

Treatment of simulated wastewater from in situ leaching uranium mining by zerovalent iron and sulfate reducing bacteria

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Received 10 August 2009; accepted 15 September 2009

Abstract: Batch and column experiments were conducted to determine whether zerovalent iron (ZVI) and sulfate reducing bacteria (SRB) can function synergistically and accelerate pollutant removal. Batch experiments suggest that combining ZVI with SRB can enhance the removal of U() synergistically. The removal rate of U() in the ZVI+SRB combining system is obviously higher than the total rate of ZVI system and SRB system with a difference of 13.4% at $t=2$ h and 29.9% at $t=4$ h. Column experiments indicate that the reactor filled with both ZVI and SRB biofilms is of better performance than the SRB bioreactor in wastewater basification, desulfurization and U() fixation. The results imply that the ZVI+SRB permeable reactive barrier may be a promising method for treating subsurface uranium contamination.

Key words: zerovalent iron (ZVI); sulfate reducing bacteria (SRB); uranium; sulfate; wastewater; basification

1 Introduction

Groundwater contamination caused by uranium mining and milling activities, especially from in situ leaching (ISL) process, is a widespread environmental problem. Due to the use of sulfuric acid as an extractant in the operation, the polluted groundwater is generally characterized by very low pH, high levels of uranium, sulfate, which can seriously threaten downstream groundwater resources. In particular, radioactive uranium commonly present as uranyl (UO_2^{2+}), is highly soluble and mobile under oxidizing conditions and poses a great human health danger. Traditional ex situ remediation approaches based on pump and treat practice are not ideal. It is necessary for researchers to look for cost-effective alternatives to treat uranium-contaminated groundwater and prevent its further migration and spread through the deep subsurface.

In recent years, the discovery that sulfate reducing bacteria (SRB) can enzymatically transform soluble U() to geochemically inert U(), a highly insoluble

uraninite, has sparked interest in in situ bioremediation of uranium-contaminated groundwater[1–3]. Engineering a permeable reactive barrier (PRB) in the subsurface zone is another approach to treating U() contaminated groundwater. Zerovalent iron (ZVI) is a reactive medium commonly utilized in PRBs, which can promote the degradation of redox-sensitive pollutants[4]. Previous studies mostly focused on the applications of ZVI to the destruction of halogenated organic compounds[5–7]. Only recently has this process been employed for the immobilization of specific metals such as Cr(), U(), and Tc() in groundwater[8–10]. Although many investigations have been reported on the use of SRB and ZVI respectively to treat uranium-bearing wastewater, literatures on the combination of biological and chemical approaches are limited. As well known, SRB can promote the electrochemical corrosion of iron based on the theory of the cathodic depolarization. It has been demonstrated that the combination of ZVI and other microorganisms can enhance reductive treatment[11–13]. The integrated system may overcome several limitations of the individual techniques with the likelihood that

some of the combined attributes may be synergistic. In this work, we made an attempt to use the ZVI+SRB flow through column reactors to treat simulated wastewater as well as to evaluate their application future in groundwater remediation.

2 Experimental

2.1 Cell culturing and harvesting

Sediment sludge was taken with a sludge sampler (Ballcheck KB, Eijkelkamp, Holland) at 1 m depth from an abandoned uranium mill tailings impoundment in Hengyang, which is the largest uranium milling tailings disposal site in China. The sediment sludge sample was transferred to a Mason jar until it was completely full and then the jar was sealed tightly to avoid any direct contact with oxygen. The sediment was stored at 4 °C up until the experimentation. After the sample was transported into the laboratory, continuous cultivation and enrichment of the mixed SRB were carried out immediately as previously described[14]. Finally, the concentration of the obtained SRB population was then estimated by standard most probable number (MPN) methods[15]. The enriched SRB contained a final concentration of approximately $5 \times 10^9 \text{ mL}^{-1}$. The enriched SRB suspension was used as standard test solutions (STS) in the following U(VI) reduction experiment.

2.2 Pretreatment of iron powder and preparation of U(VI) stock solution

The iron powder was obtained from Shanghai Chemical Co. Its composition was determined as 3.55% C, 2.09% Si, 0.97% Mn, 0.61% Cr, and 92.77% Fe. The ZVI was hand-sieved to give a size of about 75 μm . Then it was washed with 5% H_2SO_4 three times, followed by rinsing with deionized water three times and drying naturally. The goal of the pretreatment was to clean the surface to remove any (hydr)oxide layer. The standard U(VI) stock solution containing 1 mg/mL U was prepared using $\text{UO}_2\text{Cl}_2 \cdot 3\text{H}_2\text{O}$ and deaerated water in a glovebox. This stock solution was used in the following batch and column reactor experiments.

2.3 Batch reactor experiments

Three groups of U(VI) reduction experiments, including microbial reduction by SRB (group A), chemical reduction by ZVI (group B), combined reduction by ZVI+SRB (group C) were conducted anaerobically in a series of 600 mL serum bottles in the dark at room temperature. Each group set up three parallel bottles and each bottle contained 450 mL test

solutions. The components for three groups are listed in Table 1. The headspace of the serum bottles was pressurized with ultrapure N_2 (10 min), capped with butyl rubber septa and crimped with an aluminum seal. All the serum bottles were shaken horizontally at 35 rpm for 2 d, while cells were metabolically active. The samples were taken regularly using a nitrogen-purged syringe fitted with a needle and then filtered through 0.22 μm membranes. The concentrations of U(VI) in the filtrates were determined using a kinetic phosphorescence analyzer (KPA-11, Chemchek Instrument, Richland, WA). This method is specific for analyzing all forms of U(VI), with a detection limit lower than 0.1 mg/L and a standard deviation (SD) of approximately 5%. The uranium removal rates for three groups were compared.

Table 1 Components of SRB, ZVI, ZVI+SRB systems

Group No.	Volume of STS/ mL	Volume of modified postgate C/ mL	$\rho(\text{ZVI})/ (\text{g}\cdot\text{L}^{-1})$	$\rho(\text{U(VI)})/ (\text{mg}\cdot\text{L}^{-1})$	$\rho(\text{Cys})/ (\text{g}\cdot\text{L}^{-1})$
A (SRB)	450	0	0	20	3.0
B (ZVI)	0	450	2.0	20	3.0
C (ZVI+SRB)	450	0	2.0	20	3.0

2.4 Column reactor experiments

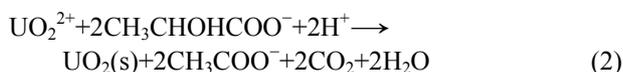
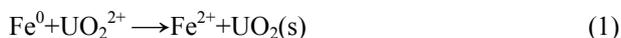
Two polyvinyl chloride (PVC) columns (64 mm in inner diameter and 1 180 mm in length) were packed with roll-front infiltration sandstone-type uranium ore, which was exploited from Wuyier Mine situated in the Yili Basin of Xinjiang, northwestern China. A gravel layer with a height of 8 cm and a grain size of 2–4 mm was placed at each column end. 5–20 g/L H_2SO_4 was dropped from the column top at a speed of 50–60 drop/min. The dropping process stopped when the U(VI) level was lower than 0.5 mg/L. The leaching residue of uranium ore in the columns was taken out and dried naturally for the subsequent column reactor experiment. Similarly, the two aforementioned PVC columns' top and bottom were filled with 8 cm high gravel (diameter of 2–4 mm). The middle part of one column was filled with the above dried leaching residue of uranium ore; the other column was filled with about 85% leaching residue of uranium ore and 15% 75 μm ZVI powder (in volume fraction). The columns were attached to a reservoir bottle that contained uranium-bearing wastewater. The initial influent was Postgate B medium. A continuous flow of 2.8–3.1 mL/min was maintained at room temperature of $(27 \pm 3) \text{ }^\circ\text{C}$. The columns were allowed to activate for 10 d, which is favorable to the formation of SRB

biofilms. After flushing Postgate B medium for 10 d, wastewater solution was introduced as the influent. The wastewater quality characteristics were described as follows (g/L): KH_2PO_4 0.5, NH_4Cl 1.0, yeast extract 1.0, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 0.1, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.0, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.002, and 70% sodium lactate 0.5. The pH was 4.66. The initial concentrations of $\text{U}(\text{VI})$ and sulfate were set as 41.33 and 4 017 mg/L, respectively. The total operation time was 30 d. The influent solution was continuously sparged with ultrapure N_2 to provide a reducing atmosphere throughout the experiment. When the reactors started, the uranium and sulfate concentrations and pH in the effluent were analyzed at regular intervals. A Dionex 500 ion chromatography system equipped with an AS14 anion exchange column and a CD20 conductivity detector was employed to measure the sulfate concentration (detection limit approximately 1×10^{-7}). The effluent pH was measured by pHs-25 type pH meter (Shanghai Leici Instrument Company, China).

3 Results and discussion

3.1 Synergistic effect of $\text{U}(\text{VI})$ fixation by ZVI and SRB

It has been reported that ZVI and SRB can remove $\text{U}(\text{VI})$ from the solution individually [16–18]. The former may occur via chemical reduction of uranyl [$\text{U}(\text{VI})$] to highly insoluble uraninite [$\text{U}(\text{IV})$] (Eq.(1)). The latter depends mainly on a biocatalyzed reduction process. During this process, SRB can grow using lactate as an electron donor and $\text{U}(\text{VI})$ as a terminal electron acceptor, converting uranyl to uraninite precipitation (Eq.(2)). It should be pointed out that in SRB system without sulfate, $\text{U}(\text{VI})$ can still be removed (Table 2). This result demonstrates that $\text{U}(\text{VI})$ is directly reduced enzymatically, rather than indirectly by H_2S produced during sulfate reduction.



However, SANI et al [19–20] suggested that hematite ($\alpha\text{-Fe}_2\text{O}_3$) and goethite ($\alpha\text{-FeOOH}$), the electrochemical corrosion products of iron surface by SRB, can reverse or impede the process of microbiological $\text{U}(\text{VI})$ reduction by SRB. So it is of significance to explore the interaction between ZVI and SRB and its influence on the immobilization of $\text{U}(\text{VI})$. As shown in Table 2, the removal rate (R) of $\text{U}(\text{VI})$ in the ZVI+SRB combining reactor is obviously higher than the total rate of two control groups with a difference of 13.4% at $t=2$ h and 29.9% at $t=4$ h. These data indicate that the combination of ZVI with SRB can enhance and

promote the removal of $\text{U}(\text{VI})$ synergistically. In other words, the positive synergistic effect from ZVI is greater than the negative antagonistic effect from electrochemical corrosion byproducts.

Table 2 Comparison of $\text{U}(\text{VI})$ removal rate for SRB, ZVI, and ZVI+SRB systems

Time/h	$R_{\text{SRB}}/\%$	$R_{\text{ZVI}}/\%$	$R_{\text{ZVI+SRB}}/\%$	$[R_{\text{ZVI+SRB}} - (R_{\text{SRB}} + R_{\text{ZVI}})]/\%$
2	9.6	42.3	81.8	29.9
4	17.4	67.3	98.1	13.4

3.2 Effluent pH changes in two column reactors

Fig.1 reflects the effluent pH changes of two reactors during the operation period. It can be seen from Fig.1 that the pH value in the two reactors increases gradually with the increase of time at early stage. This phenomenon can be interpreted by that SRB can consume H^+ and generate alkalinity through sulfate reduction under acidic pH conditions (Eq.(3)). Besides, microbial $\text{U}(\text{VI})$ reduction process was also favorable to wastewater basification (Eq.(2)). After a period of operation, the pH value of SRB reactor is kept at 6.5–7.0, whereas ZVI+SRB reactor maintains higher pH ranging from 7.2 to 8.0. This discrepancy implies that ZVI also contributes to the pH elevation for ZVI+SRB reactor. The higher pH in the latter reactor may be attributed to the release of hydroxide anion during the reaction between iron and water (Eq.(4)). Interestingly, pH for the two reactors stops rising and keeps stable at near neutral at late stage. This may be explained by that SRB has potential ability to adjust media pH to neutral, which is the optimum acidity for its growth.

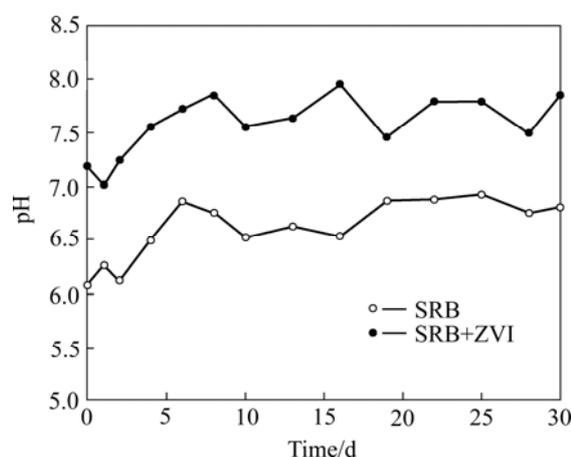
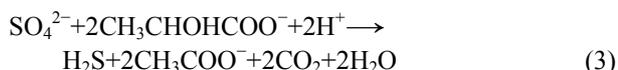


Fig.1 Effluent pH evolution in two column reactors

3.3 Effluent concentration changes of sulfate and U(VI) in two column reactors

Figs.2 and 3 reflect the concentration changes of sulfate and U(VI) in the effluent of two column reactors. It is apparent that both reactors function in an anaerobic environment that is required for the growth of SRB. During the initial period of the operation (the first 7 d), the removal rates of U(VI) and sulfate are very low. The average removal rates of sulfate reach 12.7% for SRB reactor and 36.4% for ZVI+SRB reactor; whereas the average removal rates of U(VI) achieve 43.9% for SRB reactor and 61.8% for ZVI+SRB reactor. During the middle and late period of the operation (after 9 d), two reactors run smoothly and show a better performance in desulfurization and U(VI) fixation. The average removal rates of sulfate reach 75.3% for SRB reactor and 86.2% for ZVI+SRB reactor; whereas the average removal rates of U(VI) reach 94.5% for SRB reactor and 99.4% for ZVI+SRB reactor. At the early stage of the operation, the reduction activities of U(VI) and sulfate are inhibited to a certain degree because SRB needs an acclimation period in the presence of high levels of pollutants, radioactive uranium in particular, which may be deleterious to their growth. As two reactors run, those species that can tolerate the toxicity of U(VI) gradually become dominant. This process is actually an acclimation one, resulting in the marked improvement of sulfate-reduction activity and U(VI) removal at middle and late stages.

The above results indicate that ZVI+SRB reactor has much better performance in the removal of sulfate and fixation of uranyl compared with SRB reactor (Figs.2 and 3). This finding may be related to the following factors. Firstly, ZVI is capable of raising the alkalinity of wastewater and neutralizing pH via consumption of H^+ in acidic media, thereby making it more suitable for SRB growth. Secondly, SRB-induced electrochemical corrosion of iron results in the release of cathode H_2 as well as Fe^{2+}/Fe^{3+} in the solution. It is suggested that most species of SRB can utilize H_2 as energy to stimulate its growth. On the other hand, Fe^{2+}/Fe^{3+} generated as the byproduct of ZVI corrosion process can react with H_2S from sulfate reduction to form FeS precipitate and stimulate the growth of SRB and promote sulfate removal by eliminating its toxicity or inhibitory effect[21]. Lastly, iron is a key component of hydrogenase and sufficient iron can promote its synthesis. As well known, hydrogenase-catalyzed hydrogen cycle plays a key role in electron transfer and the synthesis of bioenergy for SRB[22]. It should be pointed out that there is no evidence to date that ZVI can remove sulfate directly. Whether SRB can use ZVI directly as a slow-release electron donor to raise biomass yields needs further in-depth research.

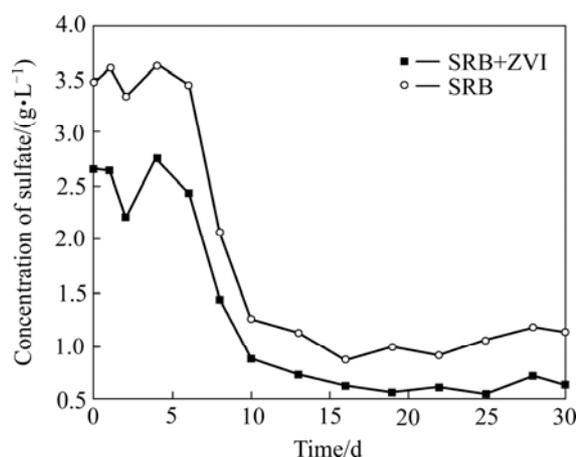


Fig.2 Evolution of effluent sulfate concentration in two column reactors

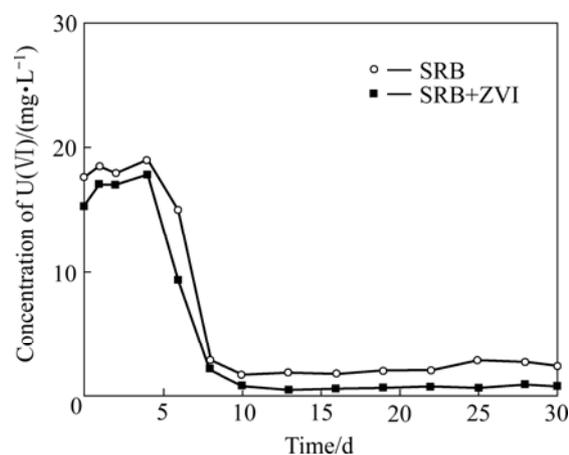


Fig.3 Evolution of effluent U(VI) concentration in two column reactors

4 Conclusions

1) The removal rate of U(VI) for the ZVI+SRB combining system is obviously higher than the total removal rate of ZVI system and SRB system in the batch tests. The contribution of SRB and ZVI to U(VI) removal exhibits synergistic effect.

2) The ZVI+SRB integrated column reactor filled with both iron filings and SRB biofilms is more effective than the SRB bioreactor in the aspect of wastewater basification, desulfurization and U(VI) immobilization. ZVI has the potential enhanced effect on the treatment of wastewater by SRB.

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(Edited by CHEN Wei-ping)