

Enhanced organic matter degradation by a sediment microbial fuel cell using hexavalent chromium as an electron acceptor

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Abstract: In this study, a sediment microbial fuel cell (SMFC) system for the simultaneous biodegradation of organic matter and detoxification of hexavalent chromium Cr(VI) was investigated. The total organic carbon (TOC) removal rate of the SMFC with Cr(VI) was 30.07%, which was significantly higher than that in a SMFC without Cr(VI) (13.74%). In the SMFC with Cr(VI), the maximum values of open-circuit voltage (OCV) and power density were 408 mV and 4.8 mW/m², respectively. During the long-term operation of the SMFC with Cr(VI), 25 mg/L of Cr(VI) were completely reduced from all four consecutive batches over 48 days. MiSeq sequencing revealed that the biofilm microbial community of the anode comprised of Bacteroidetes (42.9%), Proteobacteria (33.6%), Chloroflexi (7.5%), and Euryarchaeota (7.5%) as the predominant phyla. Compared with that of the sediment, certain families were enriched; they included Pseudomonadaceae (46.88-fold), Flavobacteriaceae (5.05-fold), and Syntrophaceae (4.48-fold), which are organic matter-degrading bacteria. These results suggest that SMFCs are useful for TOC removal and detoxification of heavy metals in remediation of contaminated lakes.

Keywords: sediment microbial fuel cell, TOC removal, hexavalent chromium removal, detoxification, microbial communities

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1 Introduction

Owing to rapid industrialization and urbanization, both sediments and water bodies are contaminated with heavy metals, polycyclic aromatic hydrocarbons (PAHs), and organic substances (Pu et al., 2017; Qu and Fan, 2010). In June 2011, a serious chromium slag contamination event occurred at Qujing in Yunnan Province. A total of 5222.38 tons of hazardous chromium slags were illegally disposed of in the local reservoir, resulting in a reservoir hexavalent chromium (Cr(VI)) concentration of 200 mg/L, which is 2000 times higher than the standard concentration (Gao and Xia, 2011). Subsequently, 300000 m³ of Cr(VI)-polluted water was discharged into the Nanpan River (the source of the Pearl River), and the outlet groundwater Cr(VI) concentration increased to 2.24 mg/L (242 times the standard concentration (Gao and Xia, 2011; Pu et al., 2017)). Previous studies have proved that Cr(VI) is highly soluble, mobile, mutagenic, and carcinogenic, and exposure to it increases the risk of asthma and cancers of the respiratory system (Mahmoud and El-Twab, 2017). However, organic substances, including alkylphenol polyethoxylates (APEs), polychlorinated biphenyls (PCBs), and PAHs, are also adsorbed by the aquatic sediment. In 2008, total PAH concentrations in the sediment of the Lanzhou Reach of the Yellow River ranged from 464 to 2621 ng/g dry weight (Xu et al., 2007). High concentra-

tions of organic matter cause a sharp increase in water algae populations and the growth of aquatic plants, so that water ventilation and levels of dissolved oxygen are reduced; the oxygen-free layer may even be destroyed, which prevents the diffusion of pollutants from the sediment to the overlying water (Xu et al., 2015b). The technologies used to remove total organic carbon (TOC) from lake sediment include traditional physical and chemical methods (dredging, ozonation, and electrochemical degradation) and new biological methods (phytoremediation, bio-manipulation, and plant and microbe interaction) (Li and Yu, 2015; Qu and Fan, 2010). Several systems have been developed to treat Cr(VI) pollution in particular. They include pilot-scale ion exchange columns, a magnetic hybrid adsorbent, a byproduct of magnesium-based wet flue gas desulfurization, and nano-scale zero-valent iron particles (Cruz et al., 2017; Fang et al., 2017; Korak et al., 2017; Shang et al., 2017). There is no doubt that these methods have many advantages, such as high removal rate or rapid reaction rate. However, there are few reports on the simultaneous biodegradation of organic matter and Cr(VI) detoxification in polluted lakes.

Recently, the sediment microbial fuel cell (SMFC) has attracted significant attention owing to its unique ability to accelerate organic matter biodegradation in contaminated sediment or soil (Yan et al., 2012). A wide range of complex organic compounds, such as naphthalene, acenaphthene,

and phenanthrene, can be degraded in the SMFC anode (Sherafatmand and Ng, 2015). Certain microbes are capable of simultaneously remediating organic matter and heavy metal pollutants. Such microbes include *Pseudomonas gessardii* (strain LZ-E), which can degrade 77% of naphthalene with an initial concentration of 800 mg/L and reduce 95% of Cr(VI) with an initial concentration 10 mg/L (Huang et al., 2016). The simultaneous degradation of organic matter and the reduction of heavy metals by microbes can be simulated in a microbial fuel cell (MFC) by accelerating the transmission of electrons. Wang et al. (2012) constructed an MFC and increased total chemical oxygen demand (COD) removal in algal organic matter from $23 \pm 4\%$ to $81 \pm 6\%$. Another MFC technology was used to reduce the Cr(VI) content of electroplating wastewater by 99.5% (Li et al., 2008). However, there have been few studies on the use of SMFCs for simultaneous organic matter biodegradation and Cr(VI) reduction in polluted lakes.

Compared with oxygen, the most common electron acceptor in MFC, Cr(VI) has higher oxidation potential (1.33 V vs 1.23 V, determined using a standard hydrogen electrode) under acidic conditions (Yang et al., 2015), and Cr(VI) can be used as an electron acceptor in the cathode of a microbial fuel cell (MFC). Therefore, the MFC is an efficient novel technology for Cr(VI) reduction (Xafenias et al., 2013), and is able to completely remove an initial concentration of 100 mg/L Cr(VI) over 150 h (at an initial pH of 2) (Wang et al., 2008). Removal rates of 99.5% and 66.2% were obtained for Cr(VI) and total chromium, respectively, during a 25 h MFC treatment, and increasing the initial concentration of Cr(VI) enhanced the power density (Li et al., 2008). However, little information is available on the use of SMFCs for the simultaneous detoxification of Cr(VI) and the biodegradation of organic matter of polluted lakes. In the present study, an SMFC system was set up for the simultaneous enhancement of organic matter degradation in the anode, and reduction of Cr(VI) in the cathode, with Cr(VI) as the electron acceptor. The inoculation sediment was collected upstream of the Lanzhou Reach on the Yellow River. We monitored the removal of TOC and Cr(VI), the generation of electricity, the microbial communities of the raw sediment, and the SMFC anode. Long-term SMFC operation was stable and effective, suggesting that SMFCs are useful for the *in situ* bioremediation of Cr(VI) contaminants and organic pollutants of lakes.

2 Materials and Methods

2.1 Sample Collection

Sediment and freshwater samples were collected from a sewage drain exit near the Yellow River in Lanzhou ($36^{\circ}06'N$, $103^{\circ}39'E$). The soil samples were collected from 10 cm beneath the surface and stored at $4^{\circ}C$ until required.

2.2 SMFC Construction and Operation

The SMFCs were constructed in 1-L beakers, which were loaded with 0.5 L of sediment and covered with 0.5 L of stream water. Graphite rods were used for the electrodes. The graphite rods had a surface area of 54.26 cm^2 (diameter: 1.6 cm; length: 10 cm), and the anode contained two graphite rods linked by copper wire. The cathode was placed 4 cm above the sediment-water interface, and contained one graphite rod. The anode and cathode were connected by copper wire with an external resistance of 1000Ω (Figure 1). The operation temperature of the SMFCs was maintained at $20 \pm 5^{\circ}C$.

Four treatments were tested as follows: (1) Cr-SMFC: 25 mg/L of Cr(VI) was added to the cathode; (2) O_2 -SMFC contained no Cr(VI) in the cathode; (3) the open-circuit Cr-SMFC contained Cr(VI) but the circuit was broken; (4) the sterilized closed-circuit Cr-SMFC contained Cr(VI), and the sediment and water had been sterilized at $121^{\circ}C$ for 1 h. The long-term SMFCs were constructed in 3-L beakers, which were loaded with 1.5 L of sediment, covered with 1.5 L of stream water, and operated for 48 days. The voltage of resistance was recorded using a multimeter. The polarization and power curves were measured at an external resistor load varying from 100 to 10000Ω , and the voltage at each resistor value was recorded when it reached a pseudo steady state (Hong et al., 2009a).

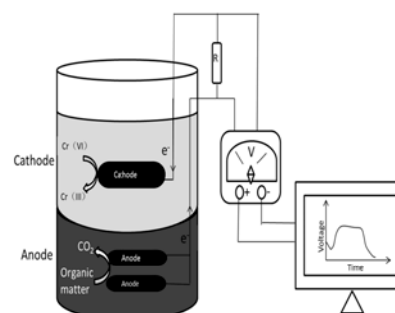


Figure 1. Schematic of the sediment microbial fuel cell (SMFC) reactor.

2.3 Analytical Methods

The TOC of the sediment was measured using a TOC solid state matrix analyzer (vario EL cube, Elementar Analysysteme GmbH, Germany). The TOC of the samples was expressed as a percentage of TOC removed (Adelaja et al., 2017). The voltage was converted into current according to Ohm's law, as described previously (Gee and Bauder, 1986). The concentration of Cr(VI) was determined using the 1, 5-diphenylcarbazide method at 540 nm, and was measured using a UV/VIS spectrophotometer (Lu et al., 2006). The Cr(VI) content in 1 g of water-soil surface soil was washed with 25 mL of phosphate-buffered saline (PBS) (pH 7.0), and determined using the 1, 5-diphenylcarbazide method. Nature withering the soil before and after washed by PBS, respectively. Following acid digestion, the content of Cr(III) and the total chromium content of 1 g of dry soil were determined by atomic absorption spectrometry.

try (Miao et al., 2015). Anode biofilm formation was investigated by scanning electron microscopy (SEM). Please refer to previous research for the details of sample preparation (Zhang et al., 2006). Finally, the samples were freeze-dried and sputter-coated with gold, then investigated by SEM (S-3400, HITACHI, Japan).

2.4 Extraction and MiSeq Sequencing of 16S rRNA Gene Amplicons

After use, the anode of the SMFC was washed with phosphate-buffered saline to collect the microorganisms; the resulting liquid was centrifuged at $7104 \times g$ for 10 min. DNA was extracted from 0.25 g (wet weight) of the raw sediments and from the anode biofilm using a PowerSoil DNA Isolation Kit (MO BIO, USA). The DNA concentration and quality were determined using a NanoDrop 2000 spectrophotometer (Thermo, China). Extracted DNA was diluted to 10 ng/ μ L and stored at -20°C for downstream use. Universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCGCYCAATTCMTTTRAGT-3'), with unique 12 nt barcodes at the 5'-end of 515F were used to amplify the V4-V5 hypervariable region of the 16S rRNA gene. Annealing temperatures and polymerase chain reaction (PCR) amplification procedures have been described previously (Wu et al., 2016). Replicate PCRs were carried out for each sample; the products were tested by 1% agarose gel electrophoresis and purified using a SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China). The sequencing samples were prepared using a TruSeq DNA kit, according to the manufacturer's instructions. The purified library was diluted, denatured, re-diluted, and mixed with PhiX (equal to 30% of the final DNA amount), as described in the Illumina library preparation protocols, and then presented to the Illumina MiSeq system for sequencing with the MiSeq Reagent Kit v2 (2 \times 250 bp) at the Environmental Genome Platform of the Chengdu Institute of Biology.

2.5 Statistical Analysis

The TOC results were subjected to statistical analysis using SPSS software (SPSS 16.0, Duncan, USA). $P < 0.01$ was considered significant.

3 Results and Discussion

3.1 TOC Removal from Sediment

The sediment used in this study was heavily contaminated with organic materials and heavy metals. The initial TOC was 2.2% (Table 1), and after 48 days, the three treatments applied (Cr-SMFC, O_2 -SMFC, and open-circuit Cr-SMFC) had removed 30.07%, 13.74%, and 7.84% of the TOC, respectively (Figure 2).

This data is consistent with previous studies showing that SMFCs can enhance the biodegradation of organic matter (Xu et al., 2015a; Yang et al., 2015). The low rate of oxygen reduction in the cathodes and electron transfer are the major limitations of the SMFC application. Our data showed that as an electron acceptor, Cr(VI) can increase the TOC removal rate by 30.07%, which is higher than the TOC degradation rate (22.1%) attributable to long-term SMFC use (operation

for 2 years) (Yang et al., 2015). With Cr-SMFC, the electrons from the organic matter biodegradation in the anode chamber are transferred to the cathode electrode, and are then immediately accepted by the Cr(VI). Therefore, the presence of Cr(VI) in the cathode stimulates electron transmission and TOC removal by the SMFC. However, TOC removal using the Cr-SMFC system in the present study (30.07%) was lower than that using an SMFC with Fe(III) oxide ($57.19\% \pm 1.52\%$) (Xu, et al., 2017). The explanation for this observation might be that Cr(VI) is highly toxic to microbial communities (Arshad et al., 2017). Moreover, Fe(II) can be re-oxidized to Fe(III) by bacteria for recycling (Xu, et al., 2017). This would explain the higher rates of TOC removal obtained using the SMFC with Fe(III) oxide compared with the Cr-SMFC system; Cr(VI) was irreversibly reduced to trivalent chromium (Cr(III)). However, considering that the annual discharge of Cr slag in China is 450,000 t (Gao and Xia, 2011), Cr(VI) is an ideal candidate for an electron acceptor in SMFC cathodes designed to enhance organic matter degradation.

Table 1. Baseline analysis of contaminated sediments.

Parameter	value
pH	6.5
Total organic carbon (%)	2.21%
	mg/kg
Rapidly available potassium	17.9112
Rapidly available phosphorus	19.2618
Copper	31.828
Zinc	68.983
Chromium	144.216
Nickel	58.71

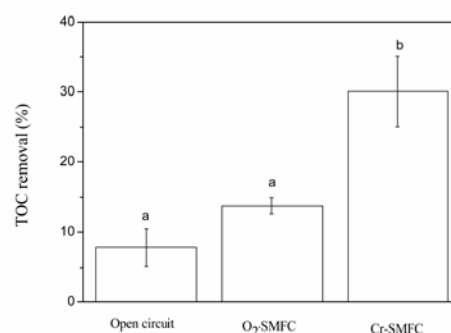


Figure 2. Total organic carbon (TOC) removal using the various sediment microbial fuel cells (SMFCs) for 48 days. Error bars represent the standard deviations from three tests. Significant differences ($P < 0.01$) between groups are indicated with letters above the bars.) reactor.

3.2 Electricity Generation and Cr(VI) Removal by SMFCs

After a stable lag period of 11 days, Cr(VI) was added to the cathode. The voltage of the Cr-SMFC increased rapidly from 52 to 110.2 mV 60 h after Cr(VI) addition, and then gradually decreased, whereas the O₂-SMFC voltage remained at 50 mV throughout the experiment. Interestingly, no electricity was generated by the sterilized Cr-SMFC (Figure 3A). The polarization curve of Cr-SMFC exhibited a maximum open-circuit voltage (OCV) of 408 mV and a maximum power density of 4.88 mW/m² (Figure 3B). The closed-circuit Cr-SMFC removed Cr(VI) 100% more quickly than the open-circuit Cr-SMFC (64.1%) over 16 days (Figure 4A). Total chromium removal was also faster with the closed-circuit Cr-SMFC than with the open-circuit Cr-SMFC (100% vs 85.2%) over 16 days (Figure 4B). After the experiment, we discovered that the soil in the water-soil interface of the closed-circuit Cr-SMFC contained 364.95 mg/kg of Cr(III) (compared with 267.80 mg/kg in the soil in the water-soil interface of the open-circuit Cr-SMFC).

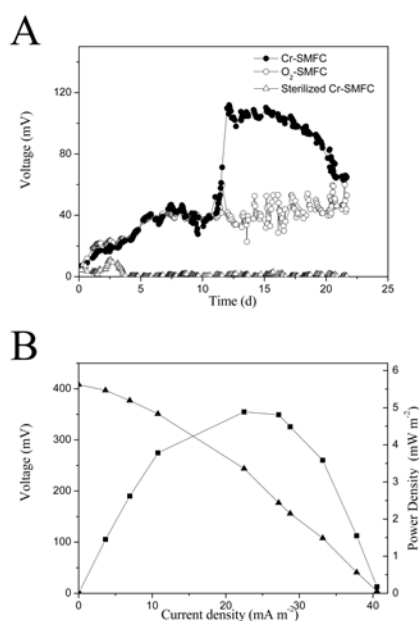


Figure 3. Electricity generation using the sediment microbial fuel cells (SMFCs). (A) Voltage generation in SMFCs with Cr(VI), without Cr(VI), and with Cr(VI) plus sterilization, with an external resistance of 1000 Ω . (B) Polarization and power density curves.

Electron acceptors play an important role in MFC electricity generation (You et al., 2006); most use oxygen, but this is complicated by low solubility, and SMFC operation has generally depended on aeration or a rotating cathode to improve the oxygen content (Xu, et al., 2017). This increases the required energy input and operating costs. Cr(VI) is a practicable electron acceptor with high solubility and oxidation potential (Tandukar et al., 2009). The voltage of

the Cr-SMFC increased by 57.8 mV immediately after the addition of Cr(VI), which may have been due to a cathode potential increase of 50–70 mV upon addition of Cr(VI) (Huang et al., 2010), promoting electron transfer between the anode and cathode. Although Xu added Fe(III) oxide to the sediment to achieve a higher TOC removal rate, the voltage of the SMFC decreased to 104 mV because the Fe(III) oxide competed with the anode electrode for electrons (Xu, et al., 2017).

Previous researchers have proposed that a typical mechanism for the removal of Cr(VI) involves adsorption, co-precipitation, ion exchange, and subsequent reduction (Cruz et al., 2017; Fang et al., 2017; Korak et al., 2017; Shang et al., 2017). The use of MFCs is a popular method of treating wastewater containing Cr(VI) because the Cr(VI) is directly reduced to Cr(III), which is far less toxic form of chromium (Huang et al., 2010). Table 2 gives details of Cr(VI) remediation as reported in previous MFC studies, and reveals that SMFC has been underused for Cr(VI) remediation in the past. In contrast to conventional MFC, in SMFC the organic matter contained in the sediment acts as the electron donor rather than acetate (Huang et al., 2010) or lactate (Xafenias et al., 2013), resulting in lower operation costs. We demonstrated that Cr(VI) removal occurred more slowly in the open-circuit Cr-SMFC than in the closed-circuit Cr-SMFC (Figure 4A and B), suggesting that the production of electricity is an efficient Cr(VI) reduction pathway (Xafenias et al., 2015). Some of the Cr(VI) may be reduced to Cr(III) by acting as an electron acceptor in SMFC, as in previous MFC studies (Huang et al., 2010). Furthermore, soluble Cr(III) was undetectable in the catholyte, and the resultant Cr(III) was present as precipitated Cr(OH)₃, which was adsorbed/or migrated from the cathode to the sediment in the present study (Figure 4C) (Huang et al., 2011). In this way, Cr(VI) is detoxified and stabilized in the sediment with minimum transfer to the water, hence our investigation of the use of SMFCs as a remediation technique for treating Cr(VI)-contaminated lakes.

3.3 Long-term Operation of the Cr-SMFC

The Cr-SMFC was operated in 4 batches over 48 days at room temperature ($20 \pm 5^\circ\text{C}$). We detected a voltage increase immediately after the addition of Cr(VI), and the voltage decreased gradually in each batch as the Cr(VI) was depleted. The maximum voltage of each successive batch decreased slightly. This clearly demonstrates that large variations in voltage generation were dependent on the presence of Cr(VI) (Figure 5A). Cr(VI) removal reached 100% approximately every 12 days (Figure 5B).

Long-term MFCs have been investigated for electricity production and sludge and wastewater treatment (Ge et al., 2013; Zhang et al., 2015). An MFC has been operated for more than 110 days to produce electricity using dairy manure as a fuel (Zhang et al., 2015). Two 1.8-L MFCs have operated on sewage sludge for almost 500 days, and an MFC using primary sludge achieved better results than one using digested sludge (Ge et al., 2013). SMFCs are particularly

Table 2. Comparison of microbial fuel cell (MFC) studies in Cr(VI) remediation.

Anode inoculum	Cathode	Initial concentration	Cr(VI) reduction	Electron donor	Cathodic pH	References
Anaerobic microorganisms	Cr(VI)-containing wastewater	100 mg/l	100% over 150 h	Acetate	2	(Wang et al., 2008)
Anaerobic sludge	Cr(VI)	204 mg/l	99.5% over 25 h	Acetate	2	(Li et al., 2008)
<i>Shewanella oneidensis</i> MR-1	Cr(VI)	10 mg/l	100% over 3 days	Lactate	8	(Xafenias et al., 2013)
Anaerobic sludge	Cr(VI)	9.5 mg/l	90% over 5 days	Acetate	8.7 – 9.3	(Habibul et al., 2016)
Cr(VI)-contaminated soil	Biocathode+ Cr(VI)	39.2 mg/l	100% over 7 h	Acetate	-	(Huang et al., 2010)
Mixed anaerobic culture	Biocathode+ Cr(VI)	20-70 mg/l	100% over 7 days	Acetate	7	(Tandukar et al., 2009)
Sediments	River water +Cr(VI)	25 mg/l	100% over 14 days	Organic of sediments	6.5	-

suitable for long-term operation. Sediments accumulate significant biomass as well as organic contaminants, and are considered an energy reservoir (Xu et al., 2015). A 100-L SMFC has been in operation for over 2 years, and could theoretically continue to operate for up to 8.5 years without external electron donors (Yang et al., 2015). Compared with other MFCs, SMFCs are inexpensive because they do not require a proton exchange membrane (PEM), and are easy to operate over a range of temperatures (10-30°C) (Hong et al., 2009b). A particular advantage of SMFCs is that they enable the simultaneous *in situ* treatment of both the sediment and overlying water.

3.4 Analysis of the Microbial Communities of the Cr-SMFC Anode and Sediment

The electricity-generated biofilm was investigated by scanning electron microscopy. The anode surface was smooth and clean before the experiment commenced, but had a rough and porous surface after 47 days (Figure 6). MiSeq sequencing was used to determine the diversity of the SMFC microbial community. The basic sequencing parameters are shown in Table 3. The initial and Cr-SMFC sediments yielded 3,319 and 1,856 operational taxonomic units (OUTs), respectively. The Good's coverage estimator indicated that the library size was sufficient to cover 74-89% of the bacterial communities. The Shannon diversity index decreased from 10.282 in the raw sediment to 7.553 in the Cr-SMFC sediment. At the phylum level, Bacteroidetes accounted for 42.9% of the total composition of the Cr-SMFC biofilm, followed by Proteobacteria (33.6%), Chloroflexi (7.5%), Euryarchaeota (7.5%), Firmicutes (3.3%), and Spirochaetes (2.5%). In the raw sediment, Proteobacteria accounted for 47.9%, followed by Bacteroidetes (17.7%), Chloroflexi (9.4%), Firmicutes (8.2%), and Euryarchaeota (3.8%; Figure 7A). The abundance of Bacteroidetes bacteria increased 2.5 times during Cr-SMFC

treatment. In contrast, the abundance of Actinobacteria decreased after Cr-SMFC treatment (2.25 vs 0.81%). Archaea from the phylum Euryarchaeota were also highly enriched by Cr-SMFC treatment (7.5 vs 3.8%); they included members of the genera *Methanospirillum*, *Methanobacterium*, and *Methanosaeta*. Furthermore, 1.98-4.14% of the reads could not be classified in any phylum, suggesting that they represent novel, uncultured bacterial strains. Families enriched after Cr-SMFC treatment included Pseudomonadaceae (46.88 times), Moraxellaceae (6.92 times), and Flavobacteriaceae (5.05 times) (Figure 7B). *Flavobacterium* was the most predominant genus (0.31%) in the Cr-SMFC anode community (Figure 7C).

To date, more than 30 pure isolates have been reported in MFC research. However, mixed culture is better suited to practical applications such as wastewater treatment (Kim et al., 2007) and organic contaminant removal (Luo et al., 2015). The main phylum detected after Cr-SMFC treatment was Bacteroidetes, at a much higher percentage than in previous reports (42.9%, compared with 9.5 (Wang et al., 2015) and 1.7-6.9% (Lu et al., 2015)). Bacteroidetes specialize in degrading high-molecular weight organic matter (Thomas et al., 2011). Members of the phylum Proteobacteria constituted the second most abundant bacteria in this study, and are often enriched on MFC anodes used for current generation (Lu et al., 2015). Most exoelectrogens, such as *Geobacter* (Wang et al., 2015) and *Pseudomonas* (Jayapriya and Ramamurthy, 2012), belong to the phylum Proteobacteria. *Flavobacterium* (0.31%) was the dominant genus observed and contains many species that can degrade harmful organic matter. Five representative strains that can degrade pentachlorophenol were assigned to the genus *Flavobacterium*. A *Flavobacterium* sp. that can degrade diazinon and parathion has also been isolated (Sethunathan and Yoshida, 1973). Several common electrogenic bacteria, including *Geobacter* (Bond and Lovley, 2003), *Pseudomonas* (Jayapriya and Ramamurthy,

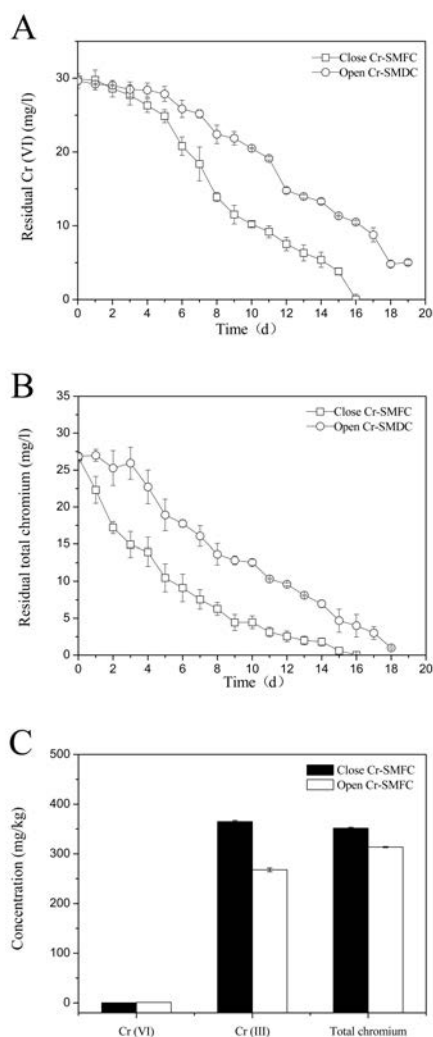


Figure 4. Cr(VI) removal using the sediment microbial fuel cells (SMFCs). (A) Cr(VI) removal in closed-circuit and open-circuit, Cr-SMFCs. (B) Total chromium removal in closed-circuit and open-circuit Cr-SMFCs. (C) Analysis of Cr(VI), Cr(III), and total chromium in the water-sediment surface soil.

2012), *Desulfobacter* (Zhao et al., 2008), and *Rhodoferrax* (Chaudhuri and Lovley, 2003), were also observed in our study (Figure 7C). Moreover, some electrochemically active bacteria are Cr(VI) resistant and have strong Cr(VI) reducing activity, such as *Geobacter metallireducens* (Bond et al., 2002; Chovanec et al., 2012); *Escherichia coli* (Liu et al., 2010; Zhang et al., 2006), and *Shewanella oneidensis* MR-1 (Wang et al., 2014; Xafenias et al., 2013). Therefore, the presence of Cr(VI) may alter the microbial community of the anode and enrich the dominant species.

4 Conclusion

In this study, we used Cr(VI) as a novel electron acceptor in an SMFC cathode. TOC removal by the Cr-SMFC was enhanced 2.19 times compared with an SMFC without Cr(VI).

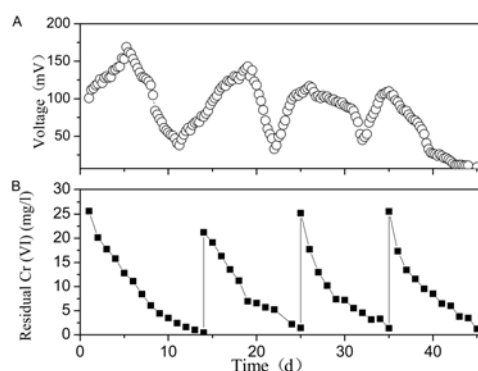


Figure 5. Long-term stability of the sediment microbial fuel cell (SMFC). (A) Voltage generation in an SMFC with a Cr(VI) concentration of 25 mg/L over 47 days. (B) Cr(VI) removal during 4 cycles of Cr-SMFC use.

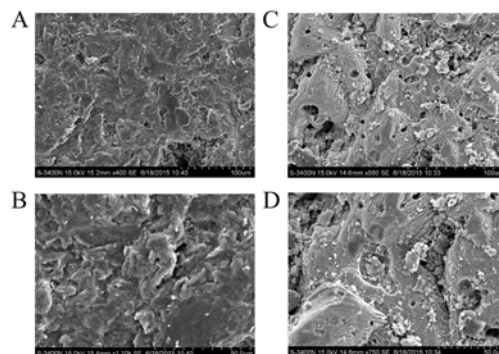


Figure 6. Scanning electron microscope images of anodes without microbes (A, B) and with microbes (C, D) at scales of 50 and 100 μm, respectively.

The maximum power density attained was 4.8 mW/m². During long-term operation, Cr(VI) removal reached 100% approximately every 12 days. Furthermore, the operation of the Cr-SMFC decreased microbial community diversity and enriched electrochemically active microorganisms in the anode, such as members of the phyla Bacteroidetes (42.9%), Proteobacteria (33.6%), and Chloroflexi (7.5%). Therefore, SMFCs have the potential to simultaneously remove Cr(VI) and bioremediate organic-rich sediment *in situ*.

Table 3. Diversity indices calculated based on a cutoff of 97% similarity of 16S rRNA sequences of 9001 reads per sample.

Sample ID	PD_whole_tree	Chao1	Coverage	OUT-num	Shannon
Sediment	248.0703	9720.899	0.745	3319	10.282
Cr-SMFC	138.533	5180.054	0.8623	1856	7.5537

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