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## EDITED BY

Bor-Yann Chen,  
National Ilan University, Taiwan

## REVIEWED BY

Bin Bian,  
King Abdullah University of Science and  
Technology, Saudi Arabia  
Hossain M. Zayed,  
Jiangsu University, China

## \*CORRESPONDENCE

Qingliang Zhao,  
qlzhao@hit.edu.cn  
Yimin Zhu,  
ntp@dlmu.edu.cn

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# Cooperative cathodes for enhanced hexavalent chromium reduction and electricity generation in bioelectrochemical reactor with simultaneous sludge degradation

Hang Yu<sup>1</sup>, Yuhui Cao<sup>1</sup>, Qingliang Zhao<sup>2\*</sup>, Zhuyuan Liang<sup>2</sup> and Yimin Zhu<sup>1\*</sup>

<sup>1</sup>Collaborative Innovation Center for Vessel Pollution Monitoring and Control, College of Environmental Sciences and Engineering, Dalian Maritime University, Dalian, China, <sup>2</sup>State Key Laboratory of Urban Water Resources and Environments (SKLURE), School of Environment, Harbin Institute of Technology, Harbin, China

A novel bioelectrochemical reactor assembled with cooperative cathodes of chemical cathode and bio-cathode (BER<sub>CC</sub>) and excess sludge as the anodic substrate obtained continuous and effective Cr(VI) reduction. Cooperative cathodes in BER<sub>CC</sub> stimulated the growth of electrochemically active microorganisms such as *Geobacter* sp. and *Shewanella* sp. in the anodic biofilm and produced  $8.21 \pm 0.64$  mg C/(L·h) more electrons than the dual chemical cathodes in the bioelectrochemical reactor with dual chemical cathodes, which enhanced the electrons for electricity generation and Cr(VI) reduction by approximately 58.3% and  $56.1 \pm 5.6\%$ , respectively.

## KEYWORDS

bioelectrochemical reactor, hexavalent chromium, cooperative cathodes, excess sludge, electron flux, electrochemically active microorganisms

## 1 Introduction

The microbial electrochemical system (MES) is an emerging platform technology that combines electrochemical systems with microbial processes. In a typical MES with a dual chamber for Cr(VI) reduction, the initial high concentrations of Cr(VI) and H<sup>+</sup> can lead to a high Cr(VI) reduction rate at the early stages of the reaction process (Gupta et al., 2017). As the reaction progresses, the consumption of Cr(VI) and H<sup>+</sup> in the cathodic chamber results in a decline in the motivation force from the cathodic reaction, which reduces the activity of electrochemically active microorganisms (EAMs) in the anodic chamber and has a negative influence on electricity generation (Xafenias et al., 2014; Li et al., 2021; More and Gupta, 2021). Similar changes have shown that the MES faces a decline in Cr(VI) reduction efficiency after cycles of operation, which limits the continuous and effective Cr(VI) reduction in the MES (Huang et al., 2021; Song et al., 2016; An et al., 2014).

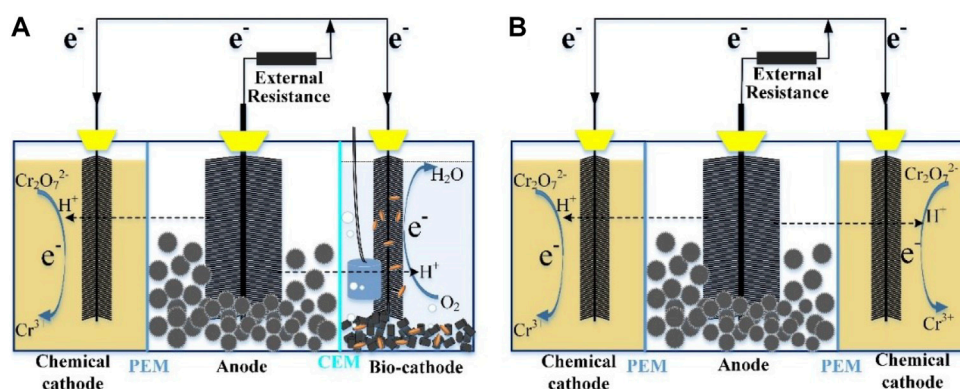


FIGURE 1  
Configurations of BER<sub>CC</sub> (A) and BER<sub>DC</sub> (B).

Using a high organic concentration of anodic substrate is one solution that supplies abundant electrons for continuous and effective Cr(VI) reduction in the MES. Sludge has been reported to supply sufficient electrons for stable and high-flow electron transfer in the MES (Jiang et al., 2009; Meng et al., 2019) and can thus create the condition of sufficient electron donors for stable and high-flow electron transfer. Maintaining high activity of EAMs is the other solution that supplies electrons for continuous and effective Cr(VI) reduction in the MES, which results in high performance of electricity generation. The continuous Cr(VI) reduction rate and electricity generation can be kept stable by the cooperative cathodes of the bio-cathode coupled to traditional dual-chamber MES, as has been shown in a previous study by Xie et al. (2011). In addition, bio-cathode could lower the cost of construction and operation of reactors (He and Angenent 2006; Rabaey and Keller 2008). Hence, coupling of the bio-cathode and chemical cathode can be regarded as the appropriate cooperative cathodes to breakthrough the limitations of continuous and effective Cr(VI) reduction in the MES, which has rarely been reported.

To obtain abundant electrons for continuous and effective Cr(VI) reduction in the MES, a novel BER assembled with cooperative cathodes of chemical cathode and bio-cathode was constructed, as shown in Figure 1A. The performances of the BER with cooperative cathodes (BER<sub>CC</sub>) and BER with dual chemical cathodes (BER<sub>DC</sub>) were compared to substantiate the feasibility of maintaining high efficiency by cooperative cathodes. Furthermore, the distribution of electron flux and the structure of the microbial community within BER<sub>DC</sub> and BER<sub>CC</sub> were analyzed. This study provides an alternative technology for the continuous and efficient remediation of Cr(VI)-contaminated wastewater and the treatment of excess sludge.

## 2 Materials and methods

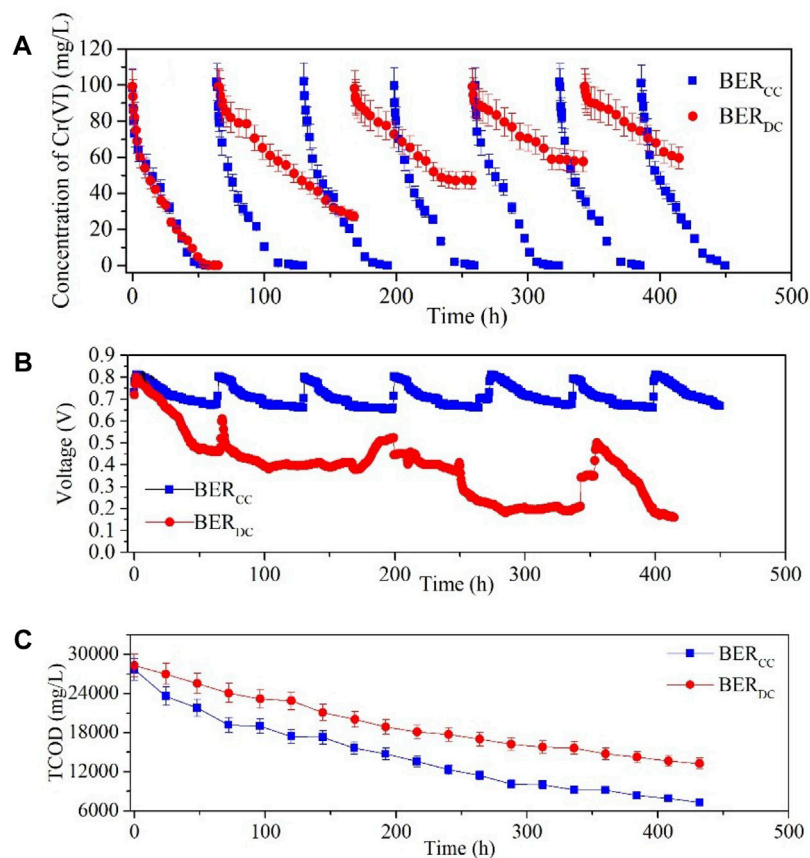
The bioelectrochemical reactor with cooperative cathodes (BER<sub>CC</sub>) consists of a cylindrical anodic chamber ( $\Phi$  80 mm  $\times$  90 mm) and two cubic cathodic chambers (60 mm  $\times$  70 mm  $\times$  90 mm each) (Figure 1A). A proton-exchange membrane (PEM) (Nafion<sup>TM</sup> 117, DuPont Co., United States) separated the anodic chamber from the chemical cathodic chamber to prevent the Cr<sup>3+</sup> produced in the chemical cathodic chamber from entering the anodic chamber, and the cation exchange membrane (CEM) (AMI-7001, Ultrex, United States) separated the anodic chamber from the bio-cathodic chamber for low cost. All of the anodes and cathodes were made of graphite fiber brushes ( $\Phi$  70 mm  $\times$  70 mm for the anode and  $\Phi$  60 mm  $\times$  70 mm for both the cathodes) and titanium wires. The BER with dual chemical cathodes (BER<sub>DC</sub>) possesses the same configuration as that of the BER<sub>CC</sub>, as shown in Figure 1B, which was applied as the control for the experiments. Both the chemical cathodic chambers were separated by the PEMs from the anodic chamber. The BER<sub>CC</sub> at the open circuit was used as the control to reflect the role of adsorption for Cr(VI) removal. The operation and analysis methods of this study are shown in the Supplementary Material.

## 3 Results and discussion

### 3.1 Performance of bioelectrochemical reactor with cooperative cathodes and bioelectrochemical reactor with dual chemical cathodes

#### 3.1.1 Performance of hexavalent chromium reduction

As shown in Figure 2A, Cr(VI) was almost completely reduced within 55.5 and 57.5 h in the BER<sub>CC</sub> and BER<sub>DC</sub>, respectively, during



**FIGURE 2**  
Variations in Cr(VI) concentration (A), voltage (B), and TCOD (C) during the operation of BER<sub>CC</sub> and BER<sub>DC</sub>.

the first Cr(VI) reduction cycle. After that, the Cr(VI) reduction rates of the BER<sub>DC</sub> gradually decreased, and it took 102.5 and 66.0 h for the BER<sub>DC</sub> to reduce Cr(VI) by  $72.5\% \pm 2.8\%$  and  $52.1\% \pm 1.8\%$ , respectively. The Cr(VI) reduction efficiency dropped to  $39.9\% \pm 1.3\%$  for the fifth cycle. Based on the abovementioned results, Cr(VI) could be entirely reduced for seven cycles in BER<sub>CC</sub>; however, it took much longer to reduce Cr(VI) in the BER<sub>DC</sub> than in the BER<sub>CC</sub> (Figure 2A). The variation in the Cr(VI) concentration in the BER<sub>CC</sub> at the open circuit revealed that adsorption was nonsignificant in the BER<sub>CC</sub> (Supplementary Figure S1). Similar results were also found by Li et al. (2018) that adsorption was nonsignificant in open-circuit microbial fuel cells. The reduction products (dark green precipitates) of Cr(VI) were found attached to the chemical cathode and sank to the bottom in the BER<sub>CC</sub> (Supplementary Figures S2A,B). Both the main constituent elements of the precipitates at the bottom of the cathodic chamber and those attached to the surface of the chemical cathode were Cr (Supplementary Figure S2; Supplementary Table S1). The main component of the precipitate in the cathodic chamber was chromium oxide (Supplementary Figure S3).

### 3.1.2 Performance of electricity generation and sludge degradation

During seven Cr(VI) reduction cycles, the voltage of BER<sub>CC</sub> was consistently above 0.672 V, and the maximum voltage reached approximately 0.813 V at the beginning of each cycle in Figure 2B. The BER<sub>DC</sub> produced a high voltage only at the beginning stage of the first cycle, and the voltage gradually decreased as the Cr(VI) reduction proceeded. During the second reduction cycle of the BER<sub>DC</sub>, the voltage first increased slightly compared to that at the end of the first cycle, but it gradually declined as Cr(VI) reduction proceeded. The tendency of voltage for the fifth cycle was similar to that for the previous cycles. After 432 h of operation, the total chemical oxygen demand (TCOD) of the sludge decreased by  $73.6\% \pm 4.4\%$  from  $27,692 \pm 1,662$  mg/L to  $7,307 \pm 438$  mg/L in the anodic chamber of the BER<sub>CC</sub> and by  $52.1\% \pm 3.1\%$  from  $27,692 \pm 1,662$  mg/L to  $13,267 \pm 796$  mg/L in the anodic chamber of the BER<sub>DC</sub>. Of the electrons produced in organic matter degradation, a great amount was used for electricity generation, and this process could accelerate the organic matter degradation of the sludge in bioelectrochemical system (BES) (Xafenias et al., 2014).

That the BER<sub>CC</sub> had a higher performance of electricity generation was the main reason for the TCOD degradation of the BER<sub>DC</sub> being higher than that of the BER<sub>CC</sub> (ref. Section 3.1.1). The current generation decreased gradually during the 200 h of operation in the microbial fuel cell (MFC) with the cathode chamber treating chromium wastewater (Kim et al., 2017). A dual-chamber MES was tested on the wastewater from an electroplating industry; the voltage increased to its maximum value after 48 h and reached its peak at 72 h, after which the voltage started to drop with time (More and Gupta, 2021). A salt bridge was used as a proton-exchange membrane of the MES to treat Cr(VI)-containing water, and the wave phenomena of the current was also found for 456 h in this system (Sophia and Sai, 2016). These results have confirmed that the configuration of the dual chamber is the bottleneck that limits the application of the MES in the treatment of industrial wastewater with chromium.

The voltage of the BER<sub>DC</sub> decreased with the operation, and the reaction of extracellular electron transfer at the anode slowed. The decrease in the performance of the MES was attributed to the reduction of the catalytic activity of the electrode (Xafenias et al., 2014). Under the same conditions, although the electrons that reacted at the chemical cathode decreased with the decrease in Cr(VI) concentration in the BER<sub>CC</sub>, the electrons that reacted at the bio-cathode increased with it. Therefore, the anodic reaction could be kept at a high rate, and the voltage could be kept at a high level. A certain number of rod-shaped EAMs existed on the surface of the anode, and the biofilm could be found on the bio-cathode (Supplementary Figures S1C,D). These indicate that the EAMs maintained high activity with the cooperative cathodes of the chemical cathode and bio-cathode.

### 3.2 Quantification of the electron fluxes

The analysis of the electron flux could reveal the advantages of cooperative cathodes in the production and utilization of electrons within the BER<sub>CC</sub>. Electron fluxes were distributed among different electron sinks (viz., current generation, exoelectrogen growth, hydrogen gas production, methanogen growth, and methane production) based on the electron balances and estimated (exoelectrogen and methanogen) growth rates in the BER as reported in a previous study (Yu et al., 2018). Most of the electrons for current generation were transferred along the anode to the cathode and participated in the cathodic reaction with a small amount of loss.

During two Cr(VI) reduction cycles of the BER<sub>DC</sub>, the average degradation rate of TCOD was  $18.42 \pm 4.03$  mg C/(L·h) [viz., mg Coulombs/(L·h)] (Supplementary Table S2), which contained  $7.23 \pm 1.94$  mg C/(L·h) for methane production,  $9.59 \pm 0.43$  mg C/(L·h) for biomass (exoelectrogen and methanogen) growth, portions for the average current production of 0.48 mA, and a Cr(VI) reduction rate of  $1.07 \pm 0.11$  mg C/(L·h). Of the electrons produced in the anodic chamber at a unit time as shown in

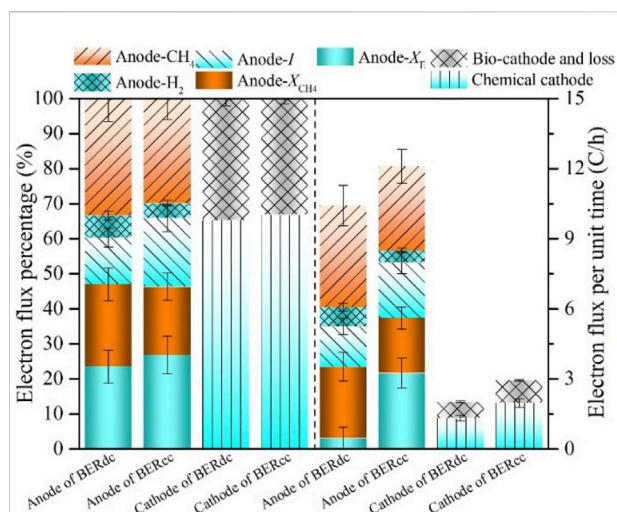
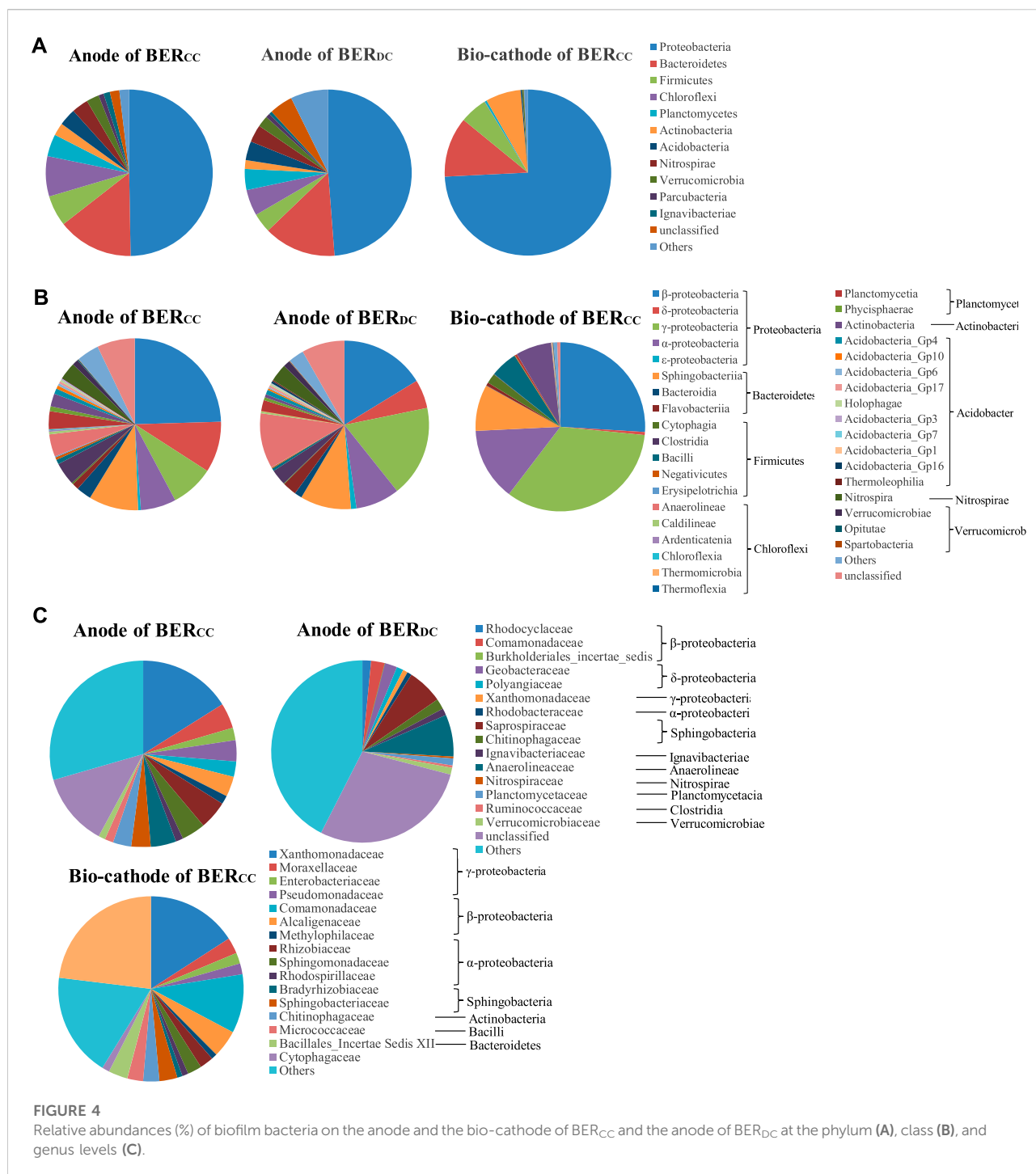


FIGURE 3

Distribution of electron fluxes among different electron sinks and the electron fluxes per unit time at the anode and cathode of BER<sub>DC</sub> and BER<sub>CC</sub> (Anode-CH<sub>4</sub>: estimated electrons used for methane production at the anode; Anode-H<sub>2</sub>: estimated electrons used for hydrogen production at the anode; Anode-I: electrons transferred from the anode to the cathode to generate current at the anode; Anode-X<sub>CH4</sub>: estimated electrons consumed for methanogen growth at the anode; Anode-X<sub>E</sub>: estimated electrons consumed for exoelectrogen growth at the anode; Bio-cathode and loss: estimated electrons transferred to the bio-cathode or loss in transfer; Chemical cathode: estimated electrons transferred to the chemical cathode).

Figure 3, the proportions of electrons for methanogen growth, hydrogen production, methane production, and current generation (transferred along the anode) were  $23.5\% \pm 2.7\%$ ,  $6.4\% \pm 0.8\%$ ,  $33.3\% \pm 3.5\%$ , and  $13.3\% \pm 1.7\%$ , respectively. A total of  $77.0\% \pm 6.9\%$  of the electrons that were transferred along the anode moved to the chemical cathodes for Cr(VI) reduction. By contrast, the average degradation rate of TCOD was  $8.21 \pm 0.64$  mg C/(L·h) higher in the BER<sub>CC</sub> than the BER<sub>DC</sub>, and the electrons for the average current were increased by 0.28 mA (Supplementary Table S2). These results are consistent with those mentioned in Sections 3.1.2.

For each cycle in the BER<sub>CC</sub>, the Cr(VI) concentration gradually decreased with the reduction of Cr(VI) by the chemical cathode, and the electrons for the chemical cathode were therefore reduced and the excess electrons transferred from the anode were used for bio-cathode. Cooperative cathodes supported the high activity of EAMs (Supplementary Figure S1) instead of gradual reduction, therefore Cr(VI) reduction could be continuously and efficiently maintained (Figure 2) at the chemical cathode for the next cycle. During two operation cycles, quantification of the electron fluxes revealed that  $8.21 \pm 4.03$  mg C/(L·h) more electrons were produced from organic matter degradation in the BER<sub>CC</sub> than the BER<sub>DC</sub>.



Of them, the electrons for Cr(VI) reduction increased by  $56.1\% \pm 5.6\%$ , and some electrons supported the current generation increase of 0.28 mA. The electrons for the chemical cathodic reaction and bio-cathodic reaction (and loss) were increased by  $51.5\% \pm 4.6\%$  and  $41.1\% \pm 3.7\%$ , respectively, in the BER<sub>CC</sub> as compared to the BER<sub>DC</sub> (Figure 3).

### 3.3 Distribution of microbial community

Microbial community analysis was used to further explore why more electrons were generated by sludge in the anodic chamber and utilized for electricity generation and Cr(VI) reduction within the BER<sub>CC</sub> than in the BER<sub>DC</sub> (Figure 4A). The proportions of Deltaproteobacteria, Clostridia, and Anaerolineae in the biofilms on

the anodes of the BER<sub>CC</sub> and BER<sub>DC</sub> were, respectively, 9.5–17, 38–54, and 85–212 times those on the bio-cathode of the BER<sub>CC</sub> which reflects the selective distribution of the EAMs on the anode (Figure 4B). Deltaproteobacteria were abundant in the anodic biofilm of the MES with high electricity generation (Bond et al., 2002; Lee et al., 2003), indicating that bacteria in relation to iron reduction and electricity generation might be present. Some bacteria belonging to *Clostridia* with bioelectrochemical activity and characteristics of Fe(III) reduction were isolated from the MFCs (Park et al., 2003). Geobacteraceae, which are well-known EAMs, can transfer electrons to the anode directly. *Xanthomonas* of the class Gammaproteobacteria is the EAM that is found in the MFCs under different operation conditions (Yu et al., 2015). The relative abundances of Comamonadaceae, Geobacteraceae, and Xanthomonadaceae were higher in the anodic biofilm of the BER<sub>CC</sub> than that of the BER<sub>DC</sub>, which implies that BER<sub>CC</sub> has a higher performance of electricity generation (Figure 4C). More effective cooperative cathodes than dual chemical cathodes have stimulated the growth of EAMs (Supplementary Table S3) and produced more electrons (ref. Section 3.2), which enhanced the performance of Cr(VI) reduction and electricity generation in the BER<sub>CC</sub> (ref. Section 3.1).

## 4 Conclusion

A novel BER with cooperative cathodes of a chemical cathode and bio-cathode (BER<sub>CC</sub>) for continuous and effective Cr(VI) reduction and electricity generation was realized. Cr(VI) could be fully reduced, and the voltage was consistently above 0.672 V for at least seven cycles in the BER<sub>CC</sub>. The TCOD of sludge decreased by 73.6% ± 4.4% after at least seven cycles. The quantification of the electron fluxes have revealed that 8.21 ± 4.03 mg C/(L·h) more electrons were produced from sludge degradation and 47.9% ± 4.3% more electrons participated in cathodic reactions in the BER<sub>CC</sub> than in the BER<sub>DC</sub>. More EAMs were found in the anodic biofilm of the BER<sub>CC</sub> than of the BER<sub>DC</sub>, of which *Geobacter* was the most abundant. This study provides an alternative technology for the continuous and efficient remediation of Cr(VI)-contaminated wastewater and the treatment of excess sludge.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/bioproject/>, PRJNA836022.

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## Author contributions

QZ and YZ participated in the conceptualization, supervision, and writing—reviewing and editing. HY participated in the conceptualization, methodology, software, and writing—original draft preparation. YC participated in the visualization and investigation. ZL participated in the data curation, methodology, and software.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.932266/full#supplementary-material>

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