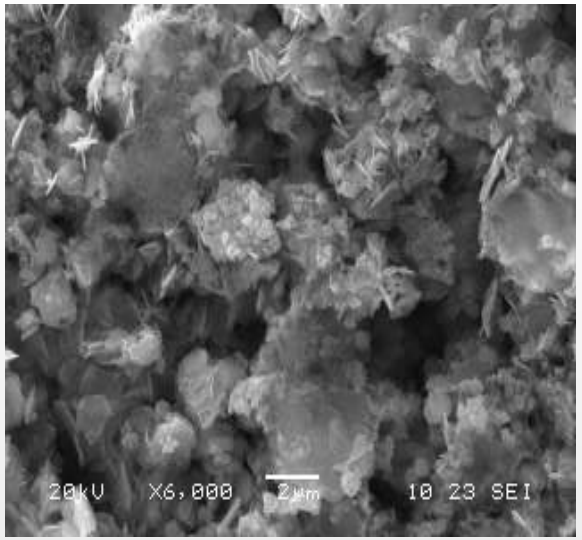
Table 2: correlation coefficient for different instances

Reaction series	Zero series	first series second series	first series second series
R^2	0.7897	0.9863	0.9788

Electronical microscope analysis of ZVI to remove U (Ⅵ)



before electronical microscope analysis



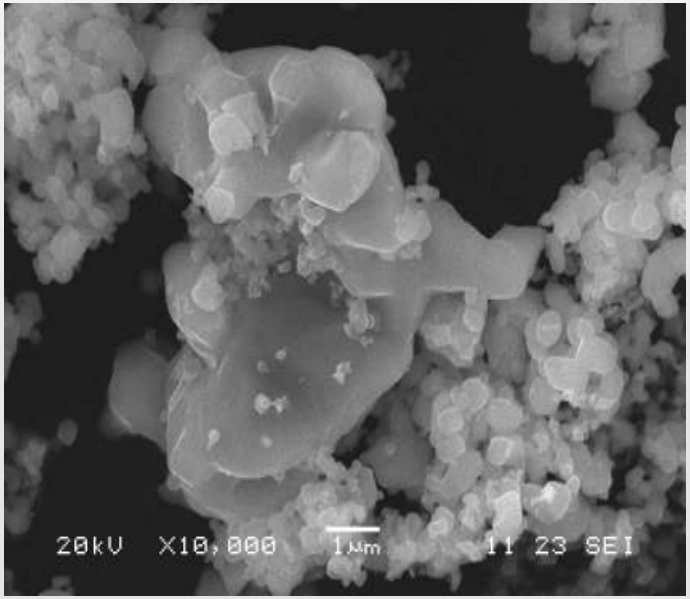
after electronical microscope analysis

The surface of iron power is smoothly, not centralized and no lamellar crystal at the reacting of beginning. The result is opposite after the reaction has finished, arborescent surface is surrounded, and more thick. It indicated that the surface iron is eroded, new substance depositted on the surface of the reduced iorn, blocking the reactant connecting active sites of ZVI,to reduce the its activity.

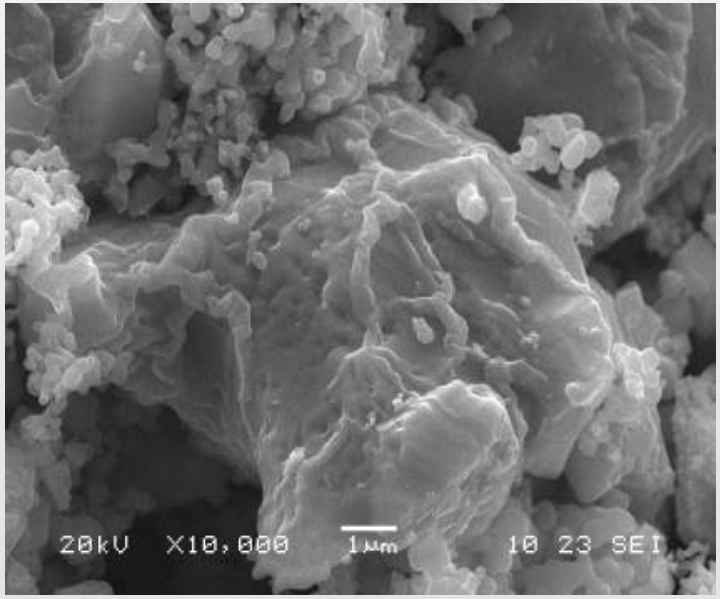
Mechanism analysis

It was obviously that hexagonal crystals and quartet crystals coexisted and it is abundant. The hexagonal crystals are α -Fe_2O_3, the main component of hematite and square crystals are magnetic hematite (Fe_3O_4), the secondly component.

ESEM of hematite having adsorpted U(Ⅵ)



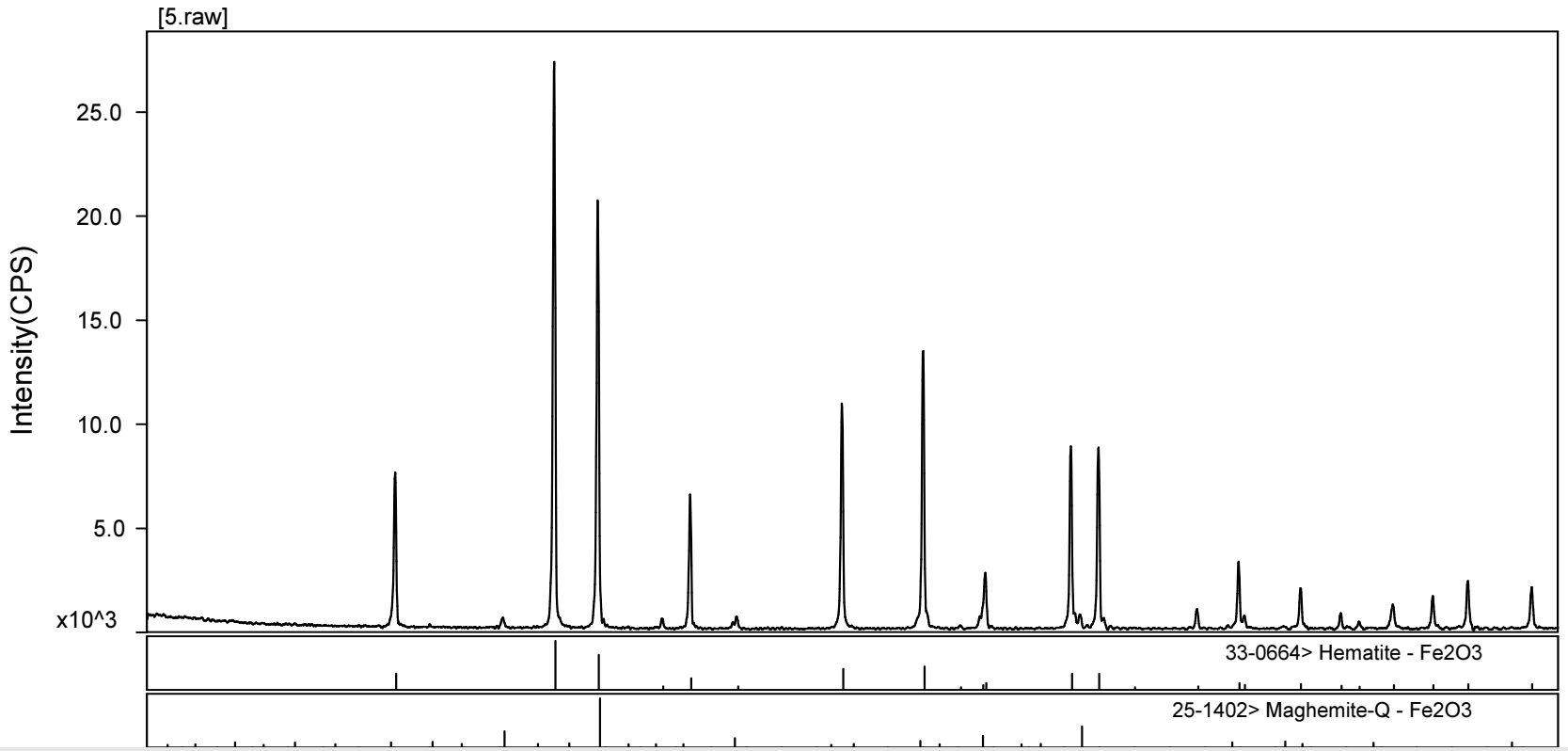
Before adsorption



After adsorption

Mechanism analysis

X-ray diffraction patterns of hematite



It can't be seen the diffraction peaks of crystals formed by uranium. It may be explained that the complex formed by U(Ⅵ) in neuter solution didn't form crystal lattice after adsorption to the surface of hematite with the function of electrostatic force.

ZVI is mainly precipitated by the electrochemical corrosion solution to restore the U (Ⅵ), and reduced U (Ⅵ) to U (Ⅳ) fixing. Electrochemical reaction of a new generation of [H], Fe^{2+}, OH have a strong reduction, etc., and can damage the structure of UO_2^{2+} to make it into a stable U (Ⅳ) , Iron hydroxide generated and its adsorption of U (Ⅵ) of sediment play a very important role. by the flocculation precipitation of iron corrosion products .

To use ZVI alone, the acidic conditions is U (Ⅵ) to remove the better, with the pH increase in, U (Ⅵ) to reduce the removal rate. When the pH = 3, the removal rate can reach 98.1%; when pH> 5, the removal rate of a sharp decline, that zero-valent iron to remove U (Ⅵ) in response to low pH conditions easier. Excess iron did not significantly improve U (Ⅵ) removal rate.

- X-ray powder diffractometer（D/MAX -2400）
- X-ray fluorescence spectrometer (XRF- 1500)
- Scanning electron microscope（LEO1450VP）

The Efficiency and Mechanism on Reduction of U（Ⅵ） by SRB

- Under anaerobic conditions，the characteristics of SRB applied to reduce U(Ⅵ) were investigated under different temperature，the concentration of U(Ⅵ), pH，and the coexisting ions.
- The results showed that the optimum reduction conditions was the temperature of 35℃，pH 7.0 and concentration of U（Ⅵ）25 mg·L-1.
- The maximum reduction capacity of SRB was 179.1 mg·g-1. Mo(Ⅵ) or Ca2＋did not affect SRB on the reduction process of U9(Ⅵ) under the concentration less than 5 g·L-1，but they strongly inhibit the process under the concentration more than 20 g·L-1.
- The main inhibition of Mo(VI) was physiological inhibition and the inhibition of Ca2＋was competitive inhibition through the stable complex formation，Ca-UO2-CO3. The results also showed that lag phase did not appear on the concentration of Ca2+ less than 5 g·L-1，but the lag phase of 24 hours appeared on the concentration of Ca2+ more than 20 g·L-1.

- The effect of pH is big to hematite remove U (Ⅵ) ,and the removal rate proves in positive correlation with pH , Under the acid conditions removal rate is quite low and only 14.8% at pH＝3,while reach 89.1% at pH＝8; Coexistence ion such as Ca^{2+} and Mg^{2+} didn't affect the adsorption of uranium by hematite significantly when its concentration is about $10^{-3}mol\cdot L^{-1}$.
- ESEM analysis the component of hematite powder indicated that the hexagonal crystals are α -Fe_2O_3 and square crystals are magnetic hematite (Fe_3O_4).The X-ray diffraction spectrometry test results of hematite after response can't be seen the diffraction peaks of crystals formed by uranium. X-ray fluorescence spectrometry analysis showed that the content of uranium is equal to that of uranium in adsorption.
- The pseudo-second-order kinetic model is appropriate to describe the adsorption process of U(Ⅵ) by hematite, and correlation coeffecient is 0.9959. The max adsorption capacity is 3.54 mg·g-1 at T= 293K. The adsorption of U(Ⅵ) by hematite fit for Langmuir equation.
- For the mixed pollutants, the repairing effect will be better by the filling material of mix material. Such as mixed hydroxyl ferric oxide, zeolite and other materials. The mixed strain to U (Ⅵ) have highly effective.

SRB to repair uranium wastewater pollution

The initial pH value of U (VI) to restore the biological effect

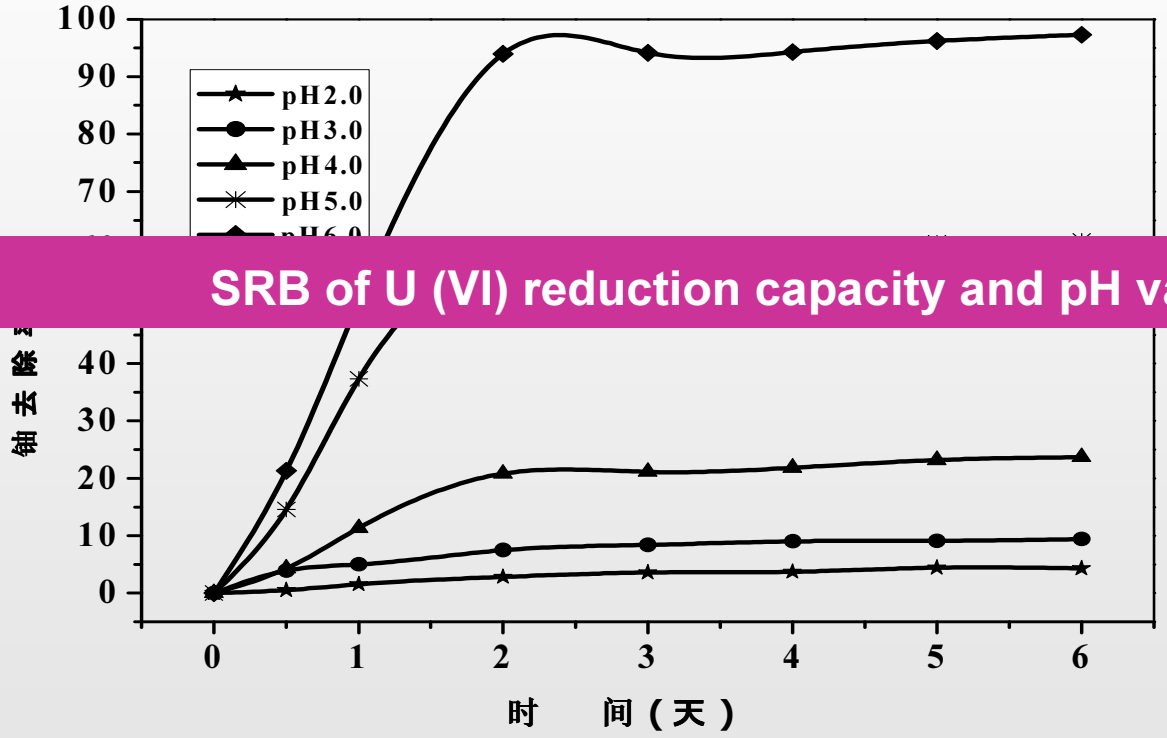


fig8 Different pH conditions, the impact of removal of uranium

SRB of U (VI) reduction capacity and pH values are related

U (VI) initial concentration 20mg / L, when the initial pH value of 2.0 and 3.0, the experimental U sedimentation rate after less than 10%; the rise in pH value, SRB of U (VI) reduction capacity. The initial pH value rose to 6.0 from 2.0 hours, U (VI) reduction rate from 4.3% to 99.2%.

SRB to repair uranium wastewater pollution

the results of SRB at co-existence of heavy metal ions to restore the sediment U (VI)

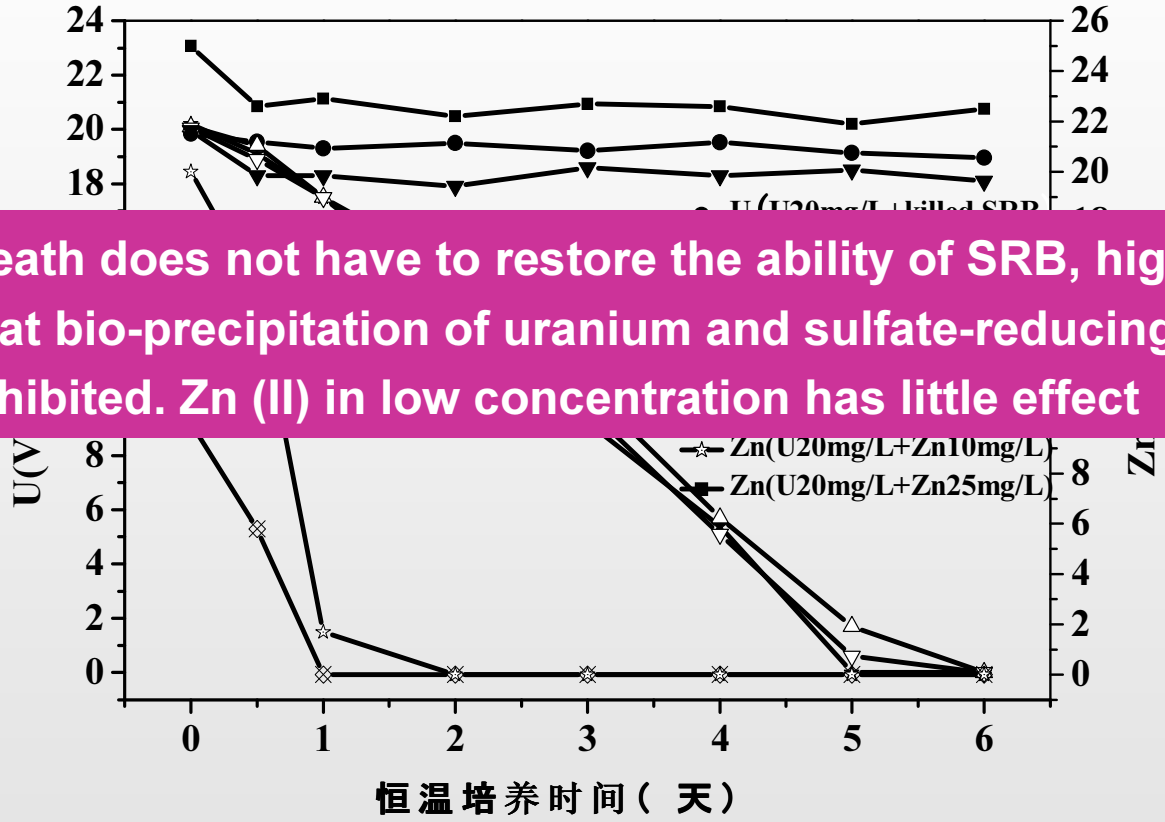


fig9 Different concentrations of Zn (II) co-exist on the impact of sedimentation U

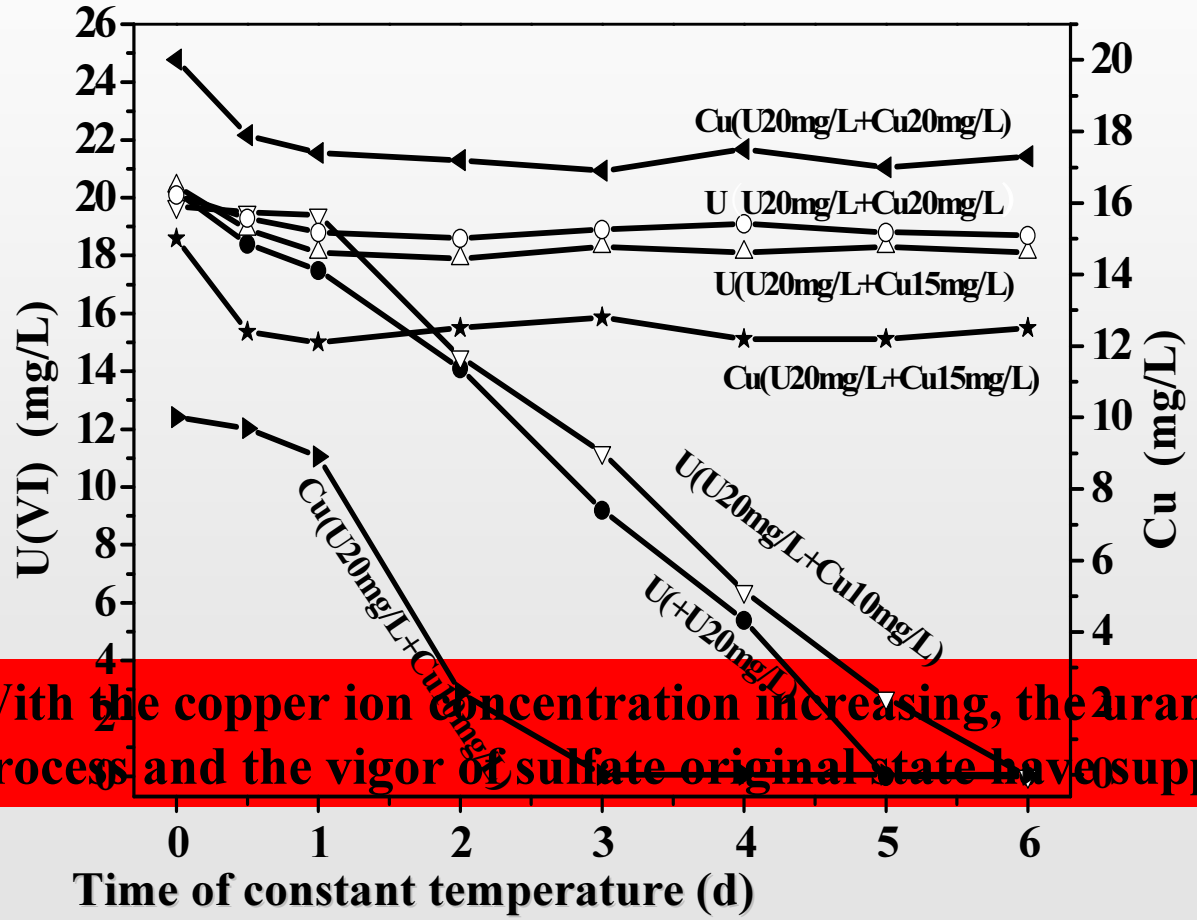
Death does not have to restore the ability of SRB, high concentrations of Zn (II) so that bio-precipitation of uranium and sulfate-reducing dynamic completely inhibited. Zn (II) in low concentration has little effect

SRB death does not have to restore the ability of U-rich, high concentrations of Zn (II) will precipitate uranium

metals). Zn (II) in the 0 ~ 20mg / L of the low concentration range of bio-precipitation process of uranium has little effect; low concentration of Zn (II) was completely removed in the 2d maintained a vigorous show that sulfate-reducing vitality.

The experiment of sulfidogenic bacteria repair the waste water of uranium

Effect of different concentration Cu(II) coexistence



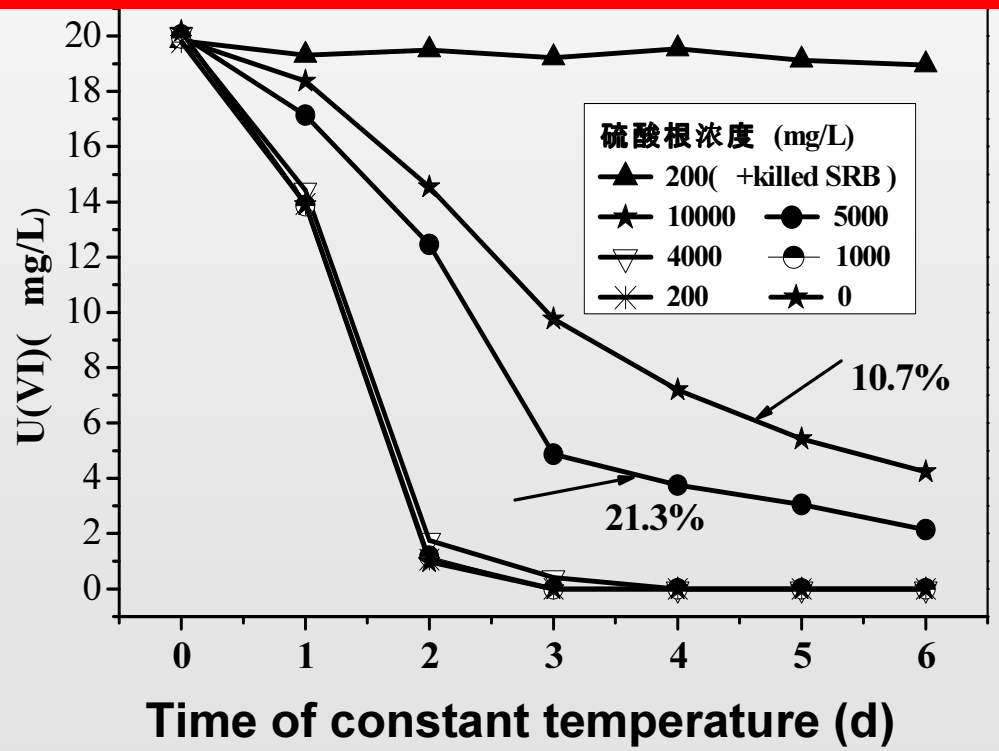
Initial concentration of Cu(II) was 10ml/L, that respond 6d with black group, U(VI) may completely precipitate gets down, but it presented the 1d slow time, and produced the H2S gas, the black precipitate of bottle base wasUO2+ CuS/UO2, With the copper ion concentration increasing, the uranium biogenic sediment process and the vigor of sulfate original state have

With the copper ion concentration increasing, the uranium biogenic sediment process and the vigor of sulfate original state have suppressed

The experiment results of sulfidogenic bacteria in repair the uranium polluted water

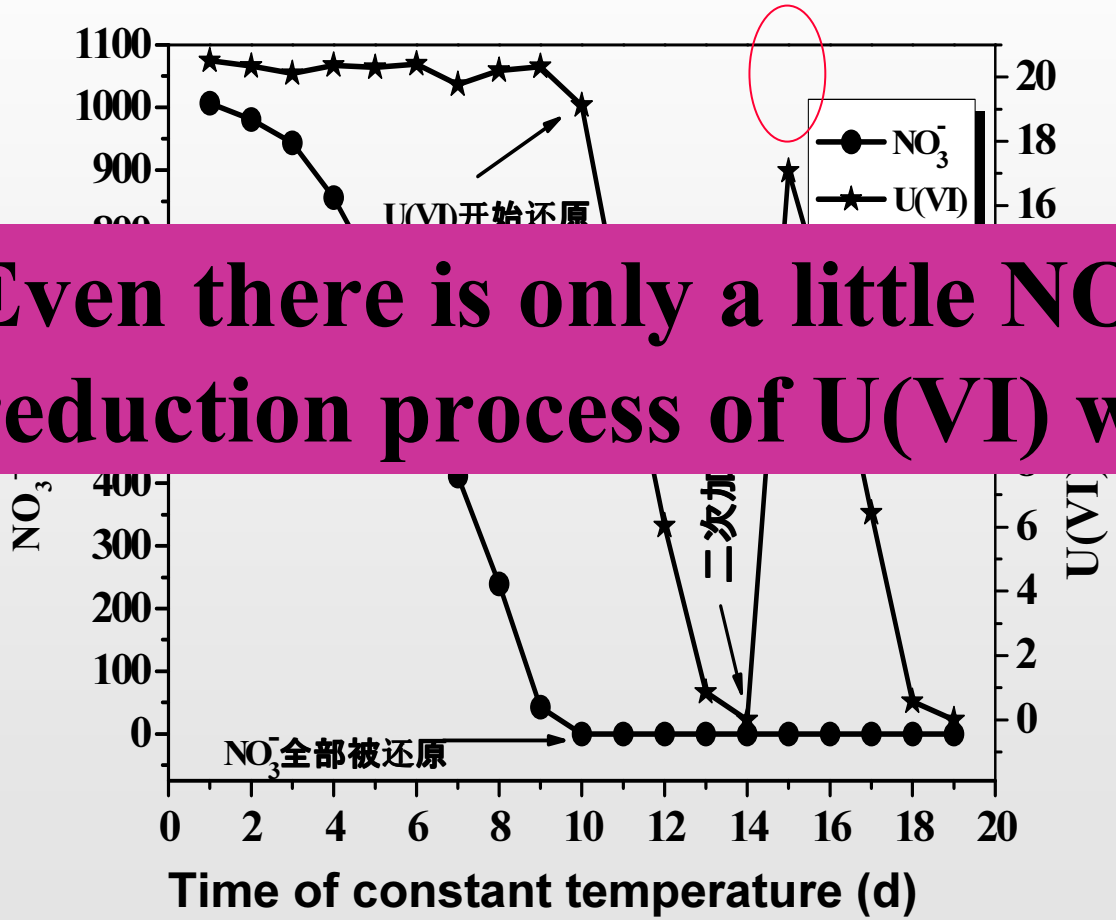
Effect of the concentration of SO_4^{2-} deoxidation precipitary the SBR

When the concentration of SO_4^{2+} was below 4000mg/L, it did not effect, but when the concentration of SO_4^{2+} above 5000mg/L, the deoxidize obviously existed inhibitory action about SBR to uranium, and it was related with the concentration of SO_4^{2+}.



The experiment results of sulfidogenic bacteria in repair the uranium polluted water

(7) Influence of SRB Reduction and Precipitation U(VI) Caused by NO3's Coexistence in Solution



The change of concentraion when NO3 and U(VI) coexist

Initial conditions：the concentraion of NO3- is1006.9mg/L ,U20.5mg/L ,before the 10th

Even there is only a little NO^{3-} in solution, the reduction process of U(VI) will be affected.

In the 14th day，NO_3 whose concentraion was 50mg/L was added, and all the NO3- was reduced 1 day later，the concentration of U rose to17.1mg/L,4 days later, all the uranium was reduced, which indicated that the reduction process of U(VI) couldn't happen even only a little NO_3 exist in the solution

Result and Discussion

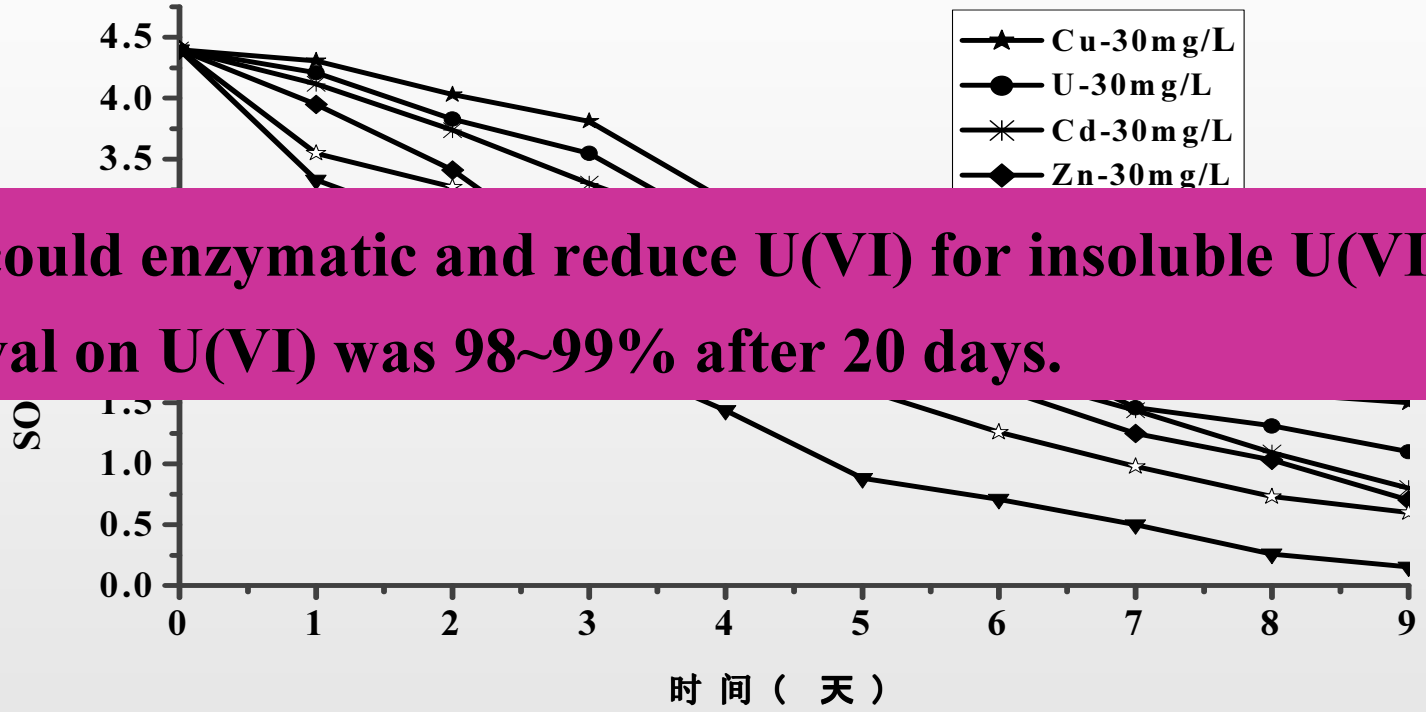
Influence of SRB's reduction ability on SO_4^{2-} caused by coexistence ions :U6+，Fe2+，Cd2+，Cu2+，Zn2+ and Pb2+

ELEMENTS	U	Cu	Cd	Zn	Pb
SO_4^{2-} REMOVAL RATE					
5mg/L	83.2%	76.3%	83.9%	84.5%	86.2%
10mg/L	81.1%	77.1%	82.8%	81.6%	85.7%
40mg/L	70.5%	62.7%	74.6%	70.4%	79.3%
50mg/L	66.8%	59.4%	67.7%	68.6%	78.5%

The toxicity sequence of heavy metal ions to SRB：$Cu^{2+} > U^{6+} > Cd^{2+} > Zn^{2+} > Pb^{2+}$

The experiment results of the reduction of U(VI) by SRB in wastewater

Effect of coexisting ions (U^{6+}、Cd^{2+}、Cu^{2+}、Zn^{2+}、Pb^{2+}) on reducing SO_4^{2-} of SRB



SRB could enzymatic and reduce U(VI) for insoluble U(VI), and the removal on U(VI) was 98~99% after 20 days.

图 1、 U、 Cu、 Cd、 Zn和 Pb30mg/L对 SRB还 原 SO_4^{2-}的 影 响

Fig.1 Effect of the concentration of the coexisting ions (U^{6+}、Cd^{2+}、Cu^{2+}、Zn^{2+}、Pb^{2+}) for 30mg/L.

The experiment results of the reduction of U(VI) by SRB in wastewater

The removal on SRB reducing SO_4^{2-} was highest (95.8%) ,when the concentration of Fe^{2+} were 50~1500mg/L, the precipitation efficency of FeS was 96.5~99%.

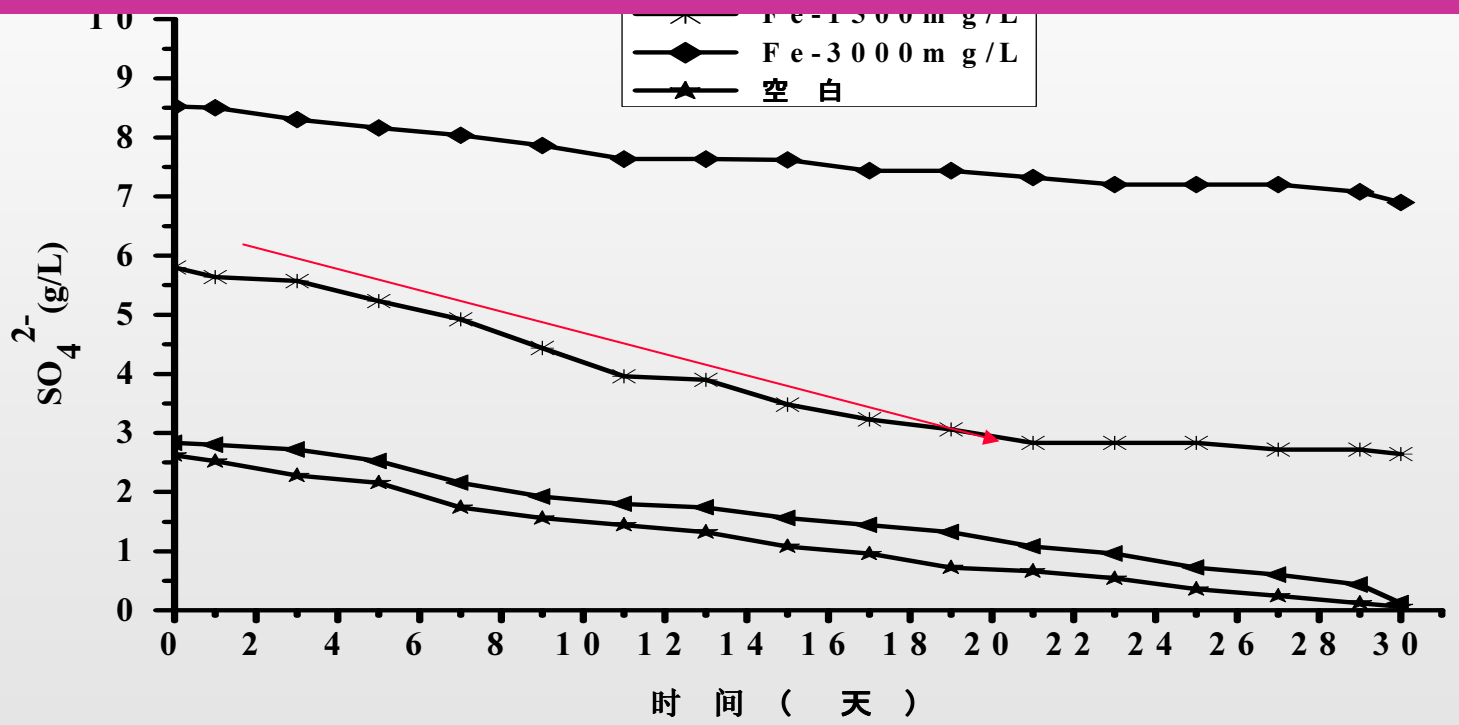


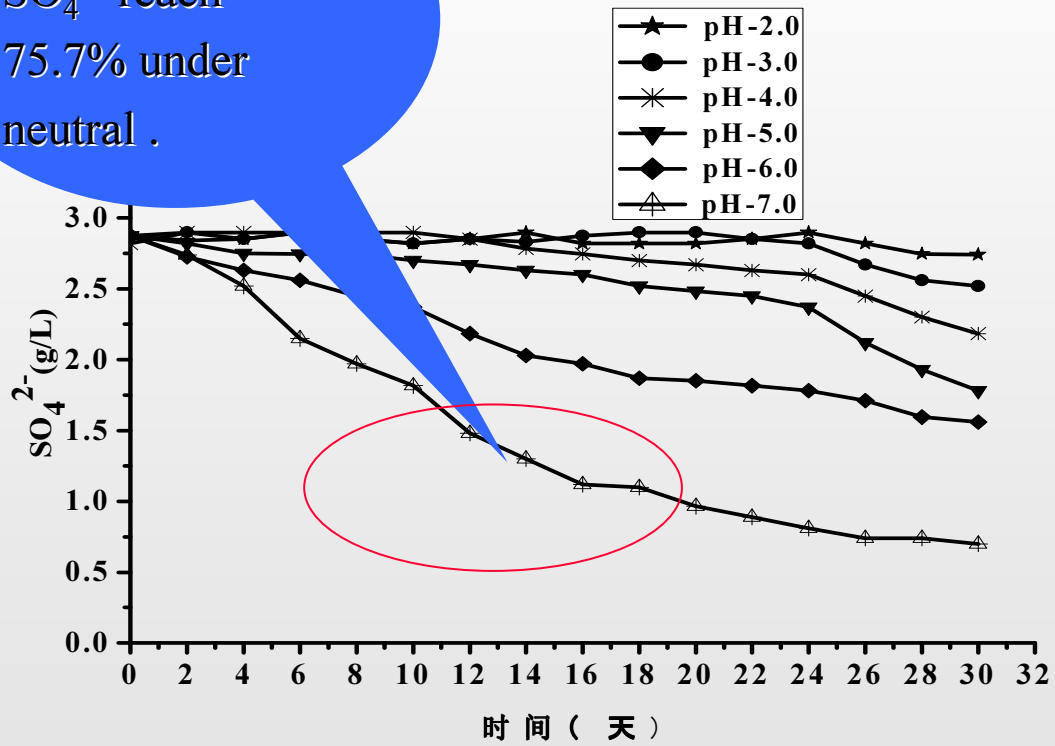
图 2 不同浓度的 Fe^{2+} 对 SRB 还原 SO_4^{2-} 的影响

Effect of the different concentration on reduction of SO42- by SRB.

The experiment results of the reduction of U(VI) by SRB in wastewater

Effect of pH on reduction efficency of SO_4^{2-} by SRB.

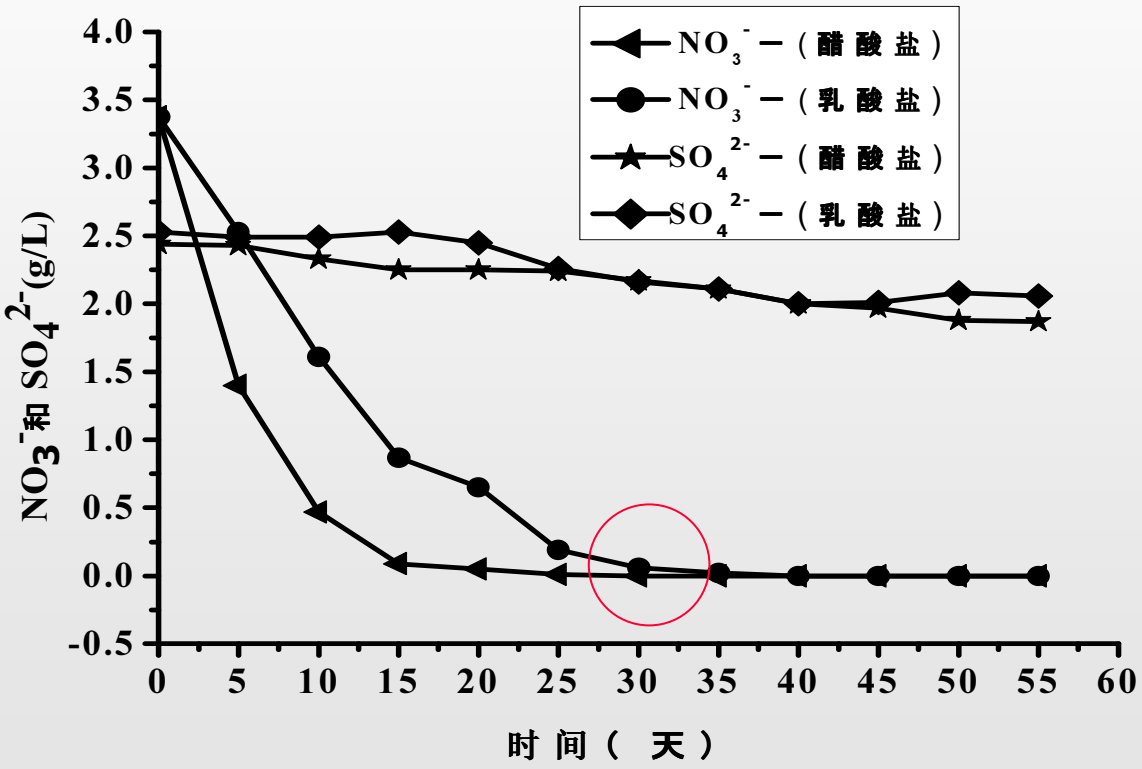
The removal of SO_4^{2-} reach 75.7% under neutral .



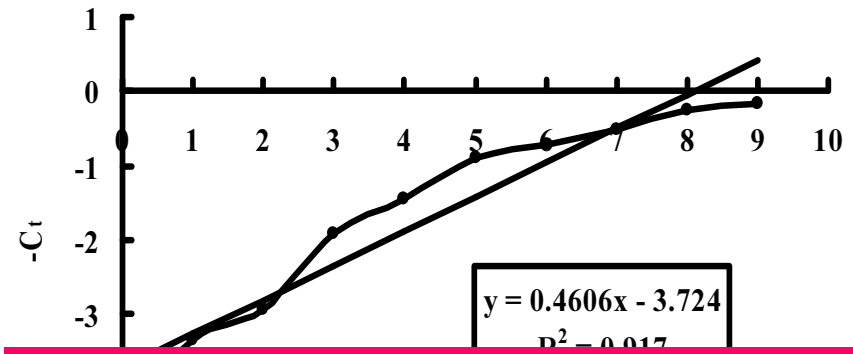
SRB had active in pH of 5.0 and 6.0, and the activity increased with increasing of pH.
The removal rate of SO_4^{2-} also grew. The removal rate of SO_4^{2-} were respectively reached to 38.1% and 45.7% at 30 days.
The max rate is 75.7% at pH of 5.0

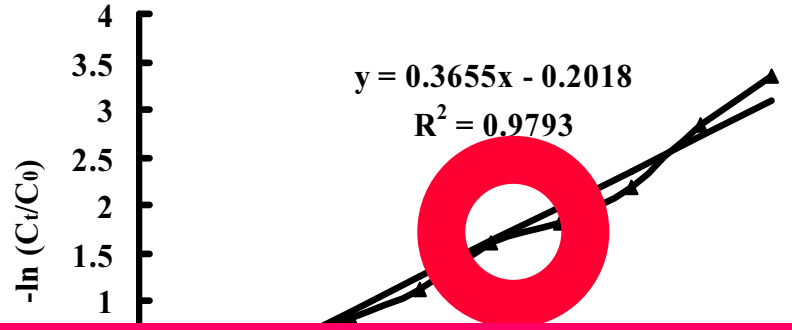
The experiment results on reduction U(VI) by SRB wastewater

Effect of nitrate of high concentration on reduction efficiency of SO42- by SRB.



The max removal rate of NO^{3-} is 440mg/L·d, with carbon source of acetate. With carbon source of lactic acid, the rate obviously reduced. Prior to 30 days, denitrifying bacteria predominate on denitrification reaction. The reduction of SO_4^{2-} began after 30 days.





The reaction of SRB deoxidize sulfate radical comply with the first order chemical reaction kinetics, R^2=0.9793

Fig.3 Fitting Chart of $-C_t$—t

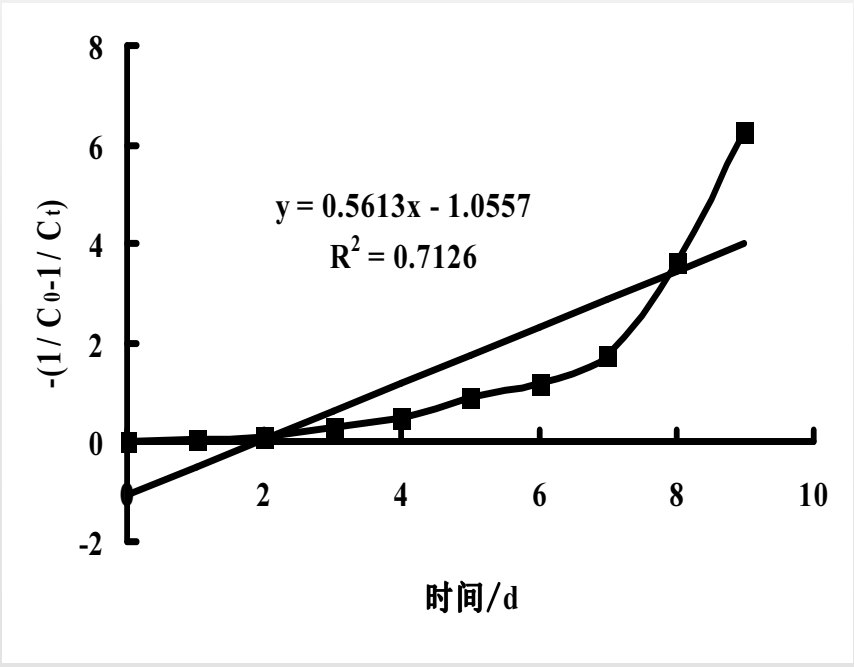


Fig.5 Fitting Chart of $-(1/C_0 - 1/C_t)$ —t

From the curve and the related coefficient of the fig, it can be seen that R^2=0.9793 of the first order reaction, linear is better.

The results of Bacillus subtilis adsorption of uranium by transmission electron microscope TEM

Wet cell before and after adsorption

TEM analyses that is an intracellular sequestration

(a) Before adsorption magnification:×0000

(b) After adsorption, magnification:×80000

- Transmission electron micrographs of uranium loaded cells revealed a dark opaque region throughout the cytoplasm indicating the intracellular sequestration of biosorbed metals the presence of accumulated radionuclides.
- It had better to use EDX analyses to identify the deposited elements.
- No significant metal accumulation in extracellular polymers.

Uranium has increased the permeability of cell membranes, once uranium ions through the membrane, the negative in cells through the chelate group, complexation, coordination etc, adsorbed the uranium acid-ion on cells.

Summary

In the treatment of radioactive wastewater with biotechnology，the concentration of nitrate is very important. Denitrificate reducing NO_3^- is the premise in uranium pollution control. Denitrifying bacteria could compete electron donor with SRB. Denitrification processes are more than sulfate-reducing activities of SRB in most cases. If the concentration of nitrate is up to 500 mg/L, sulfate-reducing activities will be inhibited. NO_3^- will impede bioreduction process of U(VI) entirely.

With the lower concentration of NO_3^- , there are bioreduction process. After U(VI) restored, readd NO_3^- will reverse the process of bioreduction of U(VI) , the restore product of U(IV)/UO_2 will oxidized and soluble in water.

When the concentration of SO_4^{2-} is more than 5000 mg/L, the inhibition function will exist in bio-sedimentation of uranium，the inhibition increased with it. If it is less than 4000 mg/L, the effect is less.

There are synergy functions between SRB and other bacteria，Mixed SRB used to deal with the mixed acid-situ leaching of uranium water is better than a single strain. The removal rate of sulfate and uranium can be reached 93.5% and 75.5%. Mixed acid method for the in-situ leaching of uranium mining waste, the proposed mixed SRB bacteria and denitrifying bacteria.

When the concentration of U(VI) is 5~50 mg/L， it can be changed into insoluble U (IV) by SRB，the efficiency are as high as 98-99%. Uranium reduction process is catalyzed by SRB involved in negative charge of lactate with the electron transfer to U (VI) to form U (IV) / UO2 enzymatic reduction. The activity of SRB in response to control by a variety of factors, pH affects the biological activity significantly, was positively correlated.

The results indicated that the concentration of U, Cu, Cd, Zn plasmas as high as 50 mg/L, SRB can grow, but be inhibited. With the increasing of concentration, the reduction rate of sulfate will decrease. The influence of Cu^{2+} is the greatest on the SRB. Lower concentration of Cu^{2+}, Zn^{2+} are easy to form sulfide precipitation, there are less influence to PRB. But when the concentration of Cu^{2+} 15mg/L and Zn^{2+} 25mg/L, the restore process would be completely inhibited. When the range of concentration of Fe^{2+} is 50~1500 mg/L, it can accelerate the reduction reaction of SRB. At the experiment range of pH, the max rate of SRB reduction happened at pH7, the removal rate of SO_4^{2-} was 75.7%.

Using both ZVI and SRB to deal with in-situ leaching of uranium waste and SO_4^{2-}, could enhance the efficiency up to 5-15%， it is indicated that between ZVI and SRB synergies, SRB is a reducing agent here and the catalyst as well.

- It is very difficult to test with purified bacterial, due to the existence of mixed strain. The effects/results of biochemical methods dealing with wastewater of acid leaching of uranium may be better.
- Using the physical and chemical properties of ZVI+ fluorspar, SRB-1 as catalytic agent, SRB-1 will synergism with ZVI, keep suitable environment for the microorganism, Such as adjusting pH or controlling the interfering ion. ZVI is excellent filling material of PRBs.
- There are both the adsorption and the catalytic effects to SBR. The efficiency of cooperative processing ZVI may enhance 5~15%.You can adjust pH about 5.5 to control the concentration of nitrate ions. SBR could alive at weak acidic and radioactive conditions.
- Optimal select microorganism and inorganic material. Carry on the pretreatment to the material or do some modifications for enhancing the performance of processing uranium.

The results of Biological isolation technology is stable

2005	influent		effluent		Removal rate %	remark
	PH	U(VI) /mg/L	U(VI)/ mg/L	PH		
6.11	2.7	17.80	0.35	6.78	98.03	
6.12.	2.6	17.42	0.17	6.85	99.02	
6.13.	2.7	16.91	0.48	6.70	97.2	
6.14.	3.6	19.61	0.20	6.78	99.0	
6.16	2.7	20.14	0.22	7.50	98.9	
6.18.	2.9	21.03	0.53	7.30	97.6	
6.20.	2.8	19.76	0.75	6.88	99.2	
6.24.	2.6	67.20	0.09	7.19	99.8	
6.28	2.7	75.10	0.07	7.21	99.0	
7.2.	2.9	66.30	0.11	7.12	99.8	
7.7.	3.1	64.70	0.23	7.03	99.7	
7.12.	3.3	19.78	0.32	7.13	98.78	
7.17.	3.2	43.2	0.45	7.15	98.95	
7.22.	3.4	7.61	0.14	6.98	98.1	
7.27.	2.9	49.1	0.26	7.03	99.47	
8.1.	3.7	47.5	0.19	7.14	99.6	
8.6	2.9	32.1	3.20	5.78	90.0	
8.11	3.7	19.1	1.44	4.89	92.5	
8.16	3.9	17.4	2.11	5.11	87.87	
8.21	4.5	101.4	0.73	7.18	99.28	
8.26	3.7	108.3	0.35	6.93	99.7	

Removal rate is above90%

The result of biochemistry interception technology

Those are average results in 2006 from mines in Yunnan Province which is not beyond the criteria specific.

program	Before biochemistry interception		After biochemistry interception	
	effusion of tailing	Concentration of excretion	effusion of tailing	Concentration of excretion
pH	2.71	3.65	2.71	7.50
U/(mg/L)	16.21	1.46	16.21	0.003

Conclusion and Suggestion 1

- **In process of a separate ZVI reduction the solubility of hexavalent uranium reduction for insoluble tetravalent uranium，acidic conditions is benefit for removal of uranium. It is the first order reaction，corrosion product of iron and iron oxide can adsorpte U(Ⅵ).**
- **Adding artificially selected strain SPR-1, the hexavalent uranium is reduced to the difficult soluble tetravalent uranium, it is a bio-chemial reaction, and the removal rate could reach 40%.**
- **Most coexisting ions, such as $U6^{+}$, Cu^{2+}, Cd^{3+}, Zn^{2+} and Pb^{2+} have inhibition on SRB, especially the Cu^{2+}. When the concentration of nitrate is more than 500mg/L or the concentration of SO_4^{2-} is more than 5000mg/L, the process of reducing will be inhibited completely. The concentration of inhibitory substances must be controlled effectively.**
- **Both the mixed flora of SRB and denitrifying bacteria should used when the uranium effluent from leaching process by the sulfuric acid method and nitric acid method at the same time.**
- ***Bacillus Subtilis* and *Citrobacter* are great amount of adsorption to uranium, more strong tolerance, more fast rate of adsorption that is more than 90% and easy to desorb. They are suit for recovering.**

Conclusion and Suggestion 2

- **uranium from the solution with lower concentration.**
- **The adsorption capability of bacteria to uranium have improved after treated *Citrobacter* by NaOH. At the zone of biological adsorption, the rates of dead thallus are better than the living ones. Carboxyl, amino and amide play an important role in the adsorption of uranium. The effect of using 4%PVA+1% sodium alginate as fixative to embed *Bacillus Subtilis* is better.**
- **The bench test and plant practice proved that the process of CITB is successful for uranium pollution control at the uranium tailings.**
- **Combined analysis of TEM and EDX, especially the specific part of thallus' biosorption, functional groups, create conditions for techniques of pretreatment and desorption agent, for improving the ability of adsorption capacity and adaptability.**
- **To isolate and purified in site bacteria, reform the bacteria. establish more advantage engineering strainod of acid resistant and radiation resistance.**
- **Study on stability genetic engineering bacteria;and ZVI and establish of biological, feasible models for directing the applying of bioabsorption method for industry.**