Effects of Electron Donors and Acceptors on Cr(VI) Removal in Biotic Fe⁰ Columns Preloaded with Microorganisms

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Abstract

The removal of Cr(VI) from groundwater by zero-valent iron (Fe^0) and microorganisms under different nutrients and electron-accepting conditions was investigated using continuously-flowed column reactors with an abiotic Fe^0 column as the control. Both abiotic and biotic Fe^0 columns were operated at low and relatively high concentrations of organic substrates (sodium acetate and humic acid) and electron acceptors (nitrate and sulfate). Sodium acetate and humic acid were found to be effective substrates for the biotic Fe^0 system to remove Cr(VI) since microorganisms were able to gain extra reducing capacity from two nutrients and simultaneously avoid the passivation effect of humic acids on Fe^0 by metabolizing them. For nitrate and sulfate, varied influences were observed in the biotic Fe^0 system. The former showed apparent inhibition on Cr(VI) removal as it competed with Cr(VI) for electrons, and the inhibition became more severe while nitrate concentrations increased. Differently, highly reactive minerals such as green rusts and iron sulfide could be generated on the biotic Fe^0 surface under sulfate-reducing conditions. They significantly enhanced Cr(VI) removal by providing more adsorptive and reductive sites. Overall, though the biotic Fe^0 column exhibited a longer life-span and higher Cr(VI) removal capacity than the abiotic Fe^0 column, the effects of organic substrates and electron acceptors should still be taken into account when applying biotic Fe^0 systems for Cr(VI)-contaminated groundwater remediation.

1 Introduction

Chromium is an important metal and finds a variety of uses in industries such as metallurgy, leather tanning, electroplating and pigment productions (GracePavithra et al. 2019). As a result of improper disposal and accidental leakages, chromium is released to soil and aquifer, causing adverse influences to most living organisms. Naturally, chromium mainly exists in Cr(VI) and Cr(III) oxidative forms. The latter species have a low solubility in water and are less mobile. In contrast, because of its oxyanion form, the Cr(VI) is weakly adsorbed onto inorganic surfaces and has significant mobility in groundwater (Richard & Bourg 1991). Moreover, Cr(VI) is considered to be a highly toxic substance that can cause human cancer by inhalation or oral exposure (Zhitkovich 2011). Therefore, Cr(VI) has been listed as a hazardous contaminant by many countries, and effective methods are required to remediate Cr(VI)-contaminated groundwaters.

To date, due to the low redox potential ($E^0 = -0.44 \text{ V}$) and environmental-friendly properties of metallic iron, Fe^0 -based permeable reactive barriers (PRBs) have been widely investigated in laboratories or field-scale sites for Cr(VI)-containing groundwater treatment (Cao et al. 2022, Wang et al. 2022). Many studies (Gheju 2011, Wilkin et al. 2005) reported that Cr(VI) can be adsorbed on Fe^0 surface and reduced to Cr(III) by accepting electrons $\text{Fe}^0/\text{Fe}^{2+}$ while Fe^0 is finally oxidized to Fe^{3+} . Meanwhile, as iron corrosion continuously progresses, Cr(VI) and resulted Cr(III) can be encapsulated or immobilized in iron oxides/hydroxides, which considerably diminishes the exposure or availability of chromium. In addition to chemical reduction and physical elimination, H_2 generated in the anoxic iron corrosion process can serve as a potential electron donor for some facultative hydrogen-consuming bacteria to metabolize and

simultaneously induce a coupled abiotic and biotic effect in $\text{Fe}^0\text{-H}_2\text{O}$ systems (Dinh et al. 2004). For example, these microorganisms can directly reduce Cr(VI) by utilizing H_2 (Battaglia-Brunet et al. 2002, Chung et al. 2006). Besides, some specific bacteria, like iron-reducing bacteria (IRB), can increase the availability of reactive sites on Fe^0 surface by cleaning up passive precipitates and generating highly reactive minerals (e.g., green rust and magnetite) (Yang et al. 2017), which can promote the removal performance as well as the utilization and life-span of Fe^0 -based PRBs. Hence, the combined use of Fe^0 and microorganisms is of great potential for in-situ remediation of Cr(VI)-contaminated groundwater as it may be more effective and long-acting (Yin et al. 2015).

Recently, this modified biotic Fe^0 system has been studied for the removal of nitrate, explosive, chlorohydrocarbon and chromium, and its superior removal performance and economical operation condition has made it become an ideal pollution control method (Niedzwiecka & Finneran 2015, Oh et al. 2001, Shin & Cha 2008, Zhong et al. 2017). These studies investigated the reaction progress between Fe^0 and microorganisms, and their synergistic mechanism in removing pollutants. Additionally, the effects of operational parameters and hydraulic conditions such as iron size, iron dosage, density of microorganisms, influent pH, flow rate of fluid and initial concentration of contaminants have also been well studied (Yin et al. 2017, Yin et al. 2016). However, little attention has been paid to the effects of coexisting matters such as natural organic matters (NOMs) and common inorganic anions on Cr(VI) removal in the biotic Fe^0 system, while the groundwater usually consists of various organic or inorganic components (Fitts 2002). Generally, the coexisting matters may affect the reactivity of Fe^0 itself and alter the growth and metabolism properties of microorganisms and their dominant communities. On the one hand, NOMs can deposit on iron surfaces and possibly diminish reactive sites in the $\text{Fe}^0\text{-H}_2\text{O}$ system (Dries et al. 2005, Liu et al. 2009b). On the other hand, NOMs can provide microorganisms with additional electrons and extra reducing capacity to promote their activity and the synergistic interaction with Fe^0 (Wu et al. 2013). Besides, the existence of some inorganic anions may serve as electron acceptors to compete with Cr(VI) for electrons and therefore misdirect the desired electron flow, which is unfavorable for Cr(VI) removal (Gu et al. 1999). Thus, it is necessary to figure out the effect of NOMs and inorganic anions and their active mechanisms on Cr(VI) removal by the biotic Fe^0 system, and which conclusion can provide a reference for the synergistic approach in practical application.

The present work was accordingly carried out to investigate the Cr(VI) removal capacity of the biotic Fe^0 system under different organic and electron-accepting conditions. In this study, continuously-flowed columns were used to simulate the Fe^0 -based PRBs. Sodium acetate (SA) and humic acid (HA) were selected as typical NOMs. Nitrate (NO_3^-) and sulfate (SO_4^{2-}) were chosen as common inorganic anions or electron acceptors in groundwater to explore their effects on Cr(VI) removal in the biotic Fe^0 system.

2 Materials And Methods

2.1 Materials

Granular iron (0.45 mm) was purchased from Shanghai Aladdin Company and applied as experimental supplies without additional treatment. Quartz sand (0.5-1 mm) obtained from Guangdong Huaneng Company was used for dispersing the iron particles and adjusting the porosity of column reactors. Before filling into columns, the sand was washed with 1 M HCl for 24 h, rinsed to neutral with deionized water, air-dried and autoclaved.

Sludge obtained from a municipal sewage plant was inoculated and cultivated in an anaerobic bioreactor (5 L) for the acclimation of Cr(VI)-reducing bacteria. The cultivation process was similar to our previous study (Zhong et al. 2017). In the start-up phase, the suspended solids (SS) and granular iron dosage were 5 and 10 g L⁻¹, respectively. The feeding Cr(VI) concentration was 10 mg L⁻¹ and a medium comprised of nutrient and trace metals was fed simultaneously (mg L⁻¹ in deionized and deoxygenated water): glucose (1000), NH₄Cl (300), K₂HPO₄ (175), MgCl₂ (75), CaCl₂ (100), NaHCO₃ (1000), CoCl₂·5H₂O (0.0001), CuCl₂·2H₂O (0.0001), MnCl₂·4H₂O (0.0001), NiCl₂·6H₂O (0.0001), NH₄MO₃ (0.0001) and ZnSO₄ (0.0001). The hydraulic retention time was kept at 1 d and the effluent Cr(VI) concentration was measured every 2 d. After 6 months of acclimation, the Cr(VI) removal efficiency was over 90%. Then this mixed anaerobic culture was added directly to the column reactors without further acclimation.

Synthetic Cr(VI)-contaminated groundwater was prepared (mg L⁻¹) by dissolving K₂Cr₂O₇ (10 as Cr(VI)), K₂HPO₄ (25), NH₄Cl (45), CaCl₂ (50), MgCl₂ (75), NaHCO₃ (1000) and the aforementioned trace metals into autoclaved, deionized and deoxygenated water based on Chinese National Standard of Groundwater (GB/T14848-93). The initial pH value was adjusted to 7.0 ± 0.1 using 0.1 M HCl or 0.1 M NaOH and the initial oxidation-reduction potential (ORP) value was measured to be 210 ± 10 mV. All chemicals used in this study were of analytical reagent grade.

2.2 Column study

The column setup and solid phase sampling processes were conducted in an anaerobic chamber (N₂:H₂=95:5) during the experiment. Four PVC columns (2 cm internal diameter×25 cm length) were used to mimic Fe⁰-based PRBs. The columns were equipped with five lateral sampling ports at a distance of 5, 10, 15, 20 and 25 cm from the inlet. They were mainly divided into four groups: (1) the abiotic Fe⁰ column, (2) the biotic Fe⁰ column, (3) the sand column, (4) the cell column. Among them, the abiotic Fe⁰ column was carefully packed with sand and iron particles through a paper slot to obtain a wholly mixed distribution. Likewise, the biotic Fe⁰ column was prepared in the same way but additionally added with the acclimated culture through a burette while packing sand and iron particles. The other two columns were used as controls: one was filled exclusively with sand and the other was filled with the mixture of sand and the acclimated culture. After packing, the columns were sealed by rubber plugs assembled with redox potential probes for real-time measurement of ORP (oxidation/reduction potential) values. All columns and materials were autoclaved for sterilization before packing. In this study, low iron dosage with a bulk density of 0.358 g cm⁻³ (iron/sand ratio of 3:10 (w:w)) was adopted, and the initial biomass concentration in the biotic columns was 400 mg L⁻¹ as volatile suspended solids (VSS). The porosities of

all columns were 0.48 ± 0.02 , and the pore volumes (PVs) were $40 \pm 1.7 \text{ cm}^3$. The synthetic Cr(VI)-contaminated groundwater was fed continuously into the columns up-flow by peristaltic pumps at a flow rate of 0.33 mL min^{-1} , resulting in a seepage velocity of 569 m year^{-1} and the required time for 1 PV was 2 h. For the effect of NOMs, 10 and 100 mg L^{-1} as COD were chosen as low to high values in the experiment of SA, while 5 and 50 mg L^{-1} as COD were selected in the effect of HA. For the effect of electron acceptors, 5 and $50 \text{ mg L}^{-1} \text{ NO}_3^-$ -N were chosen as low to high values in the effect of nitrate whereas the concentration of sulfate varied from 100 to $800 \text{ mg L}^{-1} \text{ SO}_4^{2-}$. Besides, no NH_4Cl was added to the solution at the nitrate effect experiment. The column study was operated at 25°C .

Water samples were periodically collected from the side sampling ports. Before collecting, about 1 mL of stagnant water in the port was discarded. Then 5 mL of the solution from each port was collected and filtered through a $0.45 \mu\text{m}$ filter for immediate analysis of Cr(VI) and pH value.

2.3 Analytical methods

The Cr(VI) concentration was determined via the 1,5-diphenylcarbazide colorimetric method using a UV-visible spectrophotometer (UV2300, Shanghai, China) at a wavelength of 540 nm. The total iron concentration was determined by the atomic absorption spectrometry (AA-6880F, Shimadzu, Japan). The pH and ORP values were measured using a pH meter (PHS-3C, Sanxin, China). The SS and VSS were determined following the standard methods for examining water and wastewater (Rice 2012). Ammonia, nitrite, nitrate and sulfate were determined by an ion chromatograph (Thermo Fisher, Dionex ICS-1100, USA).

At the end of the sulfate effect experiment, the abiotic and biotic Fe^0 columns were disassembled and the packing materials at a location of 15-25 cm (effluent section) from the inlet were collected immediately in the anaerobic chamber and freeze-dried, then stored in the anaerobic chamber for iron-sample pretreatment before solid phase analysis (less than 2 d preservation). Magnetic separation was used to screen out the reacted iron. The collected iron samples were then observed using a scanning electron microscope (SEM) (ZEISS Merlin, Germany) equipped with an energy-dispersive X-ray (EDX) spectrometer. The fine surface coatings of the reacted iron were collected by grinding and polishing the iron samples in the anaerobic chamber, and subsequently sieved through a 100-mesh sifter, prior to the analysis of X-ray diffraction (XRD). The XRD of the fine surface coatings was conducted using a D8 ADVANCE (Bruker, Germany) diffractometer with Cu-K α radiation at 40 kV to identify mineral phases on the reacted iron surface.

2.4 Data analysis

To provide an applicable model for engineering design, the removal capacity of Fe^0 for Cr(VI) was estimated using the migration rate of Cr(VI) front as it moved gradually to the top of the column in the continuous-flow system. According to Lai's study (Lai & Lo 2008), the Cr(VI) removal capacity (RC , $\text{mg Cr(VI) g}^{-1} \text{ Fe}^0$), can be estimated as follow (Eq. 1):

$$RC = \frac{[Cr(VI)]}{M \times 1000 \times \rho_b} \quad (1)$$

where $[Cr(VI)]$ is the influent Cr(VI) concentration. M (cm cm^{-3}) is the normalized migration rate of Cr(VI) front at a relative concentration (C/C_0) of 0.5, which is the migration rate (cm PV^{-1}) divided by the pore volume ($\text{cm}^3 \text{PV}^{-1}$). In this study, the migration rates of Cr(VI) fronts at C/C_0 of 0.5 can be calculated from the slopes of fitting lines of the PV-column length plots. And ρ_b (g cm^{-3}) is the bulk density of Fe^0 packed in the columns.

3 Results And Discussion

3.1 Cr(VI) removal in different systems

In the control sand and cell columns, the effluent concentrations of Cr(VI) increased sharply and the Cr(VI) breakthrough occurred early in the first few PV. These indicated that Cr(VI) was neither reactive nor adsorptive in the sand and cell columns.

The Cr(VI)-breakthrough curves illustrated in Fig. 1a and b show the Cr(VI) migration patterns in the abiotic and biotic Fe^0 columns. Due to the passivation of Fe-Cr precipitates, a relatively rapid breakthrough of 126 PV occurred in the abiotic Fe^0 column and the Cr(VI) removal capacity listed in Table 1 was $3.4 \text{ mg Cr g}^{-1} \text{Fe}^0$. In contrast, with the inoculation of microorganisms, hydrogen-using bacteria could improve Cr(VI) removal by catching cathodic hydrogen as electron donors and effectively accelerating iron utilization inside the column (Yin et al. 2017). Therefore, the Cr(VI) removal performance in the biotic Fe^0 column was greatly improved that its penetration time reached 218 PV and the Cr(VI) removal capacity was recorded as $5.9 \text{ mg Cr g}^{-1} \text{Fe}^0$, which were 73% and 74% higher than the abiotic one, respectively. In the following study, the data above also served as a baseline to evaluate the effects of different electron donors and electron acceptors on the removal of Cr(VI).

Table 1
Cr(VI) removal capacities in the abiotic and biotic columns with different supplements

Column	Reactive media	Supplement (mg L ⁻¹)	RC ^a (mg g ⁻¹)
Blank			
1	Abiotic Fe ⁰	-	3.4
2	Biotic Fe ⁰	-	5.9
Sodium acetate (as COD)			
3	Abiotic Fe ⁰	10	3.5
4	Biotic Fe ⁰	10	5.8
5	Abiotic Fe ⁰	100	4.3
6	Biotic Fe ⁰	100	8.4
Humic acid (as COD)			
7	Abiotic Fe ⁰	5	3.5
8	Biotic Fe ⁰	5	5.9
9	Abiotic Fe ⁰	50	3.0
10	Biotic Fe ⁰	50	6.6
NO ⁻ -3-N			
11	Abiotic Fe ⁰	5	3.2
12	Biotic Fe ⁰	5	5.0
13	Abiotic Fe ⁰	50	3.0
14	Biotic Fe ⁰	50	3.9
SO ₄ ²⁻			
15	Abiotic Fe ⁰	100	3.2
16	Biotic Fe ⁰	100	10.3
17	Abiotic Fe ⁰	800	5.4
18	Biotic Fe ⁰	800	12.4

Column	Reactive media	Supplement (mg L ⁻¹)	RC ^a (mg g ⁻¹)
^a RC is short for removal capacity of Cr(VI)			

3.2 Effect of sodium acetate (SA)

The effect of sodium acetate at 10 mg L⁻¹ and 100 mg L⁻¹ as COD is shown in Fig. 2. When a low concentration of SA (10 mg L⁻¹ as COD) was applied, there was no obvious influence on Cr(VI) removal in both columns in comparison with the control columns. However, when a moderately higher concentration of SA (100 mg L⁻¹ as COD) was added, both abiotic and biotic Fe⁰ columns demonstrated elevated Cr(VI) removal performance. In the abiotic Fe⁰ column, the Cr(VI) breakthrough occurred at 153 PV and the corresponding removal capacity was 4.3 mg Cr g⁻¹ Fe⁰. As for the biotic Fe⁰ column, it showed much remarkable improvement that the Cr(VI) removal process lasted till 364 PV and the corresponding removal capacity was calculated to be 8.4 mg Cr g⁻¹ Fe⁰. Under the condition of an adequate SA, the life-span and Cr(VI) removal capacity in the biotic Fe⁰ column were improved by 138% and 95%, respectively, compared to the abiotic Fe⁰ column. Due to the low dosage of iron, hydrogen production was limited, and the water condition was an oligotrophic type. Therefore, the addition of SA was able to meet the desire for energy and reactivate the microorganisms inside the column since SA was an excellent substrate that could be efficiently utilized by the bacteria (He et al. 2002). In the abiotic Fe⁰ system, SA did not react with the Fe⁰ and had no effect on the buffer capacity of solution. For these reasons, the abiotic Fe⁰ column did not show a conspicuous improvement on Cr(VI) removal and its slight enhancement could be explained by the microbial breeding in the column.

3.3 Effect of humic acid (HA)

Figure 3 shows the effect of humic acid on Cr(VI) removal in the abiotic and biotic Fe⁰ columns. Similar to the effect of sodium acetate, no significant changes were found when a low concentration of HA was applied (5 mg L⁻¹ as COD). When the influent HA concentration reached 50 mg L⁻¹ as COD, a slight inhibition was observed in the abiotic Fe⁰ column. The longevity of the abiotic column decreased from 128 PV to 114 PV, and the corresponding Cr(VI) removal capacity decreased from 3.5 to 3.0 mg Cr g⁻¹ Fe⁰, compared to the low concentration of humic acid. The decrease in Cr(VI) removal performance of the abiotic Fe⁰ column was attributed to the deposition of HA on the Fe⁰ surface. As the influent contained cations such as Fe²⁺/Fe³⁺ and Ca²⁺, HA could form aggregates and then deposit on iron surfaces, which inhibited electron transfer originated from Fe⁰ (Liu et al. 2009a, Mak et al. 2011a, Mak et al. 2011b). Thereby, the Cr(VI) removal in the abiotic Fe⁰ column was affected. Nevertheless, in the biotic Fe⁰ system, 50 mg L⁻¹ HA slightly promoted the removal of Cr(VI). The life-span of the biotic Fe⁰ column was extended from 224 PV to 252 PV, and the corresponding Cr(VI) removal capacity increased from 5.9 to 6.6 mg Cr g⁻¹ Fe⁰ in comparison with the condition of low HA dosage. The observation revealed that in a HA concentration of 50 mg L⁻¹ as COD, the microorganisms could use HA as a carbon source to provide needed energy for their metabolism and enhance their activity for the synergistic interaction with Fe⁰.

Besides, by utilizing HA, the deposition of HA on the iron surface might be effectively cleaned up, thus maintaining the reactivity of iron surface.

3.4 Effect of nitrate (NO_3^-)

To investigate the effect of nitrate on Cr(VI) removal, 5 and 50 mg L^{-1} NO_3^- -N were added respectively to the simulated Cr(VI)-contaminated groundwater and the corresponding Cr(VI) breakthrough curves in different systems are shown in Fig. 4. As Fig. 4 and Table 1 demonstrated, the presence of NO_3^- had a minor inhibitory effect on removing Cr(VI) in the abiotic Fe^0 column whereas it significantly inhibited the Cr(VI) removal performance of the biotic Fe^0 column. And these inhibition effects were positively related to the initial concentration of NO_3^- -N. When the nitrate concentration increased from 5 to 50 mg L^{-1} as NO_3^- -N, the Cr(VI) penetration time in the abiotic Fe^0 column decreased from 108 to 98 PV, and the corresponding Cr(VI) removal capacity decreased from 3.2 to 3.0 $\text{mg Cr g}^{-1} \text{Fe}^0$. As for the biotic Fe^0 system, the Cr(VI) removal process shortened from 184 to 132 PV, and the corresponding Cr(VI) removal capacity decreased from 5.0 to 3.9 $\text{mg Cr g}^{-1} \text{Fe}^0$. Compared to the control abiotic Fe^0 system, there were approximately 14–22% decline of operation time and 6–13% decrease of Cr(VI) removal capacity respectively, when 5 and 50 mg L^{-1} NO_3^- -N were added in the abiotic column. Correspondingly, when 5 and 50 mg L^{-1} NO_3^- -N were fed, the biotic Fe^0 column showed a 16–39% decrease in longevity and an 18–51% decline in Cr(VI) removal capacity respectively, in comparison with the control biotic Fe^0 column.

It is reported that Fe^0 can reduce nitrate to produce ammonia (NH_4^+) and nitrite or even be adsorbed by its corrosion products (Liu & Wang 2019). For further verification, the effluent concentration of nitrate and ammonia in both abiotic and biotic Fe^0 columns with 50 mg L^{-1} NO_3^- -N were also examined and their trends are shown in Fig. 4e and f. In the beginning, a decrease of NO_3^- -N and a rapid increase of NH_4^+ -N were detected in both columns. At the first 12 PV, the effluent concentration of NO_3^- -N in the abiotic Fe^0 column decreased from 48.2 to 42.9 mg L^{-1} , and the NH_4^+ -N increased simultaneously from 0 to 4.8 mg L^{-1} . For the biotic Fe^0 system, a much significant decrease of NO_3^- -N from 48.2 to 35.7 mg L^{-1} was observed, and the NH_4^+ -N increased sharply from 0 to 10.9 mg L^{-1} . These results suggested that nitrate reduction occurred in abiotic and biotic Fe^0 columns. Additionally, it was also important to find that, in the biotic Fe^0 column, a much higher reduction of nitrate was observed and the nitrate removal process was much longer than the abiotic system. Moreover, the mass of nitrogen examined in the biotic column was not conserved. This observation might also imply that the nitrate could be partially removed through the denitrification process of microorganisms. Meanwhile, under the clearance and acceleration of microbe, higher utilization of iron and more extensive production of reactive minerals could be achieved, thus resulting in a higher nitrate reduction efficiency as well as higher ammonia generation. This observation was consistent with Gandhi's study that the mixed sludge with denitrifying bacteria could effectively improve the denitrification efficiency of conventional Fe^0 systems (Gandhi et al. 2002).

Overall, the observed results indicated that nitrate could serve as an electron acceptor and react easily with Fe^0 and microorganisms. That is, this denitrification process might compete with Cr(VI) for electrons and adsorption sites so that the removal process of Cr(VI) was inhibited. Nevertheless, nitrate has been reported to be a positive anion for the $\text{Fe}^0\text{-H}_2\text{O}$ system to remove reducible contaminants since nitrate can promote the generation of magnetite, which can facilitate electron transfer in the $\text{Fe}^0\text{-H}_2\text{O}$ system (Wei et al. 2016). Those reports seem conflictive with the present study. In this experiment, as the iron dosage was low, the columns were easily passivated by Cr(VI) or nitrate in a relatively short time, which was unfavorable for NO_3^- and Fe^0 to interact fully and generate magnetite, thus resulting in an entirely inhibitory phase. But it is also important to point out that the biotic Fe^0 system is vulnerable to treat Cr(VI)-contaminated groundwater when nitrate is present.

3.5 Effect of sulfate (SO_4^{2-})

Similar to nitrate, sulfate is a common contaminant and an electron acceptor frequently found in groundwater. The effect of SO_4^{2-} on Cr(VI) removal in the biotic Fe^0 column is shown in Fig. 5. The presence of SO_4^{2-} promoted the Cr(VI) removal performance in both abiotic and biotic Fe^0 columns. When initial SO_4^{2-} concentration was set at a relatively low value of 100 mg L^{-1} , there was no difference between the abiotic Fe^0 and control columns. Whereas, as the SO_4^{2-} concentration increased to 800 mg L^{-1} , the operational life of the abiotic Fe^0 column increased to 228 PV and the Cr(VI) removal capacity was 5.4 mg Cr/g Fe^0 , which were 81% and 59% higher than the abiotic control Fe^0 column. In the biotic Fe^0 column, the Cr(VI) breakthrough occurred at 392 and 512 PV respectively, when 100 and 800 mg L^{-1} SO_4^{2-} was applied, and the corresponding Cr(VI) removal capacities were 10.3 and $12.4 \text{ mg Cr g}^{-1} \text{ Fe}^0$, respectively. In comparison with the biotic control Fe^0 column, the longevity of the biotic Fe^0 column at 100 and 800 mg L^{-1} of SO_4^{2-} were promoted by 80% and 134%, respectively, and the corresponding Cr(VI) removal capacity increased by 75% and 110%.

The presence of sulfate led to a more vigorous promotion on Cr(VI) removal in the biotic Fe^0 system than the abiotic one. On the one hand, sulfate was reported to maintain the iron reactivity by removing the passive oxide film (Su & Puls 2001). On the other hand, similar to bicarbonate, sulfate also could migrate to the interlayer position of iron hydroxides to form green rust, which were generally considered to be a reactive mineral that could serve as chemical-conductor or reductant for Cr(VI) removal (Eq. 2) (Liu et al. 2015, Van et al. 2008).

Furthermore, the existence of SO_4^{2-} could also alter the biochemical environment of the columns from the perspective of microorganisms. By examining the effluent concentration of SO_4^{2-} , a significant difference between the abiotic and biotic columns was found in Fig. 5e and f. In the abiotic Fe^0 column, the SO_4^{2-} concentration in effluent did not change significantly with time. However, the effluent SO_4^{2-} in

the biotic Fe^0 column suffered a decline first, then increased and became stable over time. This observation indicated that utilization or adsorption of sulfate by the microorganisms might occur in the biotic column. According to Watson's study, sulfate is a typical electron acceptor in water and the sulfate-reducing bacteria (SRB) are able to reduce SO_4^{2-} to S^{2-} , then interact with aqueous Fe(II) to form amorphous ferrous sulfide (FeS) or Mackinawite (Watson et al. 2000, Watson et al. 1999). Unlike Fe(III) bearing oxides, these complex minerals have a relatively large specific surface area, which can adsorb or even reduce Cr(VI) by its structural Fe(II) , thus providing abundant reactive sites for Cr(VI) removal (Van et al. 2007, Van et al. 2008). By examining the coatings on the iron surface, cocci-shaped bacteria and hexagonal-shaped minerals were confirmed on the biotic Fe^0 surface using SEM whereas the abiotic Fe^0 surface was compactly precipitated by $\text{Fe-Cr (oxy)hydroxide}$ or calcite (Fig. 6). Meanwhile, the XRD pattern result also confirmed larger quantities of various reactive minerals (e.g., mackinawite, green rust, lepidocrocite and magnetite) on the biotic Fe^0 surface than the abiotic one (Fig. 6). Those results suggested that the existence of sulfate could not only maintain the reactivity of iron by suppressing the formation of passivated precipitates, but also could be utilized by SRB to form highly reactive minerals, thereby resulting in a remarkable promotion on Cr(VI) removal in the biotic column. However, when the SO_4^{2-} concentration increased from 100 to 800 mg L^{-1} , there was no significant promotion on column longevity and Cr(VI) removal capacity in the biotic Fe^0 column, which suggested that the limited microbes and hydraulic retention times in the column might limit further utilization of sulfate.

4 Conclusions

This study has investigated Cr(VI) removal performance in a biotic Fe^0 system under different nutrient and electron-accepting conditions. According to the obtained results, sodium acetate was found to be an effective additional substrate for the biotic Fe^0 system to remove Cr(VI) . Humic acid could form passive precipitates on the iron surface and inhibit reaction from further progressing in the abiotic Fe^0 system whereas the presence of humic acid enhanced Cr(VI) removal in the biotic system since microorganisms were able to utilize it and simultaneously clean up humic acid aggregates. But for the effects of electron acceptors, varied influences were observed in the biotic Fe^0 system. Nitrate demonstrated an inhibitory effect on removing Cr(VI) as it might compete with Cr(VI) for electrons. On the contrary, sulfate could be reduced by SRB and generate highly reactive minerals such as green rust and iron sulfide, which could significantly enhance the column reactivity and Cr(VI) removal.

Based on the above results, the biotic Fe^0 system still performed more applaudable Cr(VI) removal than the abiotic system under different nutrient and electron-accepting conditions. Therefore, a mixed culture is suggested as a potential addition to conventional $\text{Fe}^0\text{-H}_2\text{O}$ systems since organic substrates (e.g., sodium acetate and humic acid) are favored by biotic treatment and provide extra energy for removing Cr(VI) . Besides, this biotic Fe^0 system can solve combined contaminations such as Cr(VI) -nitrate and Cr(VI) -sulfate contaminated groundwater. However, in order to achieve a better removal performance for

desired contaminants, the co-existence of organic substrates and electron acceptors should also be taken into account when applying biotic Fe⁰ systems.

Declarations

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Authors' contributions

Weiquan Li: Writing - Original Draft, Conceptualization, Methodology and Data curation.

Mingxi Li: Formal analysis and Software.

Weizhao Yin: Supervision, Writing.

Sihao Lv: Writing- Reviewing and Editing.

Jiawei Zhong: Formal analysis and Software.

Ping Li: Project administration.

Hong Deng: Supervision.

Jinhua Wu: Project administration and Funding acquisition.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Publish

Not applicable.

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Figures

Figure 1

Cr(VI) breakthrough curves in the abiotic and biotic Fe⁰ columns (influent Cr(VI) was 10 mg L⁻¹; 1 PV stood for 2 h operation time; seepage velocity was 569 m year⁻¹; influent pH was 7.0 ± 0.1; influent ORP

was 210 ± 10 mV)

Figure 2

Effect of sodium acetate on Cr(VI) removal ((a) and (b) were the breakthrough curves of the abiotic Fe^0 column conducted at 10 and 100 mg/L COD, respectively; (c) and (d) were the breakthrough curves of the biotic Fe^0 column conducted at 10 and 100 mg/L COD, respectively)

Figure 3

Effect of humic acid on Cr(VI) removal ((a) and (b) were the breakthrough curves of the abiotic Fe⁰ column conducted at 5 and 50 mg L⁻¹ COD, respectively; (c) and (d) were the breakthrough curves of the biotic Fe⁰ column conducted at 5 and 50 mg L⁻¹ as COD, respectively)

Figure 4

(a)-(d) Effect of nitrate on Cr(VI) removal ((a) and (b) were the breakthrough curves of the abiotic Fe^0 column conducted at 5 and 50 mg L^{-1} NO_3^- -N, respectively; (c) and (d) were the breakthrough curves of the biotic Fe^0 column conducted at 5 and 50 mg L^{-1} NO_3^- -N, respectively); (e)-(f) the concentration of nitrate and ammonia in the abiotic and biotic Fe^0 columns

Figure 5

(a)-(d) Effect of sulfate on Cr(VI) removal ((a) and (b) were the breakthrough curves of the abiotic Fe^0 column conducted at 100 and 800 mg L⁻¹ SO_4^{2-} , respectively; (c) and (d) were the breakthrough curves of the biotic Fe^0 column conducted at 100 and 800 mg L⁻¹ SO_4^{2-} , respectively); (e)-(f) Effluent concentration

of sulfate in the abiotic and biotic Fe⁰ columns ((e) and (f) were conducted at 100 and 800 mg L⁻¹ SO₄²⁻, respectively)

Figure 6

(a)-(d) SEM images of the Fe⁰ surface and corrosion products ((a) unreacted Fe⁰; (b) abiotic Fe⁰ collected from the abiotic Fe⁰ column under 800 mg L⁻¹ SO₄²⁻; (c) and (d) were the microbial colonization and

reactive minerals on the biotic Fe^0 surface collected from the biotic Fe^0 column under $800 \text{ mg/L SO}_4^{2-}$);
(e) XRD patterns of the iron coatings composed from the abiotic and biotic Fe^0 (A1 and B1 stood for abiotic and biotic Fe^0 coatings reacted at $100 \text{ mg L}^{-1} \text{ SO}_4^{2-}$, respectively; A2 and B2 stood for abiotic and biotic Fe^0 coatings responded at $800 \text{ mg L}^{-1} \text{ SO}_4^{2-}$, respectively)