Environmental Science and Pollution Research (2018) 25:6609–6618
https://doi.org/10.1007/s11356-017-0937-7

RESEARCH ARTICLE

The effect of electron competition on chromate reduction using methane as electron donor

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Received: 29 September 2017 / Accepted: 3 December 2017 / Published online: 19 December 2017

Abstract

We studied the effect of electron competition on chromate (Cr(VI)) reduction in a methane (CH_4)-based membrane biofilm reactor (MBfR), since the reduction rate was usually limited by electron supply. A low surface loading of SO_4^{2-} promoted Cr(VI) reduction. The Cr(VI) removal percentage increased from 60 to 70% when the SO_4^{2-} loading increased from 0 to 4.7 mg SO_4^{2-}/m^2-d. After the SO_4^{2-} loading decreased back to zero, the Cr(VI) removal further increased to 90%, suggesting that some sulfate-reducing bacteria (SRB) stayed in the reactor to reduce Cr(VI). However, a high surface loading of SO_4^{2-} (26.6 mg SO_4^{2-}/m^2-d) significantly slowed down the Cr(VI) reduction to 40% removal, which was probably due to competition between Cr(VI) and SO_4^{2-} reduction. Similarly, when 0.5 mg/L of Se(VI) was introduced into the MBfR, Cr(VI) removal percentage slightly decreased to 60% and then increased to 80% when input Se(VI) was removed again. The microbial community strongly depended on the loadings of Cr(VI) and SO_4^{2-}. In the sulfate effect experiment, three genera were dominant. Based on the correlation between the abundances of the three genera and the loadings of Cr(VI) and SO_4^{2-}, we conclude that *Methylocystis*, a type II methanotroph, reduced both Cr(VI) and sulfate, *Meiothermus* only reduced Cr(VI), and *Ferruginibacter* only reduced SO_4^{2-}.

Keywords Methane · Chromate reduction · Electron competition · Microorganism

Introduction

Chromium (Cr) is associated with a variety of human activities, e.g., metallurgic industry, refractory applications, and chemicals synthesis (Barnhart 1997). The dominant oxidation states of Cr in wastewater are chromate (Cr(VI)) and chromite (Cr(IV)). Cr(VI) has high solubility and toxicity and may lead to cardiovascular shock, diarrhea, vomiting, and liver and kidney necrosis in human bodies (Barnhart 1997; Dayan and Paine 2001; Kantar et al. 2008). The US Environmental Protection Agency has set the maximum contaminant level (MCL) for Cr in drinking water at 100 μg/L (USEPA 2015). However, Cr(III), the reduced form of Cr(VI), is essential to humans (Srivastava et al. 1999) and can transform into precipitates in neutral or alkaline conditions, making it easy to be removed from wastewater (Anderson and Kozlovsky 1985; Palmer and Wittbrodt 1991). Therefore, reduction of Cr(VI) to Cr(III) is a feasible method for remediate Cr-contaminated wastewater (Cheung and Gu 2007).

Having the advantage in low cost and sustainability (Lovley and Coates 1997; Zahoor and Rehman 2009),

Pan-Long Lv and Liang Zhong contributed equally to this work.

Responsible editor: Bingcai Pan

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-017-0937-7) contains supplementary material, which is available to authorized users.

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bioreduction of Cr(VI) is deemed to be a promising method for Cr removal. Chromate-reducing bacteria reported in the literature, e.g., *Staphylococcus epidermidis* (Vatsouria et al. 2005), *Desulfovibrio vulgaris* (Lovley and Phillips 1994), *Pseudomonas* sp. (McLean and Beveridge 2001), and *Enterobacter cloacae* (Waki et al. 1989), utilize organics or hydrogen (H_2) as the electron donor to mediate Cr(VI) reduction, in aerobic or anaerobic condition. Recently, Luo et al. (2015) and Lai et al. (2016a, b) reported that methane could also serve as the sole electron donor. They observed efficient bioreduction of Cr(VI) in the CH_4-based membrane biofilm reactors (MBfR), in which CH_4 was supplied through hollow fibers. This process has drawn great interest in recent years because CH_4 is an inexpensive and widely available electron donor (Lai et al. 2016a, b; Zhong et al. 2017). Furthermore, CH_4 is a potent greenhouse gas that has 25 times higher greenhouse potential than carbon dioxide (CO_2) (Hu et al. 2014; Knittel and Boetius 2009); thus, consumption of CH_4 can also reduce the global greenhouse effect.

A frequent co-existing electron acceptor with Cr(VI) is sulfate (SO_4^{2-}), which is an extensively spreading oxyanion in wastewater (Huber et al. 1997). SO_4^{2-} reduction is performed by sulfate-reducing bacteria (SRB) which are very diverse phylogenetically, spreading from ε- and δ-*Proteobacteria* to *Clostridia* (Mori et al. 2003). In the process of dissimilatory SO_4^{2-} reduction, ATP sulfurylase activates SO_4^{2-} by connecting it to phosphate radical of ATP, producing adenosine phosphosulfate (APS) (Peck 1959). APS was then reduced by an APS reductase to adenosine monophosphate (AMP) and sulfite (SO_3^{2-}). SO_3^{2-} was finally reduced to sulfide (S^{2-}) by sulfite reductase. The interactions between Cr(VI) and SO_4^{2-} reductions depend upon the microbial community structure in the CH_4-based biofilms. SRB are versatile, and some of them are able to reduce Cr(VI) through enzymes (Michel et al. 2001; Chardin et al. 2003). On the other hand, sulfite (S^{2-}) derived from SO_4^{2-} reduction (Smith and Gadd 2000) can abiotically react with Cr(VI). SO_4^{2-} also possibly inhibits Cr(VI) reduction, due to the competition for membrane space, electron donor, et al., between chromate-reducing bacteria and SRB (Tang et al. 2012a).

Microbial reduction of CrO_4^{2-} and SeO_4^{2-} has drawn great attention in recent years due to the simplicity and low cost (Lai et al. 2016a, b). Both of chromate and selenate-reducing bacteria are phylogenetically diverse. Variety of chromate-reducing bacteria, e.g., *Enterobacter cloacae* (Wang et al. 1991), *Pantoea agglomerans* (Francis et al. 2000), *Pseudomonas putida* (Park et al. 2000), *Escherichia coli* (Ackerley et al. 2004), and selenate-reducing bacteria, e.g., *Bacillus* (Fujita et al. 1997), *Sulfurospirillum* (Lenz et al. 2009), *Enterobacter cloacae* (Ma et al. 2009), and *Desulfurispirillum indicum* (Rauschenbach et al. 2010), have been isolated. The objective of this study is to study the effect of SO_4^{2-} and SeO_4^{2-} on Cr(VI) reduction in a CH_4-based MBfR by changing the loadings of SO_4^{2-} or SeO_4^{2-} in the influent. Overall, we also want to understand the mechanisms involved in the interaction of SO_4^{2-}/SeO_4^{2-} and Cr(VI) reduction by looking into the community structure change of the biofilms using electron microscope and high-throughput HiSeq sequencing technology.

Materials and methods

Startup and continuous operation

We set up the same MBfR system as described in Lai et al. (2016a, b). The core component of MBfR is the composite hollow fibers manufactured by Mitsubishi Rayon (model MHF-200TL, Mitsubishi, Ltd., Japan). The total volume of the MBfR was 65 mL, while the total membrane surface area was 58 cm^2. The liquid in the system were mixed by using a peristaltic pump at 100 mL/min. We inoculated the reactor with 10 mL of a culture that had been adapted to anaerobic oxidation of methane coupled to chromate reduction. Inorganic medium was deoxygenated and prepared as influent for the MBfR (Lai et al. 2016a, b). The medium contained the following mineral salts per liter of ultrapure water: NH_4Cl 0.05 g, $CaCl_2$ 1 mg, $NaHCO_3$ 0.3 g, $MgCl_2$ 2 mg, $MgSO_4 \cdot 7H_2O$ 2 mg, KH_2PO_4 0.2 g, $Na_2HPO_4 \cdot 12H_2O$ 0.4 g, 1 mL acid trace element solution (HCl 100 mM, 1 g of $FeCl \cdot 7H_2O$, 68 mg of $ZnCl \cdot 7H_2O$, 14 mg of H_3BO_3, 120 mg of $CoCl_2 \cdot 6H_2O$, 500 mg of $MnCl_2 \cdot 4H_2O$, 320 mg of $CuCl_2$, 95 mg of $NiCl_2 \cdot 6H_2O$ per liter), and 1 mL alkaline trace element solution (Luo et al. 2015). We stimulated the growth of the inoculum by adding Cr(VI) into the medium at 10 mg/L and recirculating the medium for 48 h. Afterwards, the influent Cr(VI) concentration was adjusted to ~ 1 mg/L, corresponding to a loading rate of 124 mg Cr(VI)/m^2-d throughout the whole experiments. To evaluate the effect of SO_4^{2-} on Cr(VI) reduction, we varied the SO_4^{2-} concentration in the influent medium: 0, 3.5, 0, 20, and 0 mg of SO_4^{2-}/L in stages 1, 2, 3, 4, and 5, respectively. Each stage was maintained for at least 2 weeks until the variation of the effluent concentrations of Cr(VI) and SO_4^{2-} were < 10%. We kept the liquid recirculation rate at 100 mL/min. The CH_4 pressure was constant at 10 psig, and the temperature was stable (29 ± 1 °C) throughout the experiments.

We did the selenate effect experiment separately in another methane-based MBfR. The Cr(VI) concentration in the influent was kept at 1 mg/L (124 mg Cr(VI)/m^2-d) throughout the whole experiments, while Se(VI) was varied at 0, 1, 0, 0.5, 0 mg/L for stages 6–10. Each stage was ended until reaching steady state (the variation of effluent concentrations of Cr(VI) and Se(VI) was < 10%). The liquid recirculation of MBfR was maintained at 100 mL/min, while the pressure of CH_4 and the temperature were kept at 10 psig and 30 °C, respectively.

Chemical analyses

We took liquid samples of the influent and effluent of MBfR using 10-mL gas-tight syringes every 2 days and centrifuged the liquid samples (15,000*g*, 10 min) to remove insoluble Cr(III) precipitates. We then filtered them immediately through a 0.22-μm membrane filter (LC + PVDF membrane, Shanghai Xinya, China). The Cr(VI) concentration was analyzed by diphenyl carbazide method (Method 3500-Cr D, APHA 1998), and the concentration of Se(VI) was determined using ion chromatography (Metrohm 833 Basic IC plus, Switzerland) equipped with an S-Supp-5 column. We determined the concentration of SO_4^{2-} using ion chromatography (Metrohm 833 Basic IC plus, Switzerland) equipped with an S-Supp-5 column. The eluent was 3.2 mM $NaHCO_3$ and 1.0 mM Na_2CO_3, and the flow rate was 0.7 mL/min. The concentrations of dissolved oxygen (O_2) were ~ 0.2 mg/L for the influent and ≤ 0.1 mg/L for the effluent measured by dissolved oxygen probe (Starter, model 300D, Ohaus Instruments Company, Germany). The pH values throughout the experiments were 7.0–7.5 measured by a pH meter (Seven Easy, Mettler Toledo, Switzerland).

Flux calculations

We calculated the removal fluxes of Cr(VI), O_2, and SO_4^{2-} (mg m^{-2} day^{-1}) according to

$$J = (S_0 - S)Q/A \quad (1)$$

in which S_0 and S are the influent and effluent Cr(VI), O_2, or SO_4^{2-} concentration (mg/L), Q is the influent flow rate to the MBfR system (L/day), and A is the membrane surface area (m^2). The CH_4 flux was calculated based on reaction stoichiometry shown in reaction 2 (for Cr(VI)), reaction 3 (for O_2), and reaction 4 (for SO_4^{2-}) (Rittmann and McCarty 2001).

$$CrO_4^{2-} + 0.749\ CH_4 + 0.107\ NO_3^- + 5.10\ H^+ \rightarrow Cr^{3+} + 0.214\ CO_2 + 3.67\ H_2O + 0.107\ C_5H_7O_2N \quad (2)$$

$$O_2 + 1.25\ CH_4 + 0.214\ NO_3^- + 0.214\ H^+ \rightarrow 0.179\ CO_2 + 1.86\ H_2O + 0.214\ C_5H_7O_2N \quad (3)$$

$$SO_4^{2-} + 1.038\ CH_4 + 0.011\ NO_3^- + 0.011\ H^+ \rightarrow S^{2-} + 2.04\ H_2O + 0.011\ C_5H_7O_2N + 0.982\ CO_2 \quad (4)$$

$$SeO_4^{2-} + 1.124\ CH_4 + 0.107\ NO_3^- + 2.104\ H^+ \rightarrow Se^0 + 2.929\ H_2O + 0.107\ C_5H_7O_2N + 0.59\ CO_2 \quad (5)$$

The maximum CH_4 flux (e^- meq/m^2 day) was calculated according to Tang et al. (2012c).

Biofilm sampling and imagining, DNA extraction, and Illumina sequencing

We cut off two ~ 5-cm-long sections from a coupon fiber at the end of each stage. One section was used for scanning electron microscope (SEM) and energy dispersive X-ray (EDS) analysis, while the other was used for DNA extraction (Lai et al. 2016a, b). We extracted DNA by using DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) (Lai et al. 2014) and measured the DNA concentration by Nanodrop spectrophotometer.

We used primers 515F (5-GTGCCAGCMGCCGCGG-3′ and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) to amplify the conserved V4–V5 regions of the bacterial 16S rRNA gene and purified the PCR products using the QIAquick PCR Purification Kit (QIAGEN, Germany). The purified amplicons were sent to Novogene Technology (Beijing, China) to process Illumina MiSeq sequencing. The data were analyzed using QIIME (version 1.9.1) pipeline as described by Lai et al. (2014). We analyzed the phylogenetic sequences against the silva (SSU115)16S rRNA database using confidence threshold of 70%, analyzed the relationship of community composition in different stages by using the principal coordinate analysis (PCoA) (Shannon et al. 2003), and unweighted UniFrac distance matrix (Lozupone et al. 2006). The alpha diversity parameters was acquired by using QIIME (version 1.9.1) pipeline and presented the results in Table S2.

Results and discussion

Effect of sulfate on chromate reduction

Figure 1a shows the concentrations of Cr(VI) and SO_4^{2-} in the influent and effluent of CH_4-based MBfR, while Fig. 1b shows the removal percentages of Cr(VI) and SO_4^{2-}. The fluxes of Cr(VI), SO_4^{2-}, and O_2 (calculated from Eqs. 2, 3, and 4) are presented in Table 1 for each stage at steady state. The maximum CH_4 flux that can be delivered through the hollow fiber at the constant CH_4 pressure (10 psig) was much larger than the actual CH_4 flux, meaning that the CH_4 supply was sufficient (Luo et al. 2015).

In stage 1 (days 0–36), when Cr(VI) was supplied as the only electron acceptor in the influent (1 mg/L of Cr(VI)), the Cr(VI) removal percentage reached ~ 25% after 48 h enrichment, steadily increased to ~ 60% at day 25, and remained stable for the rest of the stage. The EDS spectrum demonstrated the occurrence of Cr(III) precipitates, the final product of Cr(VI) reduction (Fig. 4e). The Cr(VI) removal was very similar to the pattern as in our previous research (Lai et al. 2016a, b), showing very good repeatability. However, in stage 2 (days 38–66), when ~ 3.5 mg/L of SO_4^{2-} was introduced to the

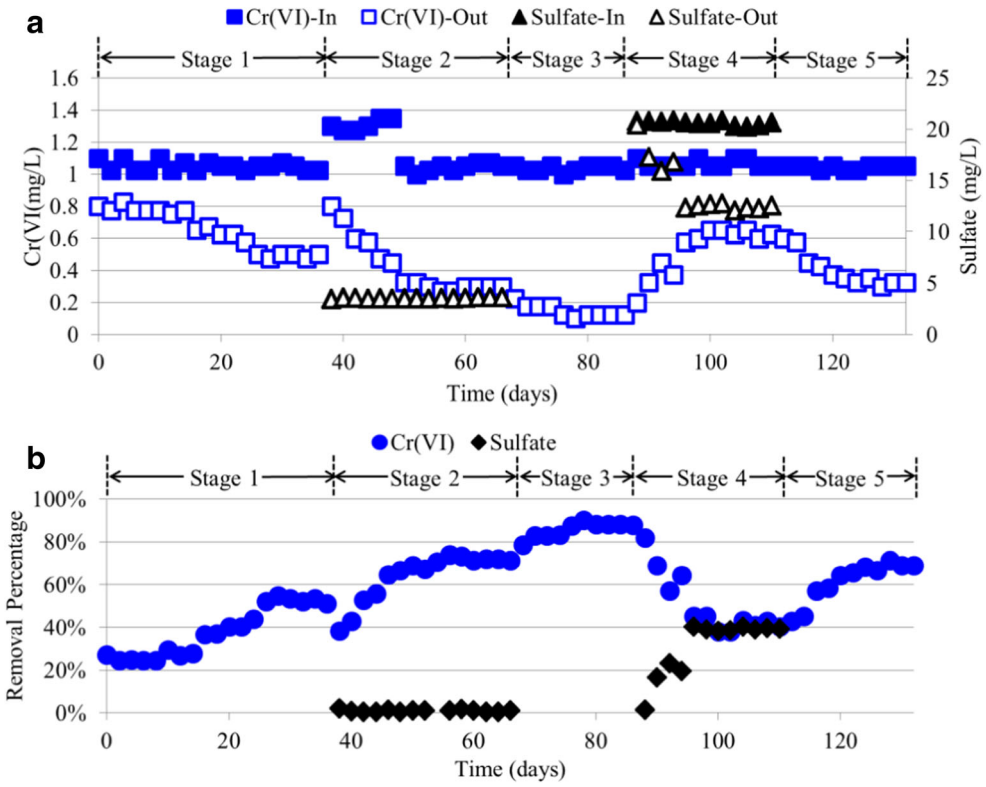

Fig. 1 **a** Cr(VI) and SO_4^{2-} concentrations in the CH_4-based MBfR throughout all five stages. **b** Cr(VI) and SO_4^{2-} removal percentages

MBfR, the Cr(VI) removal percentage did not drop but continuously increased up to ~ 70% and remained stable afterwards. Generally when a second electron acceptor is introduced, the reduction of the first fed electron acceptor (Cr(VI) in this study) will decrease due to competition for electrons and other resources. For example, Zhong et al. (2017) reported that the introduction of 280 mg NO_3^--N m^2-d dramatically suppressed Cr(VI) reduction, and Zhao et al.

Table 1 The flux of electron acceptors and methane for each stage at steady state

Stage	Cr(VI)			O_2			SO_4^{2-}			Electron donor (CH_4)	
	Surface loading (mmol/m^2-d)	Flux[a] (mmol/m^2-d)	Electron donor consumed[b] (mmol CH_4/m^2-d)	Surface loading (mmol/m^2-d)	Flux (mmol/m^2-d)	Electron donor consumed (mmol CH_4/m^2-d)	Surface loading (mmol/m^2-d)	Flux (mmol/m^2-d)	Electron donor consumed (mmol CH_4/m^2-d)	Actual CH_4 flux (mmol CH_4/m^2-d)	Maximum CH_4 flux[c] (mmol CH_4/m^2-d)
1	2.50	1.32	0.99	0.80	0.80	1.00	NA	NA	NA	1.99	57.9
2	2.49	1.78	1.33	0.80	0.80	1.00	4.65	0.04	0.04	2.37	57.9
3	2.47	2.18	1.63	0.80	0.80	1.00	NA	NA	NA	2.63	57.9
4	2.55	1.07	0.80	0.80	0.80	1.00	26.59	10.46	10.86	12.66	57.9
5	2.49	1.69	1.26	0.80	0.80	1.00	NA	NA	NA	2.26	57.9
6	2.54	1.35	1.01	0.80	0.80	1.00				2.01	57.9
7	2.51	1.47	1.10	0.80	0.80	1.00	1.51	0.86	1.70	3.80	57.9
8	2.52	2.00	1.50	0.80	0.80	1.00				2.50	57.9
9	2.40	1.54	1.16	0.80	0.80	1.00	0.81	0.67	0.92	3.07	57.9
10	2.50	2.12	1.59	0.80	0.80	1.00				2.59	57.9

[a] Calculated from Eq. 1

[b] Calculated from Eqs. 2, 3, and 4

[c] Calculated according to Tang et al. (2012)

(2011) reported that the input of NO_3^- substantially lowered ClO_4^- percent removals. No SO_4^{2-} reduction was detected in this stage, which might be due to the higher redox potential for Cr(VI)/Cr(III) (− 0.13 V) compared with that for SO_4^{2-}/S^{2-} (− 0.22 V) (Rittmann and McCarty 2001).

The improvement of Cr(VI)-reducing capability of the biofilm after SO_4^{2-} addition might be due to the accumulation of slow growing of sulfate-reducing bacteria (SRB). Michel et al. (2001) demonstrated that some SRB were able to reduce Cr(VI), using the cytochrome c or negative redox potential hemes. Cheung and Gu (2003) found that SRB were able to reduce 88% of Cr(VI) (at the initial concentration of 500 mmol/L) in 48 h. Cetin et al. (2008) also reported that in the range of 22.7–74.9 mg/L of initial Cr(VI), 99% of Cr(VI) was reduced within 2–6 days. Besides, the measured zero SO_4^{2-} removal might due to redox cycling of S in the MBfR: the final products of SO_4^{2-} reduction by SRB, sulfide (S^{2-}), might be used as the electron donor for chromate-reducing bacteria to perform Cr(VI) reduction, and S^{2-} was reoxidized to SO_4^{2-} (Arias and Tebo 2003; Zhao et al., 2013, 2014). Moreover, sulfite (S^{2-}) is an very strong reductive agent that can abiotically reduce Cr(VI) (Smith and Gadd 2000).

The Cr(VI) removal percentage further increased up to ~ 90% when SO_4^{2-} was removed out of the system in stage 3 (days 68–86). This is reasonable considering that some SRB grown in stage 2 stayed in the biofilm to reduce sulfate in stage 3.

A high concentration of SO_4^{2-} (20 mg/L, 26.59 mmol/m^2-d) gave a great impact on Cr(VI) reduction in stage 4: Cr(VI) removal dropped to ~ 45%. The sulfate removal reached ~ 40%. In this stage, the inhibition from sulfate became dominant. In stage 5, when the influent SO_4^{2-} was again returned to zero, C(VI) reduction bounced back to ~ 70%. The fact that low loading of SO_4^{2-} promoted Cr(VI) reduction and higher loading of SO_4^{2-} had a negative effect on Cr(VI) reduction is very similar to Tang's study (Tang et al. 2012a,b), in which low loading of NO_3^- (≤ 100 mg/m^2-d) promoted ClO_4^- reduction, and high loading of NO_3^- (≥ 600 mg/m^2-d) significantly inhibited ClO_4^- reduction.

Simultaneous Cr(VI) and Se(VI) reduction in the CH_4-based MBfR

Figure 2a, b shows the performance of CH_4-based MBfR to reduce Se(VI) and Cr(VI), while Table 1 shows the fluxes of Cr(VI), Se(VI), and O_2 (calculated from Eqs. 2, 3, 4, and 5) for each stage at steady state. Due to the large discrepancy between maximum CH_4 flux and actual CH_4 flux, the CH_4 supply was sufficient throughout the whole experiments (Luo et al. 2015).

In stage 6 (days 0–32), when the influent contained 1 mg/L of Cr(VI) as the sole electron acceptor, the Cr(VI) removal percentage increased slowly and achieved 53% at day 22 and remained stable in this stage. In stage 7 (days 34–58), when 1 mg/L of Se(VI) was added into the influent, the

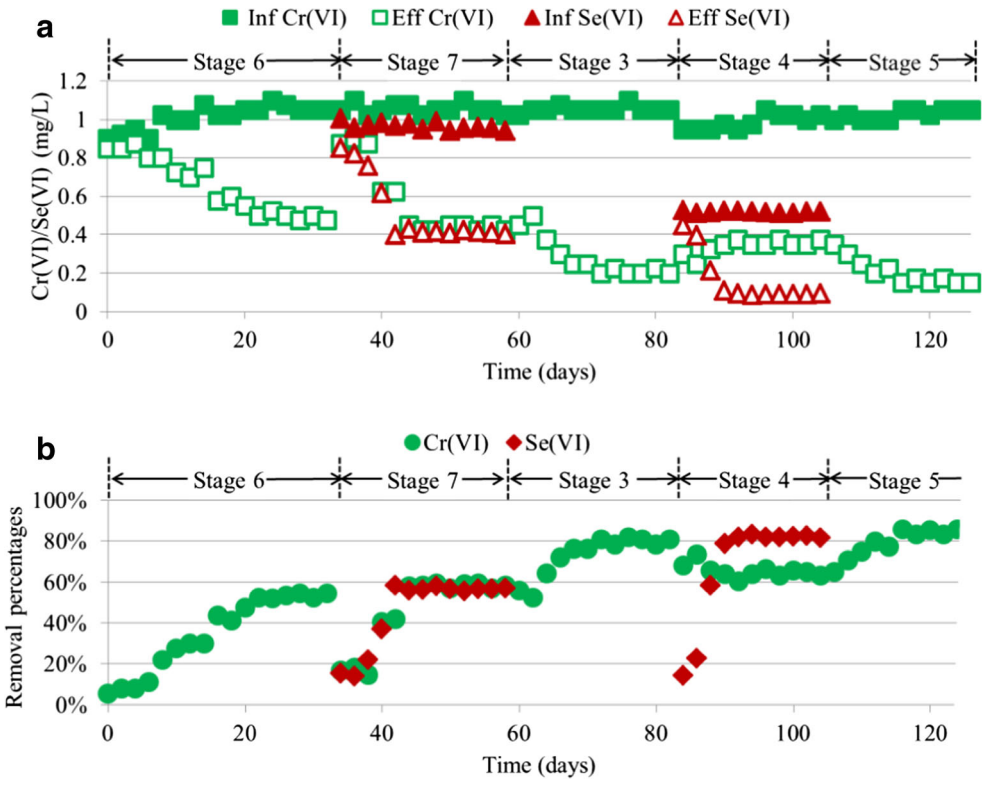

Fig. 2 **a** Cr(VI) and Se(VI) concentrations of the influent and effluent in CH_4-based MBfR for all stages. **b** Cr(VI) and Se(VI) removal percentages

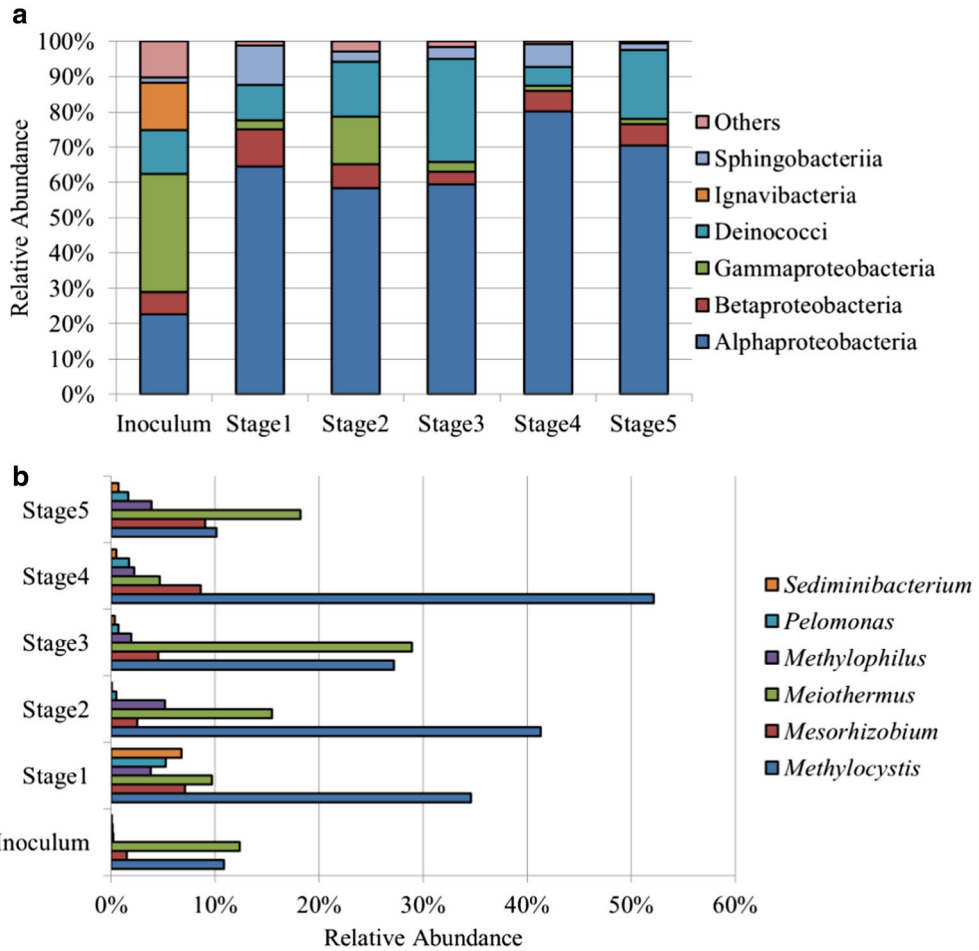

Fig. 3 The sulfate effect MBfR: relative abundances of microorganisms in the biofilms at the levels of class (**a**) and genus (**b**) for all stages

Cr(VI) removal percentage decreased to only 20%, while Se(VI) removal percentage was also ~ 20%. However, both of the Cr(VI) and Se(VI) removal percentage increased up to ~ 60% at steady state in this stage. The inhibition of Cr(VI) reduction at the start of this stage should be due to the suppression of chromate-reducing bacteria by Se(VI). Se(VI) has high toxic to organisms due to its ability to replace sulfur in sulfur-containing proteins, leading to the damage of normal function of these proteins (Fournier et al. 2010; Lemly et al. 1993). Furthermore, the reduced form of Se(VI), selenite (Se(IV)), has been reported to slowdown the growth rate of *Rhodobacter sphaeroides* (Bebien et al. 2001). The increase of Cr(VI) and Se(VI) reduction in the later phase of this stage implied the adaption of chromate-reducing bacteria to the exposure to Se(VI) and the growth of selenate-reducing bacteria in the biofilm. The similar or higher removal percentage for Cr(VI) and Se(VI) was possibly due to the higher redox potential for Se(VI)/Se(IV) (0.44 V) (Doran 1982) than that for Cr(VI)/Cr(III) (− 0.13 V) (Rittmann and McCarty 2001).

In stage 8 (days 60–82), when the influent Se(VI) returned to zero, Cr(VI) removal percentage increased to 80% at steady state. This might be because that part of selenate-reducing bacteria thriving in the biofilm in stage 7 was also able to reduce Cr(VI). Some species, e.g., *Bacillus* and *Pseudomonas aeruginosa*, have the ability to reduce Cr(VI) as well as Se(VI) (Burton et al. 1987; Lovely 1993).

In stage 9 (days 84–104), when low concentration of Se(VI) (0.5 mg/L of Se(VI)) was introduced, Cr(VI) removal percentage decreased slightly to 60% but rebounded to 80% when Se(VI) was again removed out in stage 10 (days 106–128). The Se(VI) removal percentage in stage 9 was higher than that in stage 7, as the surface loading of Se(VI) is lower in stage 9 than in stage7.

Microbial community changes in the biofilms

Figure 3a, b shows the relative abundances of phylotypes at the class and genus levels in the biofilms. *α-Proteobacteria* was the main class in the inoculum (22%) and became absolutely dominant in all biofilm samples (58–80%).

The relative abundance of *Methylocystis* was 10% of total bacteria in the inoculum, but increased to 35% with Cr(VI) addition in stage 1 and became dominant (> 52%) with high loading of input SO_4^{2-} in stage 4, suggesting that both Cr(VI) and sulfate can be used as electron acceptors for *Methylocystis* (*α-Proteobacteria*), a known type II methanotrophic

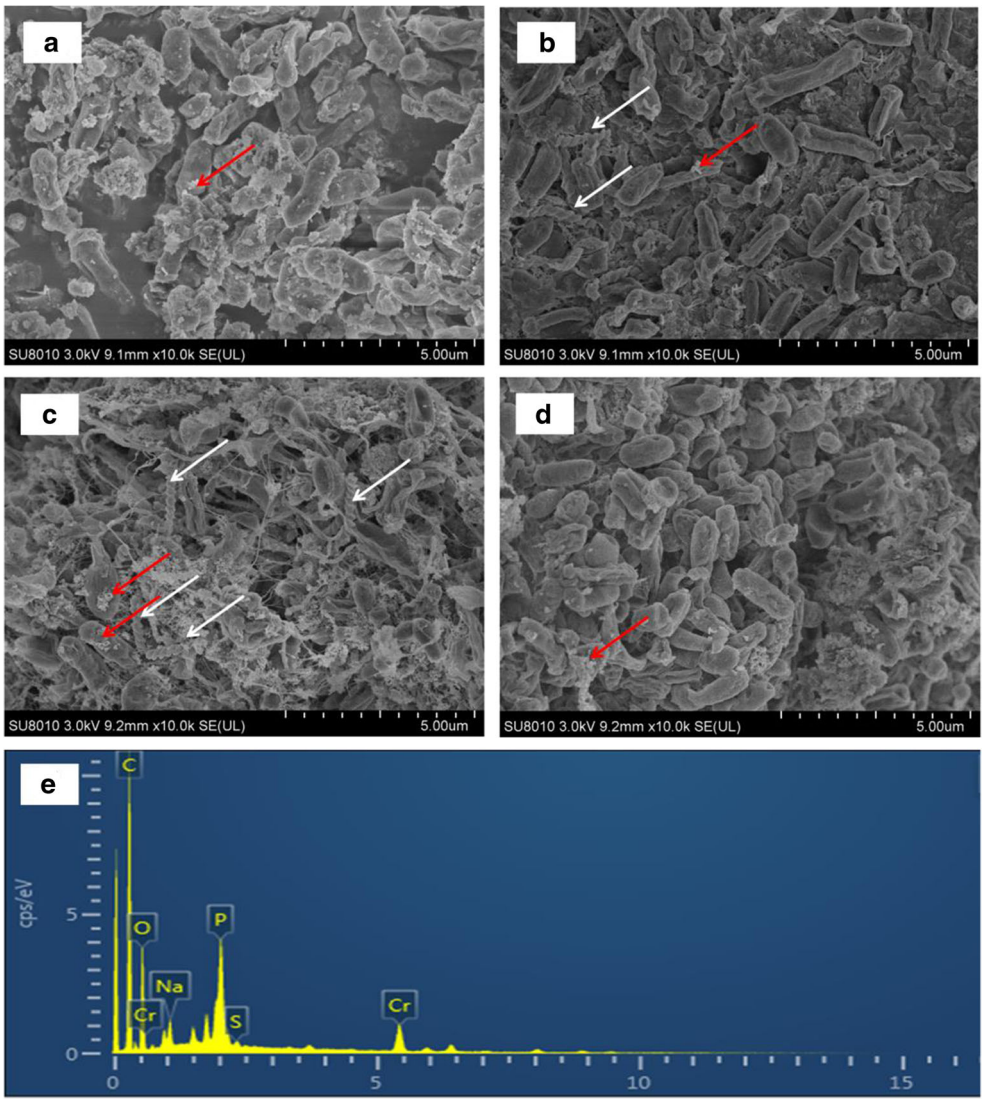

Fig. 4 The sulfate effect MBfR: SEM observations at 10000 magnification for stage 1 (**a**), stage 2 (**b**), stage 4 (**c**), and stage 5 (**d**). The white arrows indicate heliciform-shaped bacteria, which only occurred in the stages when SO_4^{2-} was in the influent. The red arrows indicate the Cr(III) precipitates, and the precipitates were identified by EDS spectrum (**e**)

bacterium using particulate methane monooxygenase to oxidize CH_4 (Dedysh et al. 2007; Yimga et al. 2003). It is reasonable that the *Methylocystis* became more dominant because of more CH_4 was consumed corresponding to higher loading of electron acceptors. The Pearson correlation (Table S1) proved that the abundance of *Methylocystis* was positively correlated with total CH_4 flux.

Meiothermus (*Deinococci*) represented 9.7% of the total bacteria in stage 1 and increased continually to 15.4 and 28.9% in stage 2 and stage 3, respectively, consistent with the increase of chromate-reducing capability of the biofilm. The abundance of *Meiothermus* decreased sharply to 4.7% in stage 4, when Cr(VI) reduction was dramatically inhibited by the high loading of SO_4^{2-}, and recovered to 18.2% in stage 5, when SO_4^{2-} was removed. Pearson correlation (Table S1) shows that the abundance of *Meiothermus* had a significantly positive correlation with the Cr(VI) flux. These data suggest that *Meiothermus* can only reduce Cr(VI), and its growth can be inhibited by SO_4^{2-}.

Ferruginibacter had the highest relative abundance in stages 2 and 4, when SO_4^{2-} was introduced into the influent (Fig. S1). *Ferruginibacter* is a potential SRB which was found in a moving bed biofilm reactor (MBBR) with sulfate reduction (Rikmann et al. 2012). Figure 3 shows the existence of helix-shaped bacteria. Since they were only observed in the biofilm samples from the stages when SO_4^{2-} was supplied, they are probably *Ferruginibacter*. SRBs are usually slow growers. The ΔG form the process of Cr(VI) reduction to Cr(III) is negative (-21.3 KJ/e^-), while that for SO_4^{2-} reduction to H_2S is positive (20.85 KJ/e^-). It explains the relatively lower abundance of *Ferruginibacter* compared to *Methylocystis* and *Meiothermus* (Fig. 4).

Figure 5 shows the unweighted PCoA based on the presence or absence of phylotypes of the biofilm samples. Stages 1, 3, and 5 were grouped together since Cr(VI) was the sole electron acceptor. Stages 2 and 4 were grouped together since both Cr(VI) and SO_4^{2-} were supplied as the electron acceptors. However, both groups had a large distance from the

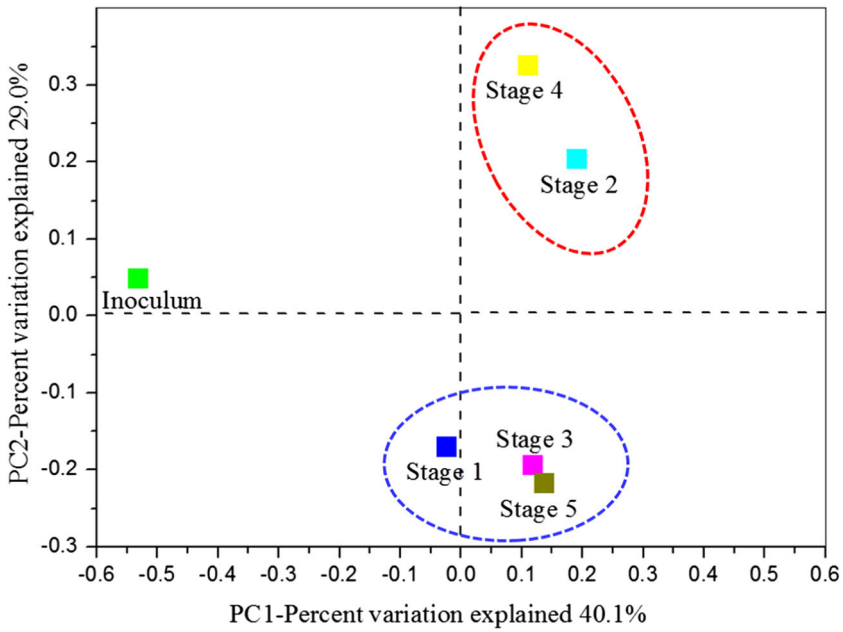

Fig. 5 The sulfate effect MBfR: PCoA based on the unweighted UniFrac analysis for microbial community. Stages 1, 3, and 5 are grouped together, while stages 2 and 4 are grouped together. All of the biofilm samples are distinct from the inoculum

inoculum. The unweighted UniFrac analysis showed a similar pattern (Fig. S2), further demonstrating that the microbial community structure in the biofilms was greatly shaped by the introduction of SO_4^{2-}.

The microbial community structure of the selenate and chromate MBfR was addressed in the SI.

Conclusion

We found the Cr(VI)-reducing activity of biofilms in a CH_4-based MBfR was greatly influenced by the introduction of SO_4^{2-}. Low concentration of input SO_4^{2-} promoted Cr(VI) reduction, while high concentration of input SO_4^{2-} inhibited Cr(VI) reduction, although the CH_4 supply was sufficient. The addition of 1 mg/L of Se(VI) into the influent suppressed Cr(VI) reduction at first, but promoted Cr(VI) reduction afterwards. Returning input Se(VI) to zero further improved Cr(VI) reduction. The introduction of 0.5 mg/L of Se(VI) slightly inhibited Cr(VI) reduction, although Cr(VI) removal percentage recovered when Se(VI) was removed again. Hiseq sequencing technology showed that *Methylocystis*, a type II methanotroph, was involved in CH_4 oxidation and reduction of both Cr(VI) and SO_4^{2-}, while the other two dominant species could only reduce one electron acceptor: *Meiothermus* only reduced Cr(VI) and *Ferruginibacter* only reduced SO_4^{2-}. The unweighted PCoA and UniFrac analysis proved that the microbial community in the biofilms was greatly shaped by the introduction of SO_4^{2-}. While selenate was co-existed, *Meiothermus* was proposed to reduce both of Cr(VI) and Se(VI), while *Methylophilus* implied the intermediate metabolites was involved in the electron transfer in the CH_4-based biofilm. In future, we will keep working on the electron competition among different electron acceptors, the electron transportation between electron donor and acceptor through different functional microorganisms when CH_4 was supplied as the sole electron donor by applying fluorescence in situ hybridization (FISH), metagenomic, metatranscriptome, metaproteomics, and other advanced technologies. Elucidating the effect of electron competition will greatly help us to explain the mechanism involved in the CH_4-based MBfR and apply it for practical use.

Acknowledgments The authors greatly thank the "Natural Science Funds for Distinguished Young Scholar of Zhejiang Province (LR17B070001)," the "Fundamental Research Funds for the Central Universities (2017XZZX010-03)," and the "National Natural Science Foundation of China (Grant No. 21377109, 21577123)" for their financial support.

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