



Synergetic Effects of Zero-valent Iron and *Morganella morganii* on the Removal of Cr(VI) from Wastewater

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ABSTRACT

Zero-valent iron permeable reactive barrier (ZVI-PRB) has attracted great attention for its high efficiency and low cost in the in-site remediation process. However, passivating oxide layers are usually yielded and covered on the surface of ZVI, which shorten the service life of PRB. In this study, *Morganella morganii* subsp was used to hydrolyse the passivating oxide layers on ZVI. The hydrolysing condition was optimized. The highest Cr(VI) removal efficiency was achieved with glucose of 4 g/L, yeast powder of 6 g/L and pH = 7. With *Morganella morganii* subsp, the maximum removal quality of ZVI was increased from 19.96 mg/g to 24.35 mg/g, indicating *Morganella morganii* subsp could improve the reduction ability of ZVI. SEM-EDS analysis also showed that *M. morganii* subsp could promote the surface corrosion of ZVI and adsorb Cr simultaneously.

INTRODUCTION

Chromium is a common heavy material widely used in metallurgy, electroplating, printing and dyeing, tanning and other industries (Kimbrough et al. 1999, Ngah & Hanafiah 2008). It usually exists in hexavalent [Cr(VI)] and its trivalent forms [Cr(III)] (Lin et al. 2018). Cr(III) is considered to be non-toxic and less soluble for it usually precipitates as hydroxides, oxides or oxyhydroxides. On the contrary, Cr(VI) is acutely toxic and carcinogenic (Cefalu et al. 2004). Meanwhile, it is highly soluble and mobile, resulting in carcinogenic, mutagenic and teratogenic effects on animals, plants and human beings (Owlad et al. 2009). Cr(VI) has been listed as one of the eight most harmful chemicals to the human body, and it is also one of the three internationally recognized carcinogenic metals (Miretzky & Cirelli 2010). Therefore, it is essential to remove Cr(VI) from wastewater before its discharge into the environment. Many technologies, including adsorption, ion-exchange, reduction, precipitation and membrane filtration have been developed for Cr removal (Mu et al. 2015, Wanner et al. 2012).

Recently, permeable reactive barrier (PRB), a site in-situ remediation technology, has attracted great attention for its low cost, environmental benign and no extra energy consumption in the remediation process (Bonneville et al. 2006). There have been developed a great deal of practical engineering cases in Europe and North America. Zero-valent iron (ZVI)-based permeable reactive barriers (PRBs) as the

most widely used filler in engineering (Childers et al. 2002). ZVI could reduce Cr(VI) to Cr(III) and coprecipitated Cr(III) from wastewater in the form of FeCr_2O_4 , $\text{Cr}(\text{OH})_3$ and $\text{Fe}(\text{OH})_x$ (Crichton et al. 1987). However, passivating oxide layers are yielded and covered on the surface of ZVI, which reduce the active site of Fe(0) and shorten the service life of PRB. Currently, the service life of PRB has attracted widespread attention. To overcome this drawback, two strategies have been carried out (Gorby et al. 2006). One is preventing the formation of mineral precipitate, the other is removing the passive film from the surface of ZVI with acid, hydrogen or sodium borohydride. However, some studies have shown that pickled zero-valent iron is more likely to be oxidized, leading to more serious passivation problems (Lovley et al. 2004). Ultrasound is another commonly used deactivation method. It removes iron oxide or iron hydroxide on the surface of ZVI by physical force. At the same time, the iron oxide blocked in the pore can also be removed, providing more active sites for the reaction (Lu et al. 2002). However, it was found that ultrasound cannot completely remove the iron oxides on the surface of zero-valent iron, thereby restore the reactive activity of ZVI.

Microbial reduction is a mild and environmental friendly method (Banerjee et al. 2017). Many microorganisms, such as *Geobacter* and *Shewanella*, are capable of reducing Fe(III) to Fe(II) (Cao et al. 2010). It was found that the addition of Fe(III) in the solution could increase the removal efficiency of Cr(VI) (Mohatt et al. 2011, Xu et al. 2005). The direct

reaction process could be broken down by the presence of Fe(III), thereby the toxicity of Cr(VI) to microorganisms was alleviated. Thus, it is supposed that microorganisms could be used to remove the passive film on ZVI through reducing Fe(III) to Fe(II).

In the present study, a strain which could reduce Fe(III) in a non-strict anaerobic environment was screened to promote the reactivity of ZVI. The reduction condition was optimized and the mechanism was revealed. Our study will provide technical support for the PRB remediation of polluted groundwater.

MATERIALS AND METHODS

Microorganisms: *Morganella morganii* subsp was separated from anaerobic reaction tank in Shaoxing wastewater treatment plant (Shaoxing, Zhejiang, China), then identified with PCR. The culture was stored in the LB slope at 4°C.

Chemical reagents and instruments: Reduced iron powder (ZVI) and potassium dichromate ($K_2Cr_2O_7$) were purchased from Shanghai Chemical Reagent Purchasing and Supply Station and Shanghai Jinlian Fine Chemical Plant, respectively. The other chemical reagents were collected from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The morphology of the particles was observed by JSM-6360LV scanning electron microscope (SEM) of Japan Electronics (JEOL) Company, and elemental analysis (EDS) was performed with Oxford X-act spectrum. Total chromium (Cr) in solution was measured using Prodigy XP inductively coupled plasma emission spectrometer (ICP-AES) (Leeman Company, American).

Screening of non-strict anaerobic dissimilatory Fe(III) reducing bacterium: Nutrient medium containing 2.5 g/L $NaHCO_3$, 10 g/L glucose, 0.25 g/L NH_4Cl , 0.1 g/L KCl , 0.1 g/L $NaCl$, 0.04 g/L KH_2PO_4 , 0.2 g/L $MgCl_2 \cdot 6H_2O$ and 1 g/L yeast extract was prepared and sterilized under high pressure at 115°C for 20 min. 50 mg/L $K_2Cr_2O_7$ was added into the nutrient medium through filtration with 0.22 μm filter. Two mL of the culture and 0.2 g of the reduced iron were added to the 100 mL nutrient medium containing 50 mg/L $K_2Cr_2O_7$ in the 250 mL shake flask. The shake flask was incubated at 30°C under aerobic (150 rpm shaking), anoxic (static) and anaerobic (N_2) conditions for 48 h. Every 24 h, 2 mL sample was obtained from the flask followed by centrifugation at 8000 rpm for 10 min. The concentration of Cr(VI) in the supernatant was determined with 1,5-diphenylcarbohydrazide spectrophotometric and the removal rate was calculated using Eq. 1. The strain which with the highest removal rate was screened.

$$\text{Removal rate(\%)} = (C_0 - C_{48}) / C_0 \times 100\% \quad \dots(1)$$

Where, C_0 and C_{48} are the concentrations of Cr(VI) at 0 and 48 h.

Optimization of removal conditions: Glucose, sucrose, sodium acetate and soluble starch were selected as the carbon source using the basal culture medium without glucose, respectively. The optimum carbon source was confirmed by measuring the concentration of Cr(VI) per 24 h. Then, the optimal content of best carbon source (2, 4, 6, 8, 10 g/L) was assessed. Yeast powder, peptone, chloramine and urea with best carbon source were applied for the selection of the optimal addition of nitrogen source. The concentration of optimal nitrogen source (2, 4, 6, 8, 10 g/L) was also studied. The pH of the medium was adjusted to 4, 5, 6, 7 and 8. Samples were obtained every 24 hours. The Cr(VI) content was measured to obtain the optimal pH.

Analysis of the acceleration effect of *M. morganii* subsp on ZVI: The strain was inoculated in the optimal medium containing $K_2Cr_2O_7$ under optimal conditions with ZVI and incubated at 30°C for 72 h. Samples were obtained each 12h and the content of Cr(VI) was determined. The change of the Cr(VI) concentration in solution was fitted to first-order and second-order reaction kinetics equations to calculate k_1 , k_2 , R_1^2 and R_2^2 .

$$C_t / C_0 = \exp(-k_1 t) \quad \dots(2)$$

$$C_t = C_0 / (1 + k_2 C_0 t) \quad \dots(3)$$

Where, C_0 and C_t were the concentration of Cr(VI) at time 0 and t . k_1 , k_2 were the coefficients of first-order and second-order reaction kinetics equations, respectively. R_1^2 , R_2^2 were the correlation coefficient of first-order and second-order reaction kinetics equations.

Adsorption capacity of ZVI and ZVI in microbial/ZVI system were measured to fit the first and second-order reaction kinetics equations to calculate k_α , k_β , R_α^2 and R_β^2 .

$$\frac{dq}{dt} = k_\alpha (q_e - q) \quad \dots(4)$$

$$\frac{dq}{dt} = k_\beta (q_e - q)^2 \quad \dots(5)$$

Where, q and q_e are the adsorption capacity of ZVI and ZVI in microbial/ZVI system. k_α , k_β are the coefficients of first and second-order reaction kinetics equations. R_α^2 , R_β^2 are the correlation coefficients of first and second-order reaction kinetics equations, respectively.

Mechanism analysis: ZVI and the spores of *M. morganii* subsp were added into the prepared optimal medium containing 200 mg/L $K_2Cr_2O_7$ under 150 rpm shaking at 30°C for 72 h. The concentration of Cr(VI) and pH were determined every 12 hours. The total Cr was analysed using ICP-AES.

Fe(II) was measured with ferrozine. The total iron content was determined after addition of hydroxylamine hydrochloride, and the content of Fe(III) could be calculated. The samples were centrifuged at 8000 rpm and the supernatant was discarded. The mixture of ZVI, microorganism and microorganism/ZVI was obtained after frozen dried. The microstructure of iron and microorganism was observed using SEM, and the composition of elements was analysed with EDS.

RESULTS AND DISCUSSION

Optimization of reduction conditions: The type and concentration of carbon source, nitrogen source and pH were

optimized to obtain the optimal reaction conditions for the removal of Cr(VI) by *M. morganii* subsp-ZVI synergistic system in anoxic conditions. As demonstrated in Fig. 1, the best carbon source was glucose of 4 g/L, the optimal nitrogen source was yeast powder of 6 g/L and the best pH was 7.

Analysis of the acceleration effect of *M. morganii* subsp on ZVI: Removal of Cr(VI) with ZVI, *M. morganii* subsp and *M. morganii* subsp-ZVI was studied and presented in Fig. 2. The removal of Cr(VI) by ZVI was faster in the first 12 hours and slower in the last 36 hours. Another interesting finding was that *M. morganii* subsp could remove Cr(VI) effectively from the solution, and the removal rate increased

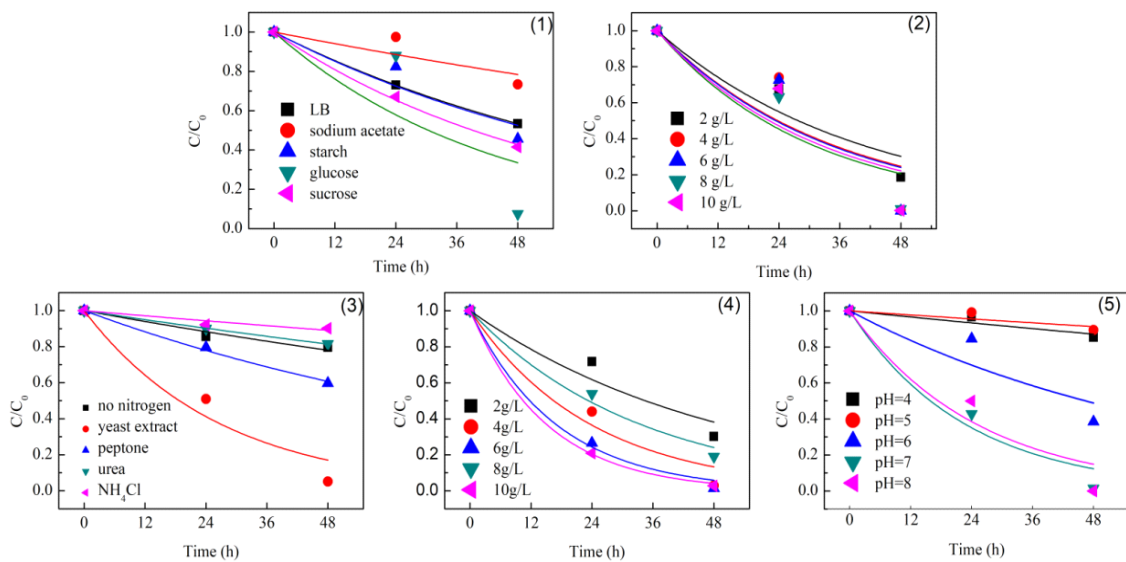


Fig. 1: Effects of carbon source, nitrogen source and pH on the removal of Cr(VI).

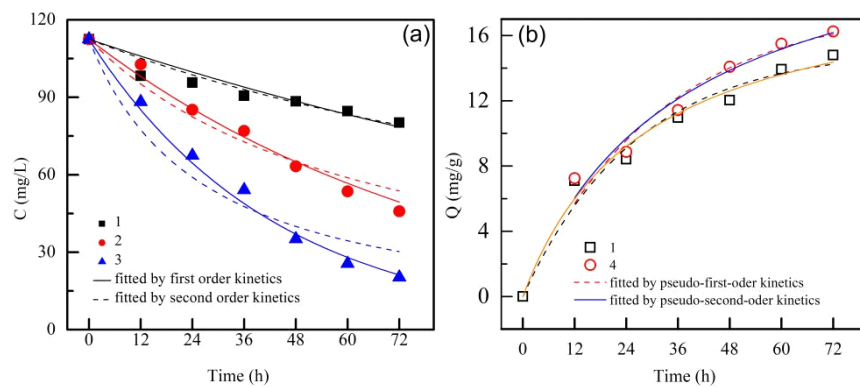


Fig.2: Removal of Cr(VI) with ZVI, *M. morganii* subsp and *M. morganii* subsp-ZVI. (a): fitting the first and second order kinetic equations; (b) fitting the pseudo first and second order kinetics equation; 1: ZVI; 2: *M. morganii* subsp; 3: *M. morganii* subsp-ZVI; 4: ZVI in *M. morganii* subsp-ZVI system.

Table 1: Kinetic fitting results of first and second order kinetics.

	First order kinetics		Second order kinetics	
	$k_1(\text{h}^{-1})$	R_1^2	$k_2(\text{h}^{-1})$	R_2^2
1	0.005	0.824	0.00583	0.874
2	0.011	0.987	0.0152	0.964
3	0.023	0.989	0.0377	0.927

between 36 h and 48 h in the *M. morgani* subsp-ZVI system, indicating that synergistic reaction may have occurred in this system (Luu et al. 2003).

First and second kinetic equations were fitted to the process to calculate the removal rate. As shown in Table 1, in the ZVI reaction process, the second-order kinetic equation fit the process better. While the first-order kinetic equation was more consistent with *M. morgani* subsp and the *M. morgani* subsp-ZVI system, which further indicated that the removal mechanism was changed when both of them exist (Niggemyer et al. 2001). Both of the fitting results implied that as compared with single action systems, the reaction rate of the synergistic system was dramatically

enhanced. Its reaction rate was faster than the sum of the two single systems, which also confirms the existence of synergism reaction.

In order to further investigate the function of *M. morgani* subsp in the reduction system, the removal of Cr(VI) by ZVI before and after the presence of *M. morgani* subsp was evaluated (the calculation method was the same as calculating adsorption ability). Afterwards, the data were fitted to pseudo first and pseudo second order dynamic equations, respectively. It can be seen that the pseudo second order kinetic equation was fitted better as the correlation coefficients R^2 were 0.980 and 0.949. The fitting results also indicated that the removal of Cr(VI) by ZVI was significantly heightened in the presence of *M. morgani* subsp, resulting in the removal amount increased from 19.96 mg/g to 24.35 mg/g.

Analysis of the change of Cr(VI)/Cr(III) content: As illustrated in Fig. 3, Cr(VI) was converted to Cr(III) with the conversion rate gradually decreased and subsequently tended to be stable during the process of removal of Cr(VI) with ZVI. Nevertheless, the total chromium content slightly changed. In addition, no obvious fixing effect on Cr with

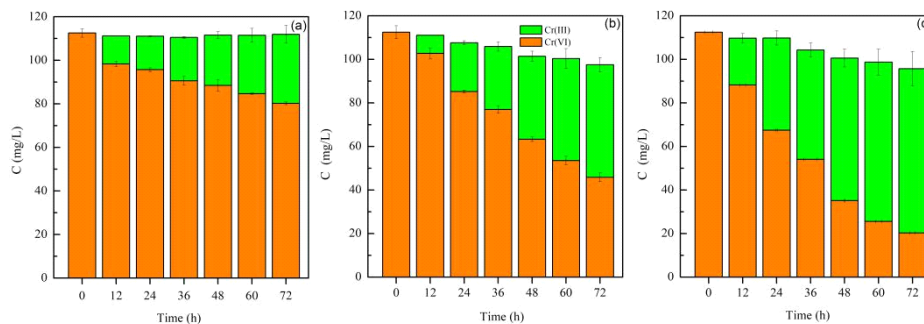


Fig. 3: Change of Cr(VI) and Cr(III) during the reduction process.
(a) ZVI (b) *M. morgani* subsp (c) *M. morgani* subsp-ZVI

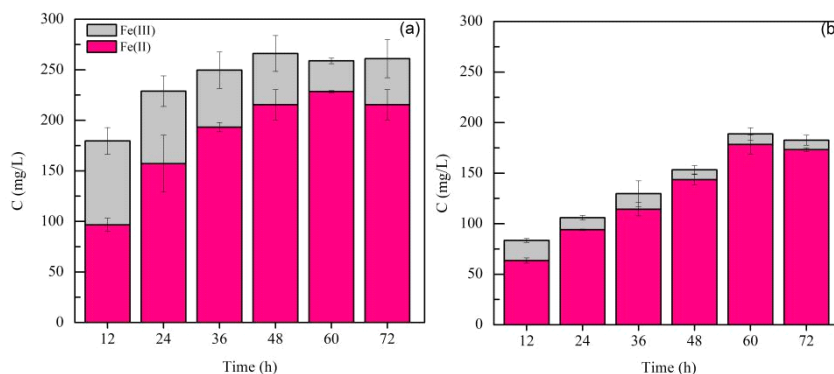


Fig. 4: Change of Fe(II) and Fe(III) during the reduction process.
(a) ZVI; (b) *M. morgani* subsp-ZVI

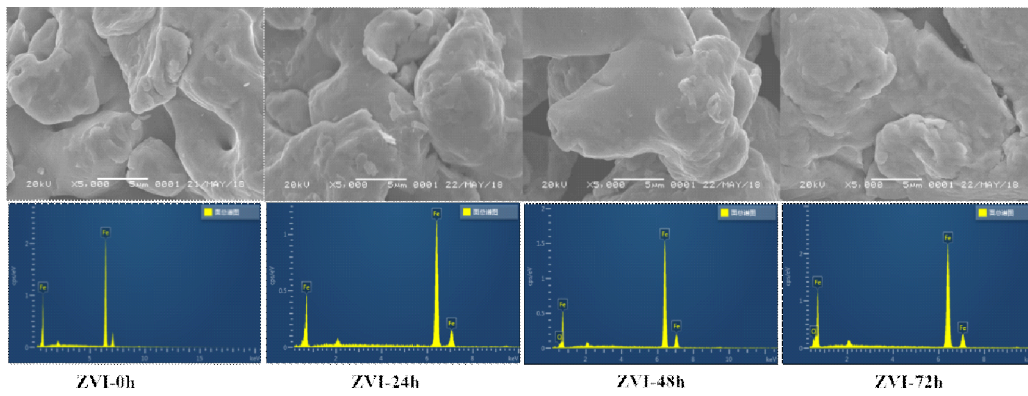


Fig. 5: SEM-EDS images of ZVI during the reduction process.

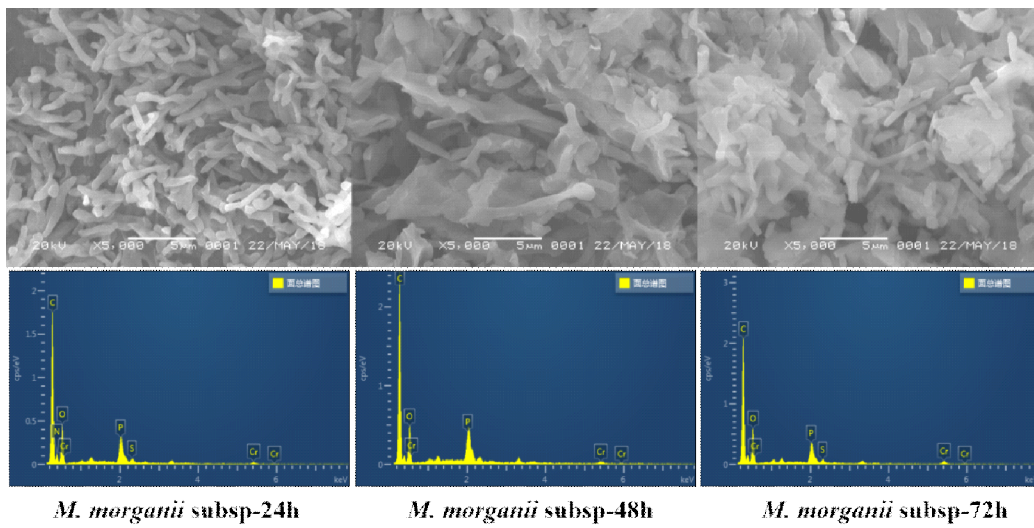


Fig. 6: SEM-EDS images of *M. morgani* subsp during the reduction process.

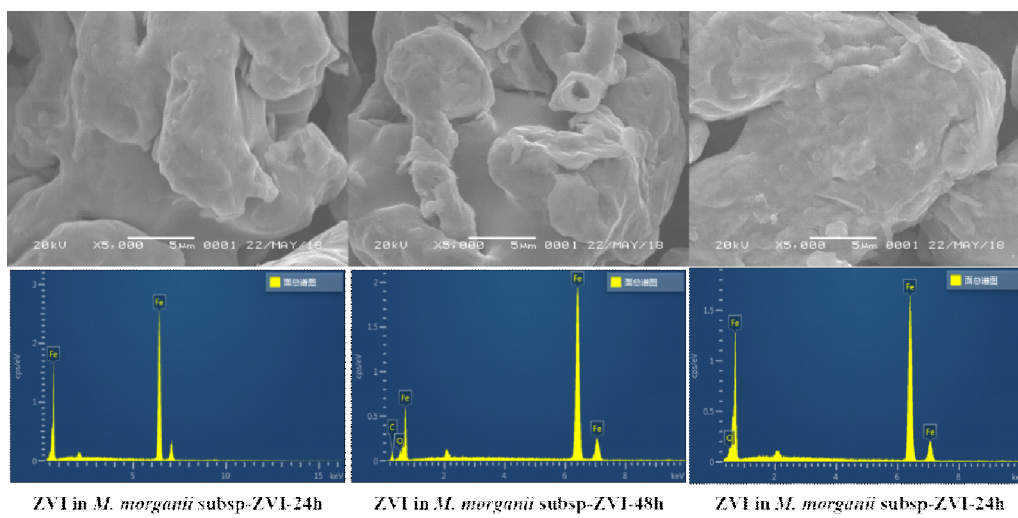


Fig. 7: SEM-EDS images of ZVI during the *M. morgani* subsp-ZVI reduction process.

Table 2: Kinetic fitting results of pseudo first and second order kinetics.

	Pseudo-first-order			Pseudo-second-order		
	q_e (mg/g)	k_a (h ⁻¹)	R_a^2	q_e (mg/g)	k_p (h ⁻¹)	R_p^2
1	15.24	0.038	0.970	19.96	0.0375	0.980
2	17.86	0.032	0.932	24.35	0.0276	0.949

ZVI was observed in this process. By contrast, during the removal of Cr(VI) by *M. morganii* subsp, the total chromium content was reduced attributed to fixation or adsorption. Besides, a part of Cr(VI) was reduced to Cr(III), which manifests that *M. morganii* subsp has competence in reducing Cr(VI). It must be mentioned that in the process of synergistic removal of Cr(VI) by *M. morganii* subsp-ZVI, not only the total chromium content was reduced, but also a large amount of Cr(VI) was converted into Cr(III), and the toxicity reduced remarkably. Hence, *M. morganii* subsp can drastically promote the reduction of Cr(VI) by ZVI, with capability of fixing Cr simultaneously.

Analysis of the change of Fe(III)/Fe(II) content: The variations of Fe(II) and Fe(III) during the reduction process of ZVI and *M. morganii* subsp-ZVI synergistic system are presented in Fig. 4. In the ZVI system, the content of Fe(II) and total Fe increased with the progress of the reaction. Nonetheless, the trend of Fe(III) content was unstable, probably due to the formation of Fe(III) and Fe(OH)_x precipitation (Shelobolina et al. 2004). By contrast, in the synergistic system, the content of Fe(II) and Fe(III) in the solution was remarkably lower, especially Fe(III), associated with the reduction by *M. morganii* subsp. Moreover, since the Fe(II) content decreased inconspicuously and Fe(III) content increased slightly, we speculate that *M. morganii* subsp can reduce Fe(III) to Fe(II) in solution.

SEM-EDS analysis: SEM-EDS analysis was further carried out to verify the mechanisms in the reaction process. As shown in Fig. 5, SEM images recorded at high magnification clearly shown that the surface of ZVI was corroded. Meanwhile, EDS exhibited the element on the surface of the ZVI was mainly Fe at 0 h and 24 h, and it is noteworthy that the O element was detected at 48 h, indicating the formation of corrosion like Fe₂O₃. However, Cr was not detected during the whole process, which implies that there was no large amount of Cr deposited on the surface of ZVI.

Fig. 6 represents the surface characteristic and basic element of ZVI during the *M. morganii* subsp-ZVI reduction process. It can be seen that no significant changes of morphology took place on *M. morganii* subsp and it grew well with the progress of the reaction, suggesting that it can adapt to Cr(VI) pollution environment. What is more, the primary elements on the surface of microorganisms at 24 h, 48 h and

72 h were Fe, O, S. A large amount of Cr was detected, revealing that the microorganisms have a immobilization effect on Cr, which majorly comes from the adsorption of Cr by extracellular polymers secreted via microorganisms.

SEM-EDS images of ZVI in the *M. morganii* subsp-ZVI reduction process are demonstrated in Fig. 7. High-magnification SEM images display that with the progress of the reaction, corrosion appeared on the surface of the ZVI in the *M. morganii* subsp-ZVI system, and the degree of corrosion was more serious than that of the ZVI single system, which maybe the reason for the increased amount of adsorption. However, Cr was not detected on the surface of the system during the whole process, implying that Cr was not deposited on the surface of ZVI, so the fixation of Cr was mainly from microorganisms.

CONCLUSION

The synergistic action of ZVI and microorganisms on Cr(VI) removal in wastewater was evaluated in the study. The optimal conditions for removing Cr(VI) with *M. morganii* subsp-ZVI system was glucose of 4 g/L, yeast powder of 6 g/L and pH = 7. Since the maximum removal of Cr(VI) from ZVI increased from 19.96 mg/g to 24.35 mg/g before and after the addition of *M. morganii* subsp, it can be found that *M. morganii* subsp could obviously improve the efficiency of Cr(VI) reduction by ZVI under anoxic conditions. The speculation is also confirmed by SEM-EDS. The results have shown that *M. morganii* subsp could promote the surface corrosion of ZVI and adsorb Cr simultaneously.

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