Treatment of Acid Wastewater Containing Uranium by Sulfate Reducing Bacteria

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Abstract. Sulfate Reducing Bacteria (SRB) strain form and its sulfate-reducibility was identified after enrichment, separation and purification of SRB from some anaerobic sludge of a uranium mine. The growth of SRB was stabilized 72 hours after inoculation. The influence of inoculation quantity, pH, temperature, SO_4^{2-} concentration on the growth of SRB was discussed. Sulfate treatment effect with 6.5g/L SO_4^{2-} concentration was 51.8% in 15 days and 69.1% in 30 days by SRB, respectively. Treatment effect of sulfate was 64% and 83%, the one of uranium was 60% and 82.3% from simulated wastewater by SRB and SRB+Fe^0, respectively.

Introduction

Nowadays sulfuric acid leaching is mainly applied for uranium extraction techniques, including in-situ leaching, heap leaching and stope leaching. However, some heavy metal and non-radioactive heavy metal will unexpectedly obtained during uranium extraction process, which lead to contamination of surface water and groundwater, especially dissolved radioactive nuclides will transport for in-situ leaching and stope leaching. Currently the main methods for treatment of acid uranium containing wastewater include chemical precipitation, ion exchange, evaporation, absorption and microbiological technology (Yang et al. 1994; Yu et al. 2002; Pan 1984; Hou 2003; Junta-Ross and Hochella 1996; Tang et al. 2003; Huang and Zheng 2002) among which microbiological technology is gradually being paid attention to due to advantages of low cost and decreased secondary pollutant. Hence Sulfate-Reducing Bacteria (SRB) is used for treatment of acid uranium containing wastewater in microbiological process.

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Principal of treatment of acid uranium containing wastewater by SBR

SRB is kind of anaerobic bacteria which reduce sulfates through dissimilation, and normally SRB, with diverse shape and nutrition, use sulfates serving as electron acceptor during organic dissimilation (Hu and Gu 2003; Janssen et al. 1995).

Under initial anaerobic condition, SO_4^{2-} is reduced to H_2S by SRB in sulfate dissimilation process, only if electron supplier i.e. organic carbon source add to wastewater could SO_4^{2} biological reduction happens.

Reactions in initial anaerobic stage (Su and Li 2005):

(1) Fermentation

Orgnic molecule i.e. sucrose transforms to lactic acid.

$$2CH_3CHOHCOOH+SO_4^{2-}\rightarrow 2CH_3COO^{-}+2HCO_3^{-}+2H_2S \quad (1)$$

It is necessary for the following SO_4^{2-} reduction, for only fermentation product i.e. pyruvate and lactic acid can be used as carbon source for SBR.

(2) Bacteria respiration

SBR use lactic acid to produce acetic acid.

$$2CH_3CHOHCOOH+ SO_4^{2-}\rightarrow 2CH_3COO\text{-}+2HCO_3\text{-}+2H^2S \quad (2)$$

Reaction (2) could also be shown as below.

$$2C+SO_4^{2-}+H_2O\rightarrow H_2S+2HCO_3^{-}$$

There are mainly four pathways for SO_4^{2-} biological reduction on heavy metal treatment: (1) According to reduction by catabolized sulfate, SBR reduce SO_4^{2-} to H_2S, U (VI) in wastewater to U (IV) sediment (Bertine 1970; Bonatti et al. 1971; Lovley et al. 1993; Tebo and Obrazsova 1998; Lovley 2003), heavy metal with high valence to low valence state sediment; (2) Heavy metal ion in wastewater will be attenuated by reaction with H_2S from pathway above, which is a significant pathway for heavy metal treatment by SO_4^{2-} biological reduction; (3) Reduction from SO_4^{2-} to S^{2-} will increase pH of wastewater and further be favorable to eliminate heavy metal ion in the form of hydroxide sediment; (4) Certain heavy metal will be removed by reaction with CO_3^{2-} that obtained from CO_2 in SBR metabolism process (Ma et al. 2003).

Sample Collection, Cultivation, Isolation, Purification

Anaerobic sludge collected from certain uranium tailing pond was put into Hungate anaerobic tube containing Germany No.DSMZ2075 culture medium under 35℃ (Su and Li 2005) in constant temperature incubator. After 7 days, sludge in Hungate anaerobic tube turned black with strong rotten egg odor, which showed well-grown of SBR. Then 1ml supernatant was transferred to newly prepare anaerobic culture medium for second purified cultivation, after that, Hungate roll

tube isolation purification (Pan et al. 2007) was carried out until purified bacteria strain was obtained, as showed in Fig.1.

Hungate roll tube isolation → Culture bacteria → choosing single colony → Second roll tube

Fig.1. Isolation & Purification of SRB

After purification, there were single bacterial colony with scattered distribution and uniform shape at tube wall, which showed small black globular, raised in surface and stretched all around. The bacterial strain was identified to be Gram-negative, spore staining negative, sing polar flagellum and the cells were observed to be swinging moved, tiny rod but curved shape, as showed inFig.2.

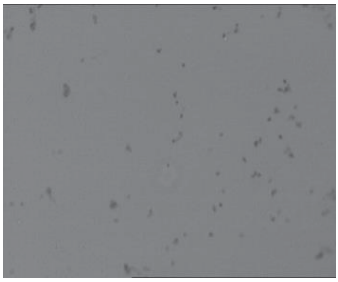


Fig.2. Isolated SRB

Being injected into liquid culture medium, the bacterial strain from above experiment turn PbAc test paper black at its logarithmic growth phase, which illustrated strong capacity in sulfate reduction, therefore, the bacterial strain was proved to be SRB (Wan et al. 2003).

Study on growth factor

Character of SRB growth

Measurement of OD_{605} of bacterial suspension by spectrophotometer demonstrated positive correlation (Shen et al. 1998) between OD_{605} and bacterial growth. Prior to measurement, bacterial suspension was diluted to 100 times as before to avoid effect of black sediment.

Growth curve

Considering the inconvenience brought by preparation of Germany No.DSMZ 2075 culture medium, formula (Chen et al. 2006) from Chengdu methane bureau under national agricultural department was adopted for growth factor study. With certain bacterial strain had inoculated into fresh uniform liquid culture medium, growth of bacterial strain experienced lag phase, logarithmic phase, steady phase and dissipation phase under adequate condition. Thus there achieved growth curve of bacterial strain with horizontal axis incubation time and vertical axis OD_{605}, which reflected variation tendency of bacterial strain.

Fig.3 showed the growth curve of isolated SRB, it is obvious that SRB went into logarithmic phase rapidly, and steady phase in 64h culure.

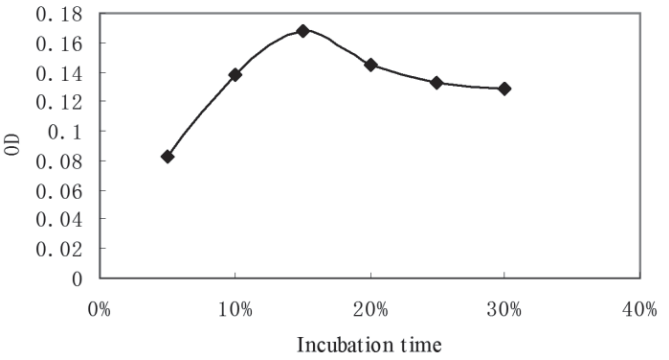


Fig.3. Growth curve of isolated SRB

Effect of inoculation quantity on SRB growth

Inoculation quantity was another factor affecting SRB growth in duration of logarithmic phase. Large inoculation quantity led to shorter logarithmic phase and competition between bacterial strain. Conversely, low inoculation quantity led to less stronger competition.

SRB was inoculated into 50ml liquid culture medium with 5%, 10%, 15%, 20%, 25%, 30%inoculation quantity respectively, OD605nm was measured after 7 days culture under temperature 35℃. Results in Fig.4 indicated a maximum OD605nm at inoculation quantity 15%, which was be adopted in the following study.

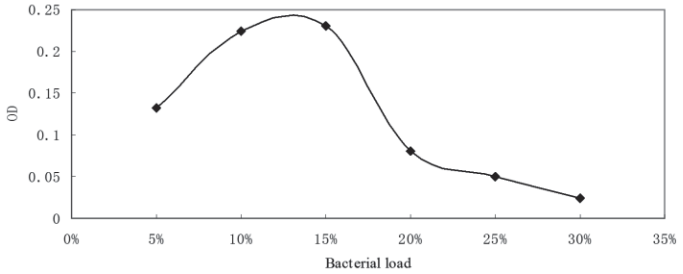


Fig.4. Effect of inoculation quantity on SRB growth

Effect of pH on SRB growth

In order to identify optimum pH of SRB growth, formula from Chengdu methane bureau was improved by adjusting pH at 2.0、2.5、3.5、4.5、5.5、6.5、7.5 with HCl (0.5mol/L). OD605nm measurement was carried out every 24h 7 days later to identify optimum pH for SRB growth. Fig.5 showed optimum pH for SRB was 6.5.

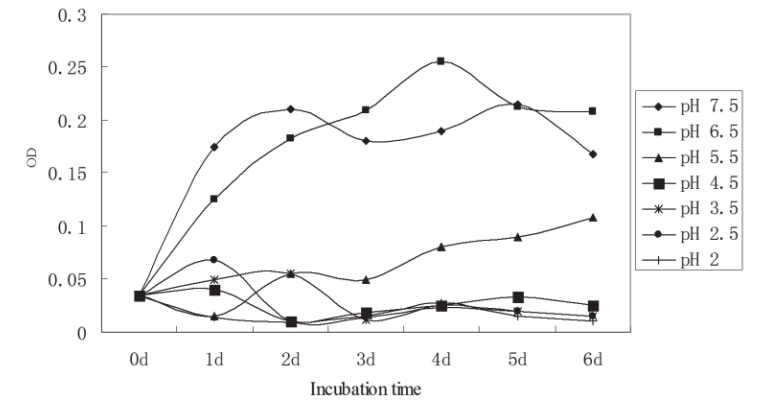


Fig.5. Effect of pH on SRB growth

Effect of temperature on SRB growth

Temperature is an important parameter for anaerobic sulfates reduction that will affect the metabolic activity and growth rate of SRB. SRB include two types, that are majority of mesophilic and the rest of thermophilic, of which mesophilic SRB are likely to achieve optimum growth between 28～38℃ ,and thermophilic is 54～70℃, maximum of 56～85℃. Related research (Li and Su 2000; Laryr and

Barton 1995) revealed that activity of SRB would suffer nearly no inhibition and observable inhibition at temperature of 31～35℃ and below 30℃ respectively.

Effect of SO_4^{2-} concentration on SRB growth

In order to identify relationship between SO_4^{2-} concentration and SRB growth, liquid culture medium was adjusted by setting SO_4^{2-} concentration at 1.5、2、3、4、5、6、7、8、9、10g/L level with unchangeable proportion of $MgSO_4 : Na_2SO_4$=1 : 2. Accordingly optimum SO_4^{2-} concentration on SRB growth was identified based on OD605nm measurement taken every 24h after 7 days growth. Results showed optimum SO_4^{2-} concentration on SRB growth was 4~6g/L, see Fig.6.

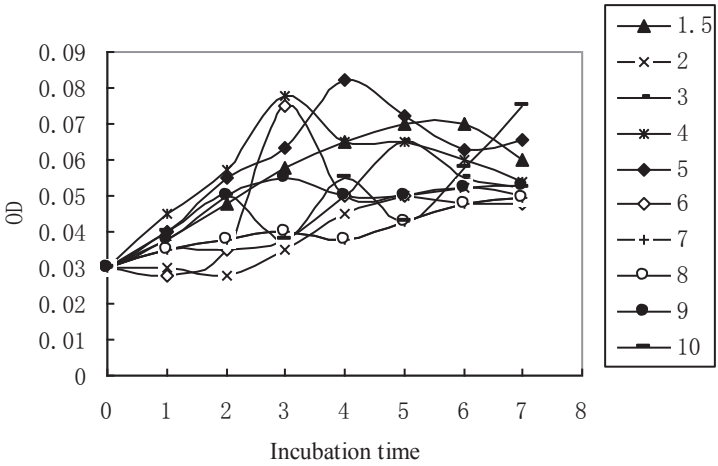


Fig.6. Effect of SO_4^{2-} concentration on SRB growth

Effect of SO_4^{2-} concentration on sulfates reduction rate

In order to identify effect of SO_4^{2-} concentration on sulfates reduction rate, liquid culture medium was adjusted by setting SO_4^{2}- concentration at 3.0、5.7、6.5、7.4、11.1、16.4、17.0g/L level with unchangeable proportion of $MgSO_4 : Na_2SO_4$=1 : 2. Sulfates reduction rate was calculated according to analysis on SO_4^{2-} concentration 15days and 30 days later. Effect of SO_4^{2-} concentration on sulfates reduction rate was shown in Fig.7.

As displayed in Fig.7,sulfates reduction rate was between 41.4～51.8% for 15 days culture and 43.7～69.1% for 30 days culture. Moreover, sulfates reduction rate reached maximum at SO42- concentration 6.5g/L for both 15 day and 30 days

culture. This was in line with the conclusion that SRB was at its optimum growth with SO_4^{2-} concentration of 4~6g/L.

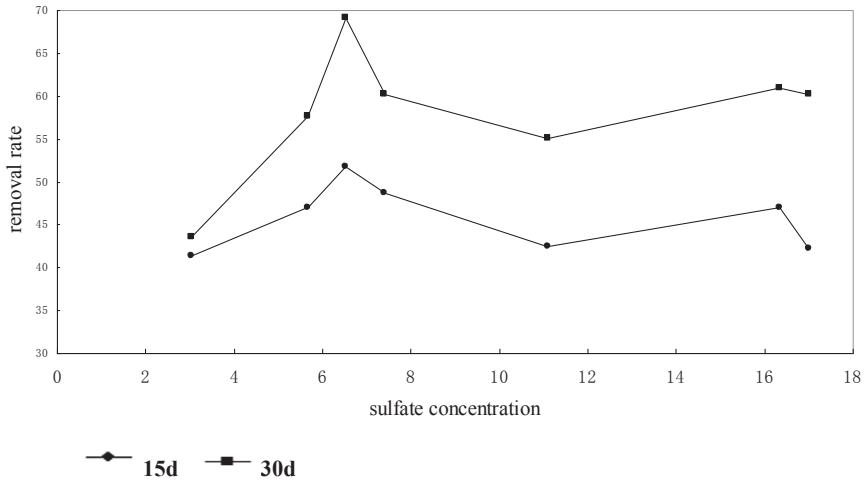
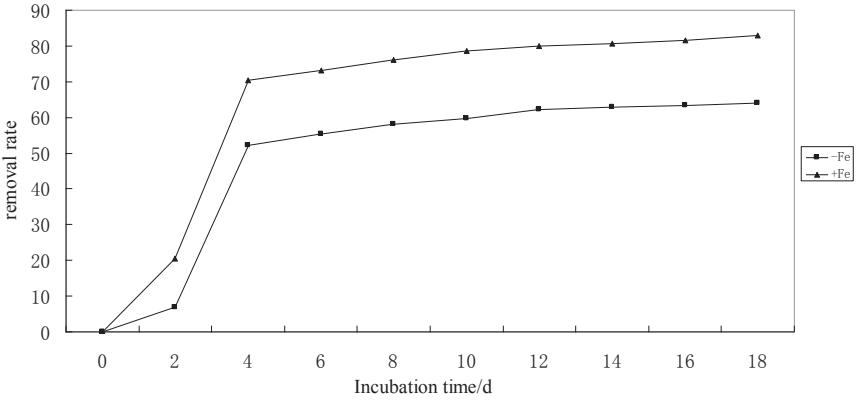


Fig.7. Effect of SO_4^{2-} concentration on sulfates reduction rate

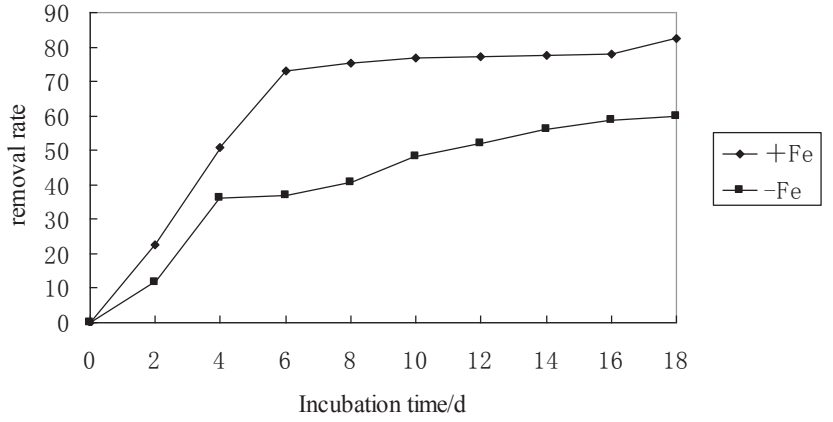
SRB on uranium wastewater treatment

In order to explore SRB and coupling effect of both SRB and zero-valent iron(ZVI) on treatment efficiency of SO_4^{2-} and U, two set of wastewater simulation was conducted with one set 15% SRB inoculation and another set 15% SRB plus 0.3g ZVI. Uranium wastewater in this trial was prepared in lab with pH value 6.5, SO_4^{2-} concentration 2g/L and U concentration 3mg/L, culture medium was still follow the formula of Chengdu methane bureau. Results can be seen in Fig.8,9.



-Fe:SRB + Fe:SRB+Fe^0

Fig.8. SRB and coupling effect of both SRB and ZVI on treatment efficiency of SO_4^{2-} in wastewater



-Fe:SRB + Fe:SRB+Fe^0

Fig.9. SRB and coupling effect of both SRB and ZVI on treatment efficiency of U in wastewater

Results indicated that SO_4^{2-} concentration was 640mg/L, treatment efficiency was 64% and U concentration 1.2mg/L, treatment efficiency 60% for 15% SRB culture 18 days later; SO_4^{2-} concentration was 340mg/L, treatment efficiency was 83% and U concentration 0.53mg/L, treatment efficiency 82.3% for 15% SRB plus ZVI culture 18 days later.

In summary, ZVI promotes SRB growth in following aspects:

ZVI's consumption of H^+ under acid condition increased pH of wastewater, which accelerated SRB growth;

Hydrogen served as energy source of SRB and further promoted SRB growth;

Fe^{2+}、Fe^{3+} generated from ZVI reduction, not only promoted SRB growth, but also eliminated inhibition on SRB by sediment formation with H_2S;

ZVI weakened toxicity of SRB by decreasing heavy metal concentration in reduction process. Therefore, ZVI promoted SRB growth and strengthened its capacity on wastewater treatment and heavy metal removal.

Conclusions

SRB is widely spread, easily isolated, simply culture method, steps into steady phase in only 64h. Uranium wastewater treatment by SRB is a prospect technique with desirable efficiency of 64% and 60% for SO_4^{2-} and U;

Factors affecting SRB growth include inoculation quantity, pH, temperature, SO42- concentration. Study show optimum growth for SRB under condition of inoculation quantity 15%, pH value 6.5, temperature 35, SO_4^{2-} concentration of 4~6g/L.

Treatment of uranium wastewater by coupling effect of both SRB and ZVI achieved removal efficiency of 83% and 82.3% for SO_4^{2-} and U, which revealed ZVI promoted SRB growth and strengthened its capacity on wastewater treatment and heavy metal removal.

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