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The remediation of chromium (VI)-contaminated soils using microbial fuel cells

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Abstract

Chromium (VI) is a priority pollutant in soil and water and poses serious threats to the environment. Microbial fuel cells (MFCs), as a sustainable technology, have been applied to treat heavy metal-contaminated wastewater. To study MFC application in soil remediation, the red clay soil and fluvo-aquic soil were spiked with Cr(VI) and packed into cathode chamber of MFCs, which were then operated at external resistances of 100 and 1000 Ω for 16 days, with open circuit condition as a control treatment. After the operation, the concentration of dissolved Cr(VI) in supernatant and total Cr(VI) in soil were obviously decreased. Soil type and external resistance significantly affected the current, removal efficiency of Cr(VI) and cathode efficiency.
Reducing external resistance improved the removal efficiency. The red soil generated a higher current of MFCs, but showed a lower removal efficiency and cathode efficiency than those of fluvo-aquic soil, implying that the red soil may contain more electron acceptors that competed with Cr(VI) reduction reaction. Our study demonstrated that MFC based technology has the potential to remediate Cr(VI)-contaminated soil and the efficiency varied between soil types and can be improved with high current.

**Keywords**

Chromium, External resistance, Microbial fuel cells, Remediation, Soil type
Introduction

Chromium (VI) is discharged into the environment through industry including the manufacture of stainless steel, welding, painting and pigment application, electroplating and other surface coating processes (Liu et al., 2011; Hemambika et al., 2013). Cr(VI) is considered as a priority pollutant due to its carcinogenic and mutagenic effects (Fonseca et al., 2012). Cr(VI) exerts heavy stress on soil microbial diversity, activity and biomass (Chrysochoou et al., 2013), and enters the food chain (Dhal et al. 2013). Therefore, appropriate remediation practices are necessary to remove Cr(VI) from soil. Techniques that have been frequently employed to remediate Cr(VI)-contaminated soils include flushing, land filling, solidification/stabilization, chemical reduction, bioremediation and electrokinetic remediation (Fonseca et al., 2012). Most of the physical and chemical approaches are generally costly and have the disadvantages of high energy input and secondary pollution. Although bioremediation is an economical and environmentally friendly strategy, it still has some limitations, e.g. plant uptake is a long time remediation (Olayinka and Ipeaiyeda, 2011), few microorganisms could survive high level of Cr(VI). When Cr(VI) concentration in soil exceeded 200 mg/kg, the ability of microorganisms to reduce Cr(VI) is significantly weakened (Jeyasingh and Philip, 2005).

As an reduction-oxidation system, microbial fuel cells (MFCs) are recently becoming popular in treating wastewater, including Cr(VI)-contaminated wastewater (Huang et al., 2011; Wang et al., 2008). Electrochemical active microbes degrade organic compounds and transfer...
electrons to anode. The electrons then flow through a conductor to the cathode where Cr(VI) acts as an acceptor and is reduced to Cr(III) (Wang et al., 2008; Huang et al., 2011). Therefore, the treatment process does not need energy input, rather it produces electrical power. In addition, the benefits of using MFCs involve many aspects: clean, safe, sustainability and ease in operating (Ghangrekar and Shinde, 2007).

To date, MFCs were mostly studied in wastewater treatment, whereas its feasibility in remediating heavy metal-contaminated soil has not been studied. Soil is a more complex medium compared with water. In soil, many variables can affect the behavior of metal ions and MFC operation. For example, soil pH and adsorption can affect the ion activity (Wang et al., 2008; Tao et al., 2011). Soil contains NO$_3^-$, Fe(III), SO$_4^{2-}$ and humic substances (Keller et al., 2009), which can act as electron acceptors or shuttles and then affect current generation. As a result, the remediation of Cr(VI)-contaminated soil using MFCs may depend largely on soil properties. In addition, changing external loading is a general method to adjust current and thus control the reaction rate on electrodes. However, whether the high current is connected with effective soil remediation is hardly known.

In the present study, we hypothesized that soil electron acceptors, soil pH, soil adsorption and current strength can affect the remediation efficiency. To testify the hypothesis, Cr(VI)-contaminated red clay soil and fluvo-aquic soil were remediated by using MFCs under different external loadings. The aim of the study was to evaluate 1) the feasibility of MFCs to
remediate Cr(VI)-contaminated soils, 2) the influence of soil type and external resistance on remediation.

Material and Methods

Soil Sampling

Two soil samples, a red clay soil and a fluvo-aquic soil, were collected in October 2012 at a depth of 0-20 cm of the farmland from Yingtan City (28°15′N, 116°55′E) and Xiamen City (24°27′N, 118°06′E), China, respectively. The soil was air-dried and sieved to 1 mm for the measurement of soil properties and Cr(VI) spiking experiment. The physical and chemical properties of the soil samples were analyzed according to Lu et al. (2000) and were shown in Table 1.

Preparation of Cr(VI)-contaminated Soils

Each soil matrix was prepared by adding the appropriate amount of dissolved potassium dichromate (K$_2$Cr$_2$O$_7$) solution to 1 kg of soil and agitating the mixture to give an initial concentration of 1000 mg/kg Cr. The moisture content of the soil samples was kept at 30% (w/w). Afterwards, each soil was respectively incubated in a 1 L beaker at room temperature in dark for 2 months. To keep the moisture content, a lid was loosely covered on each beaker and distilled water was added to compensate for the moisture loss every 2 days. After incubation, the Cr(VI)-contaminated soils were air-dried and sieved to 1 mm again, then stored for the
remediation experiments with MFCs. The total Cr and Cr(VI) in the red clay soil were 1009 and 255 mg/kg respectively, those in the fluvo-aquic soil were 1030 and 550 mg/kg, respectively. The difference of Cr(VI) content between the two soil types was because the red soil enhanced the reduction capacity of Cr(VI) to Cr(III) at low pH (Dhal et al., 2013).

*MFC Setup and Remediation Experiment*

Thirty-six dual chamber MFC reactors were constructed with plexiglass. Each MFC reactor comprised two same rectangular chambers (each chamber with a dimension of 5.0 × 5.0 × 5.0 cm and a working volume of 0.125 L). The two chambers airproofed with rubber plug were separated by a cation exchange membrane (QQ-YLM, Qianqiu Group, China). To serve as the electrodes, two porous carbon felts (each 4.0 × 3.5 cm, 8.5 mm thickness, Haoshi Carbon Fiber Co., China) were placed horizontally in the anode and cathode chambers, respectively. The anode chamber was filled with the medium (0.12 L, pH 7.0) which contained the following (per L): NaH$_2$PO$_4$$\cdot$2H$_2$O, 3.32 g; Na$_2$HPO$_4$$\cdot$12H$_2$O, 10.32 g; CH$_3$COONa, 2.0 g; NH$_4$Cl, 0.31 g; KCl, 0.13 g; MgSO$_4$$\cdot$7H$_2$O, 0.2 g; CaCl$_2$, 15 mg; MnSO$_4$$\cdot$H$_2$O, 20 mg and 12.5 mL of trace elements solution (Lovley and Phillips, 1988). The electrochemical bacteria inoculated in the anode chamber were from another MFC effluent (20%, v/v) that had 3 months of stable operation. In the start-up phase, the cathode chamber was filled with potassium ferricyanide [K$_3$Fe(CN)$_6$, 50 mmol/L] in phosphate buffer solution (0.12 L, pH 7.0) as electron acceptor.
During the start-up phase, the anode and cathode were connected with an external resistance of 1000 Ω. The voltage data over the external loading were recorded at an interval of 5 min using a data acquisition module (USB-7660, ZTIC Co., China). After 2 months of domestication, the voltage over the external resistance was stable (655 ± 2 mV). Then, the K₃Fe(CN)₆ solution in the cathode chamber was completely removed. The 36 MFCs were equally shared by four soil treatments with 9 MFCs for each soil treatment: 1) Cr(VI)-contaminated red soil; 2) non-contaminated red soil; 3) Cr(VI)-contaminated fluvo-aquic soil, and 4) non-contaminated fluvo-aquic soil. For each treatment, 40 g of soil (dry weight) was packed into a cathode chamber. 50 mL O₂-free distilled water which was purged with N₂ at a rate of 0.1 L/min for 2 hr was added to each cathode chamber to keep soil waterlogged. After clarification of the supernatant, the cathode was half immersed in the water layer and half buried in the soil. For each soil treatment, 3 MFCs were randomly selected to connect with 100 Ω external resistances, 3 MFCs with 1000 Ω external resistance and 3 MFCs were left at open circuit as control. Schematic of designed MFC treating Cr(VI)-contaminated soils was shown in Figure 1. All MFCs were operated at a constant temperature of 30°C. During 16-day remediation, 80 mL medium of anode chamber was replaced with fresh medium every 4 days. The internal resistance of MFCs was measured by electrochemical impedance spectroscopy using an AutoLab workstation (PGSTAT302N, Netherlands) (Xiao et al., 2013). The remediation experiment was conducted in July and August 2013.
**Cr(VI) Measurement**

The supernatant and soil in cathode chamber was separately collected by centrifugation at 5000 g for 15 min. Soil total Cr(VI) was extracted using an alkaline digestion (Na$_2$CO$_3$ and NaOH) based on USEPA method 3060A (James et al., 1995). The concentrations of Cr(VI) in the supernatant and digestion solution were determined using 1,5-diphenylcarbazide method according to 7196A of USEPA (Singh et al., 2012).

**Calculations and Statistical Analysis**

Current density $J$ (mA/m$^2$) was calculated by Eq. (1):

$$J = \frac{U}{RA}$$  \hspace{1cm} (1)

where $U$ is the voltage (mV); $R$ is the external resistance (Ω); $A$ is the projected surface area of the anode (m$^2$).

Removal efficiency (RE, %) was calculated by Eq. (2):

$$RE = \frac{Cr_{OC} - Cr_{MFC}}{Cr_{OC}} \times 100\%$$  \hspace{1cm} (2)

where $Cr_{OC}$ is the Cr(VI) concentration at open circuit condition; $Cr_{MFC}$ is the Cr(VI) concentration after operation of a MFC with 100 Ω or 1000 Ω external resistance.

Cathodic efficiency (CE, %), as the ratio of electrons transferred to Cr(VI) and electrons flowing through a MFC, was calculated as follows (Xafenias et al., 2013):
where \( F \) is Faraday’s constant (96,485.3 Coulombs/mole electron); \( M \) is atomic weight of Cr (52 g/mol); \( n \) is the moles of electrons transferred per mole of Cr(VI) reduced to Cr(III) (3 moles/mol); \( m \) is soil quality (0.04 kg) or supernatant volume (0.05 L) in the cathode; \( C_1 \) is the Cr(VI) concentration in the soil (mg/kg) or supernatant (mg/L) at open circuit; \( C_2 \) is the Cr(VI) concentration in the soil (mg/kg) or supernatant (mg/L) after the operation of MFCs with 100 \( \Omega \) or 1000 \( \Omega \) external resistance.

The effects of soil type, external resistance and Cr(VI) addition on quantity of electrons were analyzed using three-way ANOVA. The effects of soil type and external resistance on Cr(VI) concentration and cathode efficiency were analyzed using two-way ANOVA. All statistical tests were performed using SPSS (version 18.0, USA). Differences were considered significant at \( P < 0.05 \) by using Duncan test.

**Results and Discussion**

**Electrical Properties in the Remediation Using MFCs**

In China, the emissions of Cr slag exceed 450 000 tons annually (Gao and Xia, 2011) and a total area of approximately 50 000 km\(^2\) is affected by Cr contaminant (Zheng et al., 2011). The Cr(VI)-contaminated areas are mainly located in the eastern, central and southern regions (Yang...
et al., 2013; Cheng et al., 2014). The red clay soil and the fluvo-aquic soil are two widespread and typical soil types in the Cr(VI)-contaminated area. Some traditional remediation techniques have been applied to remediate these Cr(VI)-contaminated soils (Fonseca et al., 2012). Unlike these approaches, the remediation process using MFCs does not need energy input, instead generates electric power.

In the present study, the current generated by MFCs of all treatments sharply decreased at the beginning, then maintained relatively stable during the following 12 days (Figure 2). The quantity of electrons was analyzed for the first 4 days and the following 12 days (from day 5 to day 16). The quantity of electrons in red soils were higher than those in fluvo-aquic soils under the same Cr(VI) addition and external resistance, and those of MFCs for the same soil type increased as external resistance decreased from 1000 Ω to 100 Ω (Figure 3). Besides, compared with the non-contaminated soils, Cr(VI) addition enhanced quantity of electrons at the same level of external resistance (Figure 3). The differences reached the significant level \( P < 0.05 \). The three-way ANOVA showed that soil type, external resistance and Cr(VI) addition significantly affect the quantity of electrons (Supplemental Table S1). The addition of Cr(VI) decreased internal resistance of MFCs, and the red soil showed lower internal resistance than that of fluvo-aquic soil (Table 2).

The present MFC system that remediated Cr(VI)-contaminated soils in the cathode chamber depends on generation of electrons from the degradation of organic carbon in anode chamber.
The results suggested that MFC is an economical and sustainable way for soil remediation. In this study, an artificial medium was used in the anode chamber as electron donor. To further improve the sustainability and reduce the cost, the medium can be replaced with other organic-rich material, such as food wastewater (Oh and Logan, 2005), swine wastewater (Min et al., 2005) or domestic wastewater (Fan et al., 2008).

**Soil Type Affect Remediation**

Considering that the current experienced two steps, drop step for the beginning 4 days and stabilization step for the following 12 days of MFC running (Figure 2), the soil samples were analyzed for the removal efficiency of Cr(VI) during the drop stage (1-4 days) and stabilization stage (5-16 days) to understand the remediation process in MFCs. At day 4 and day 16, soil total Cr(VI) concentrations in two soils exhibited the order: open circuit condition > with 1000 Ω > with 100 Ω (Figure 4). The dissolved Cr(VI) concentration in the supernatant showed a similar trend except that Cr(VI) concentration in the supernatant of the red soil did not obviously decrease in closed circuit MFCs during the first 4 days (Figure 4). Two-way ANOVA showed the significant effect of soil type on Cr(VI) concentrations in the supernatant and soil (Supplemental Table S2).

During the 16 days operation of MFCs, the red soil generated more electrons than fluvo-aquic soil (Figure 3), however, the removal efficiency of Cr(VI) and cathode reaction in
the fluvo-aquic soil were much higher than those in the red clay soil (Figure 4). Although the red soil is much acidic than fluvo-aquic soil, there seems to be several main factors leading to the lower removal efficiency of Cr(VI). Firstly, the red soil may contain more electron acceptors than fluvo-aquic soil, such as Fe(III). These acceptors competed for Cr(VI) reduction and weakened the remediation efficiency but enhanced the electricity generation. It can be also implied by our findings that without Cr(VI) addition, the red soil generated higher current than fluvo-aquic soil. In addition, the cathode potential of the red soil without Cr(VI) addition was around 480 mV, significantly higher than 360 mV of the fluvo-aquic soil (data not shown). The competition of electron acceptors with Cr(VI) reduction in the red soil could also be responsible for the undetected removal efficiency during the first 4 days. Secondly, the red soil contains more clay, which may block the migration of Cr(VI) to cathode surface through soil adsorption (Reddy et al., 1997), and inhibited Cr(VI) reduction in MFC since the reduction of heavy metals including Cr(VI) (Li et al., 2008), Cu(II) (Wang et al., 2010), Ag(I) (Choi and Cui, 2012), and Hg(II) (Wang et al., 2011) can occur on cathode surface.

Effect of External Resistance on Remediation efficiency

External resistance is considered as an important factor affecting MFC performance by controlling the electron flowing from the anode to the cathode, and further influencing the reduction rate of heavy metals on the cathode (Tao et al., 2011). When MFCs were applied in the wastewater treatment, lower external resistance resulted in higher removal rate of Cu(II) (Tao et
al., 2011), sulfide and vanadium (V) (Zhang et al., 2010), and faster decolorization of dye (Sun et al., 2009) and denitrification (Clauwaert et al., 2007). Similarly, our study also showed that external resistance significantly affected Cr(VI) concentrations in the remediation process (Supplemental Table S2), and lower external resistance improved remediation efficiency. After 16 days of operation, the removal efficiency of Cr(VI) in the fluvo-aquic soil reached 99.1% with 100 Ω and 64.3% with 1000 Ω, respectively, much higher than those in the red clay soil (62.7% with 100 Ω and 50.4% with 1000 Ω) (Figure 5). The removal efficiency of Cr(VI) in the supernatant of the fluvo-aquic soil was also higher than that of the red clay soil (Figure 5).

Cathode efficiency (CE) for fluvo-aquic soil was significantly higher than the red clay soil in both stages (1-4 days and 5-16 days) (Figure 6). External resistance has also significant effect on CE of soil total Cr(VI) and dissolved Cr(VI) in supernatant except that the external resistance has no effect on CE of soil total Cr(VI) during the first 4 days (Supplemental Table S3). These results suggested that regulating external resistance is a promising way to enhance remediation of Cr(VI)-contaminated soil in MFCs.

The results of this study clearly showed that MFCs based technique has the potential to remediate Cr(VI)-contaminated soil where bioremediation would be ineffective. Generally, the high Cr(VI) concentration (more than 200 mg/kg) in soils severely inhibits the metabolic activity of microbes (Jeyasingh and Philip, 2005), and the physiological process of plants (Oves et al., 2013), finally weakening bioremediation. For remediation with MFCs, there is no contact...
between microbes and Cr(VI) due to separation of ion exchange membrane. Therefore, MFCs based technique can be used to remediate soils contaminated by high Cr(VI) concentration.

Conclusions

This study provides an experimental evidence that MFCs can serve as a potential and sustainable approach to remediate Cr(VI)-contaminated soil. This remediation process does not need energy input. The remediation efficiency varies between soil types studied but can be improved by lowering the external resistance. Further study is needed to assess the reliability of the MFC based technique with different soil types and with different heavy metals. To make the remediation applicable, the MFC reactor should be expanded in scale while the cost should be reduced.

Acknowledgements

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References


Table 1 Physico-chemical properties of the red clay soil and fluvo-aquic soil

<table>
<thead>
<tr>
<th>Analysts</th>
<th>Red clay soil</th>
<th>Fluvo-aquic soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.80</td>
<td>7.04</td>
</tr>
<tr>
<td>Organic matter (g/kg)</td>
<td>8.70</td>
<td>14.25</td>
</tr>
<tr>
<td>Total nitrogen (g/kg)</td>
<td>0.31</td>
<td>1.89</td>
</tr>
<tr>
<td>Alkali-hydrolyzable nitrogen (mg/kg)</td>
<td>14.26</td>
<td>78.25</td>
</tr>
<tr>
<td>$\text{NH}_4^+$-N (mg/kg)</td>
<td>15.37</td>
<td>46.42</td>
</tr>
<tr>
<td>$\text{NO}_3^-$-N (mg/kg)</td>
<td>0.66</td>
<td>3.29</td>
</tr>
<tr>
<td>Total phosphorus (g/kg)</td>
<td>0.24</td>
<td>0.54</td>
</tr>
<tr>
<td>Available phosphorus (mg/kg)</td>
<td>0.56</td>
<td>1.98</td>
</tr>
<tr>
<td>Available Fe (mg/kg)</td>
<td>25.43</td>
<td>15.93</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>38.23</td>
<td>21.02</td>
</tr>
</tbody>
</table>
Table 2: Internal resistance (Ω, means ± SE, n = 3) of MFCs with external resistance of 100 Ω tested at day 4 and day 16 after MFCs operation.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Cr(VI) treatment</th>
<th>Day 4</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red soil</td>
<td>Without Cr(VI)</td>
<td>593.6±64.9</td>
<td>358.5±21.1</td>
</tr>
<tr>
<td></td>
<td>With Cr(VI)</td>
<td>441.5±51.1</td>
<td>313.1±20.7</td>
</tr>
<tr>
<td>Fluvo-aquic soil</td>
<td>Without Cr(VI)</td>
<td>873.4±48.5</td>
<td>662.6±69.4</td>
</tr>
<tr>
<td></td>
<td>With Cr(VI)</td>
<td>557.6±16.9</td>
<td>424.3±24.0</td>
</tr>
</tbody>
</table>
Figure 1 Schematic of designed MFC treating Cr(VI)-contaminated soils. The electrons produced by anaerobic bacteria in the anode were transferred via an external resistance to the cathode, where they reduced Cr(VI) to Cr(III) in the cathodes.
Figure 2 Current density generated from the red clay soils (0 and 255 mg/kg of Cr(VI)) and the fluvo-aquic soils (0 and 550 mg/kg of Cr(VI))
Figure 3 The quantity of electrons generated during the first 4 days and the following 12 days of operation from different soil types under 100 Ω or 1000 Ω external resistance and with or without Cr(VI) addition. Values are means ± SE (n = 3)
Figure 4 Cr(VI) concentrations in the soil and supernatant tested 4 days and 16 days after MFCs operation. Values are means ± SE (n = 3)
**Figure 5** Removal efficiency of Cr(VI) in the soil and supernatant during 16 days operation with different soil types under 100 Ω or 1000 Ω external resistance. Values are means ± SE (n = 3)
Figure 6 Cathodic efficiency during the first 4 days and the following 12 days of operation from different soil types under 100 Ω or 1000 Ω external resistance. Values are means ± SE (n = 3)