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# Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets

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**Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets**

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1 **Enhanced sulfide removal and bioelectricity generation in microbial**  
2 **fuel cells with anodes modified by vertically oriented nanosheets**

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15 **Abstract**

16 Anode materials and structures are of critical importance for microbial fuel cells  
17 (MFCs) recovering energy from toxic substrates. Carbon-fiber-felt anodes modified  
18 by layers of vertically oriented TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> nanosheets respectively were applied  
19 in present study. Enhanced sulfide removal efficiencies (both over 90%) were  
20 obtained after 48 h operation, with maximum power densities improved by 1.53 and  
21 1.36 folds compared with MFCs with raw carbon-fiber-felt anode, respectively. The

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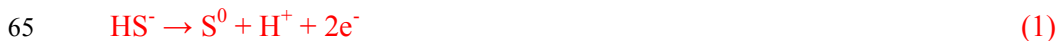
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4 22 modified anodes provided more active sites for microbial adhesion with increasing  
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6 23 biomass densities. High-throughput 16S rRNA gene sequencing analysis also  
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8 24 indicated the increase of microbial diversities. Bacteroidetes responsible for  
9  
10 25 bioelectricity generation with *Thiobacillus* and *Spirochaeta* dominating sulfide  
11  
12 26 removal were found in the MFCs with the modified anodes, with less anaerobic  
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14 27 fermentative bacteria as Firmicutes appeared. This indicates that the proposed  
15  
16 28 materials are competitive for applications of MFCs generating bioelectricity from  
17  
18 29 toxic sulfide.

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24 30 **Keywords:** Sulfide; Nano-sheets; Microbial fuel cells; Microbial community;  
25  
26 31 Carbon-fiber-felt

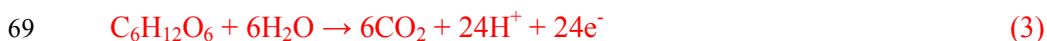
## 27 28 29 32 **1. Introduction**

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32 33 Sulfide is a hazardous, corrosive and odorous substance that often occurs in  
33  
34 34 industrial wastewaters, especially in effluent from viscose rayon industries,  
35  
36 35 petrochemical plants and tanneries [1,2]. Sulfide is toxic to human health, with studies  
37  
38 36 showing that sulfide is particularly harmful to cytochrome c oxidase and causes cell  
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40 37 hypoxia [3]. Thus, sulfide-contaminated wastewater must be treated thoroughly  
41  
42 38 before discharge into the environment. Although common physical-chemical methods,  
43  
44 39 such as adsorption and chemical oxidation, can remove sulfide, they are costly to  
45  
46 40 implement and require high energy inputs [4]. In contrast, biological processes  
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48 41 provide an environmental-friendly alternative for sulfide removal from both liquids  
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50 42 and gases under ambient environmental conditions [5-7].  
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4 43 Microbial fuel cells (MFCs) are nowadays recognized to be renewable, clean  
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6 44 energy sources that can generate bioelectricity during wastewater treatment [8,9].  
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8 45 Numerous pollutants have been successfully handled with energy recovery based on  
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10 46 MFC technology, including organics and metals [10-12]. Additionally, anode  
11  
12 47 materials and structures are particularly important regarding the performance of  
13  
14 48 MFCs in removal of toxic substances: properly designed anodes can help electricigens  
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16 49 to collect electrons from anolyte and thus sustain bioelectricity generation while  
17  
18 50 tolerating harmful substrates [13]. Carbon-based anodes are most frequently used [14]  
19  
20 51 and further gains in performance arise from coating them with conducting polymers  
21  
22 52 or metal oxides [15,16]. Nowadays, hydrothermally synthesized layers of vertically  
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24 53 oriented metal oxides nanosheets such as  $\text{TiO}_2$  and  $\text{Fe}_2\text{O}_3$  with activated carbon (AC)  
25  
26 54 powder in situ on the surface of carbon paper exhibit excellent behavior as MFCs'  
27  
28 55 anodes as they have high biocompatibility, support mass transport, have large surface  
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30 56 area for adhesion of bacteria, and provide direct pathways for electrons movement to  
31  
32 57 the electrode [17,18]. Furthermore, carbon fiber felt is more promising as anodic  
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34 58 material for scale-up of MFCs because it offers a more suitable support for bacteria  
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36 59 attachment and growth, higher mechanical strength, more active sites for the chemical  
37  
38 60 reactions [19-21], while its performances after being modified by layers of vertically  
39  
40 61 oriented metal oxides nanosheets are rarely reported. **Studies have been carried out on**  
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42 62 **sulfide removals using MFCs [4,22,23]. In anode chambers, sulfide acts as electron**  
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44 63 **donor and it is biochemically oxidized based on Equ. (1) and (2), with anodic**  
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46 64 **electrodes as electron acceptors:**  
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67 Commonly, co-substrate such as glucose is supplied as co-existing electron donor  
68 [4], which is oxidized as Equ. (3),



70 Moreover, other contaminants with higher redox potentials such as nitrate may be  
71 presented with sulfide [22]. It can also act as competitive electron acceptor, which is  
72 reduced as Equ. (4),



74 Then electrons flow to cathode via external circuits. In cathode chambers, oxygen  
75 and potassium ferricyanide are most frequently used as electron acceptors and they  
76 are reduced as Equ. (5) and (6) [23,24],



79 While most of these studies focus on co-existing pollutants removals [4,22,24-26],  
80 operating factors investigations [27] and commercial electrodes comparisons [28].

81 Little attention has been paid to date on modified carbon-based anode especially for  
82 carbon fiber felt to enhance bioelectricity generation and sulfide removal in the  
83 context of accumulation of microbes in anode chambers with toxic matrix.

84 The present research explores carbon-fiber-felt anodes modified with two kinds of  
85 metal oxides ( $\text{TiO}_2$  and  $\text{Fe}_2\text{O}_3$ ) nanosheets respectively that are vertically oriented on  
86 the surface. Enhanced performances in aspects of power outputs and sulfide removals

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4 87 are investigated for MFCs equipped with these anodes, compared with unmodified  
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6 88 one. The amounts and species of accumulated microbes are also analyzed. This work  
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8 89 provides a potential alternative for treating sulfide-contaminated wastewater by MFC  
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10  
11 90 technology with proposed anodes.

## 91 **2. Materials and methods**

### 92 **2.1 Preparation of anodes and fabrication of MFCs**

93 TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> sols were synthesized in the laboratory following a previous report  
94 [17,18]. Clean carbon-fiber-felt materials (3 mm thickness, 4 cm length and width,  
95 provided by Beijing Evergrow Resources CO., LTD, China) were immersed in the  
96 TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> sols for 10 min and dried at 80 °C. After that, the materials were  
97 calcined for 30 min in a tubular furnace at 350 °C so that a TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> seed  
98 layers formed on the surfaces of the carbon-fiber felts. A Teflon-lined stainless steel  
99 autoclave (50 mL in volume) filled with 40 mL of aqueous solution of 10 M NaOH  
100 and 0.2 g of AC powder was placed in an oven at 180 °C for 24 h. After the  
101 carbon-fiber-felts had cooled down to room temperature, the modified carbon-fiber  
102 felts were rinsed with ultrapure water to remove AC, followed by soaking with 0.1 M  
103 hydrochloric acid for 1 h, then washed to neutral with deionized water and dried at  
104 80 °C. Sequentially, the samples were calcined at 550 °C for 1 h in a N<sub>2</sub> atmosphere  
105 [17,18].

106 Three H-type MFCs with cylindrical chambers (250 mL for each) were fabricated  
107 as previously described [29]. The two chambers for each MFC were divided by a

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4 108 proton exchange membrane (Nafion117#, Dupont, USA) with a surface area about 4  
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6 109  $\text{cm}^2$ . The MFCs were equipped with  $\text{TiO}_2$  nanosheets modified carbon-fiber-felt anode  
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8 110 (MFC- $\text{TiO}_2$ ) and  $\text{Fe}_2\text{O}_3$  nanosheets modified carbon-fiber-felt anode (MFC- $\text{Fe}_2\text{O}_3$ ),  
9  
10 111 respectively, as prepared above, with surface area of  $16 \text{ cm}^2$ . Raw carbon-fiber-felt  
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12 112 anode without modification was also employed as control (MFC-CF). All cathodes  
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14 113 were made of ordinary carbon-fiber-felt with the same size as anodes. The anode and  
15  
16 114 cathode were connected across a  $100 \Omega$  external resistor. Each anode chamber was  
17  
18 115 inoculated with 25 mL anaerobic granular sludge from an up-flow anaerobic sludge  
19  
20 116 blanket (UASB) reactor treating high strength sulfate wastewater. The anolyte  
21  
22 117 included the following (per L): 0.75 g of  $\text{C}_6\text{H}_{12}\text{O}_6$ ; 5.62 g of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ; 6.15 g  
23  
24 118 of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ; 0.31 g of  $\text{NH}_4\text{Cl}$ ; 0.13 g of  $\text{KCl}$ ; 1.25 mL of vitamin solution;  
25  
26 119 and 12.5 mL of trace mineral element solution. Sulfide was added to the anode  
27  
28 120 solution in the form of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  with concentration of  $60 \text{ mg L}^{-1}$  to facilitate the  
29  
30 121 comparison [30]. The catholyte included the following (Per L): 9 g of  $\text{KH}_2\text{PO}_4$ , 8 g of  
31  
32 122  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$  and 0.05 g  $\text{K}_3[\text{Fe}(\text{CN})_6]$ .

## 33 34 35 36 37 38 39 40 41 123 **2.2. Experimental procedures**

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44 124 Morphology and composition of the modified anodes were analyzed first. Then  
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46 125 successful start-up of all MFCs was realized by refreshing anolyte every 2 days. After  
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48 126 that, the power outputs and sulfide removals in a typical cycle (48 h) were evaluated,  
49  
50 127 noting that most of the sulfide was removed within that time. Electrochemical  
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52 128 measurements were also undertaken for the three types of MFC. After 3 months  
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4 129 operation with refreshment of analyte every 2 days, ultrasonic was employed to  
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6 130 collect the bacteria attached to the surfaces of the anodes, and the samples then used  
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8 131 for high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). All  
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10 132 experiments were carried out at room temperature ( $22 \pm 2$  °C) for practical application  
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13 133 consideration and easy comparison with existing studies [22]. For each condition,  
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16 134 trials were carried out in triplicate and average results from the three MFCs were  
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18 135 reported.

### 136 **2.3. Analytical methods and data representation**

137 Measurement of chemical oxygen demand (COD) was undertaken based on  
138 digestion with potassium dichromate in concentrated sulfuric acid for 2 h at 150 °C.  
139 Sulfide was determined according to the methylene blue method ( $n = 665$  nm) [31].  
140 The indication of “sulfide” described all species ( $H_2S$ ,  $HS^-$ , and  $S^{2-}$ ). Total organic  
141 carbon (TOC) was monitored by Multi N/C 3000 TOC analyzer (Analytik Jena AG,  
142 Germany). Sulfate was measured by ion chromatography (ICS-1600, Dionex, the  
143 USA). pH was measured using a pH-201 meter (Hanna, Italy). The biomass on the  
144 anode surface was determined using the phospholipid analysis as previously described  
145 and the biomass density was expressed as the mass of phosphorus normalized by  
146 anode volume ( $48$  cm<sup>3</sup>) [32]. The morphology and composition of modified anodes  
147 were analyzed by a JEOL JAX-840 scanning electron microscope (SEM) operating at  
148 20 kV with an energy dispersive X-ray (EDX).

149 Voltage measurements were taken using a voltmeter throughout the test. The

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4 150 polarization curve and power outputs were obtained by varying the external resistance  
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6 151 over the range from 10 to 5000  $\Omega$ . For each point on the polarization curve, readings  
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8 152 were taken when pseudo-steady-state conditions was established, which might take  
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11 153 several minutes or more [4,33,34]. MFCs operated at least twice under each resistance  
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13 154 to ensure the repeatability of power outputs. Power density was normalized to the  
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15 155 single-side projected surface area of the anode. Cyclic voltammetry (CV)  
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18 156 measurement was carried out at a scan rate of 2  $\text{mV s}^{-1}$  in the range of -1 V to +1 V  
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21 157 using an electrochemical workstation (VMP3, Bio-Logic Science Instruments, France)  
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23 158 with Ag/AgCl as reference electrode. Electrochemical impedance spectroscopy (EIS)  
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26 159 measurement was conducted over a frequency range of 100 kHz to 1 MHz with an AC  
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28 160 signal of 10 mV amplitude. All electrolyte solutions were deaerated by high-purity  
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31 161 nitrogen for at least 15 minutes prior to the measurement [32]. Coulombic efficiency  
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33 162 (CE) was calculated as reported previously [4].

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36 163 Molecular biology analysis was performed to obtain the characteristics of the  
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38 164 microbial population. Total genomic DNA was collected, pooled, and amplified  
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41 165 according to previous procedures [35]. Then a mixture of amplicons was used for  
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43 166 high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). Raw  
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46 167 sequencing data were deposited in the NCBI Sequence Read Archive with access  
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48 168 number of SPR067096 and were analyzed according to Hao et al. [35].  
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### 169 **3. Results and discussion**

#### 170 **3.1 Characteristics of the modified anodes**

171 It was observed that the modified carbon-fiber-felt surfaces were covered with  
172 dense layers, comprising vertically oriented nanosheets on top, which formed vertical  
173 pores through the layer, compared with raw carbon-fiber-felt anode (Fig. 1a, Fig. 1b,  
174 Fig. 1c). This structure improved the roughness of the anode surface, which was  
175 similar with previous studies [17,18] and meant that the 3D open porous structure was  
176 favorable to biofilm growth as well as permeability of electrolyte, substrates, and  
177 electron mediators [36]. These vertically oriented nanosheets extending upward from  
178 the surface of carbon-fiber-felts could provide direct pathways for electrons to transfer  
179 from exoelectrogens in the biofilm to the carbon-fiber-felts. Moreover,  
180 carbon-fiber-felts with larger specific surface areas were selected as the basic material  
181 in present study instead of plain carbon paper, which would provide more sites for  
182 microbes' attachment [17,18]. The compositions of the modified anodes were  
183 examined using EDX and the corresponding elements (Ti, Fe, O) were detected (Fig.  
184 1d), indicating that the anodes were well modified with metal oxides. It was  
185 interesting to note that some metal oxides could not only enhance the interfacial  
186 electron transfers in MFCs [17] but also stimulate the growth of chemoautotrophic  
187 and heterotrophic bacteria using solar energy [37]. This implied that the proposed  
188 modified anodes were promising and ready for following experiments.

### 189 3.2 Bioelectricity generation by the MFCs

190 Polarization curves were obtained using closed-circuit MFCs during 48 h operation  
191 (Fig. 2a). MFC-TiO<sub>2</sub> exhibited the highest maximum power density of  $607.8 \pm 16.1$   
192  $\text{mW m}^{-2}$  at current density of  $1591.38 \text{ mA m}^{-2}$  with  $150 \Omega$  external resistances,  
193 followed by MFC-Fe<sub>2</sub>O<sub>3</sub> with this value of  $537.6 \pm 14.8 \text{ mW m}^{-2}$  at current density of  
194  $1296.15 \text{ mA m}^{-2}$  with  $200 \Omega$  external resistances, 1.53 and 1.36 folds higher than the  
195 maximum power output of MFC-CF ( $396.1 \pm 11.7 \text{ mW m}^{-2}$  with  $400 \Omega$  external  
196 resistances), while the maximum power output of MFC-CF was slightly higher than  
197 that obtained from previous study, where maximum power output of  $283 \text{ mW m}^{-2}$  was  
198 obtained in dual chamber MFCs (300 mL net volume for anode chamber) with 300  
199  $\text{mg L}^{-1}$  initial sulfide concentration [25]. The results indicated that the modified  
200 anodes with the TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> nanosheets vertically oriented on the surface of  
201 carbon-fiber-felt could increase the power output of MFCs by providing more sites for  
202 microbes' attachment and more direct electrons transfer pathways [17,18].

203 Anode potentials which dominated the above difference were also monitored  
204 during this period (Fig. 2b). The anode potentials of the MFC-CF were lower than  
205 those obtained with sulfide free substrate due to lower redox potential of sulfide  
206 [38,39]. Additionally, the sulfide added to the anodic solution and the sulfate produced  
207 through sulfide oxidation acted as a soluble redox mediator, which could promote  
208 electron transfer from the bacterial cells to the anode surface [40]. The anode  
209 potentials of both MFC-TiO<sub>2</sub> and MFC-Fe<sub>2</sub>O<sub>3</sub> were further lower than those of  
210 MFC-CF. It should be noted that previous results had shown that the lower negative

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4 211 anode potential could demonstrate better activity of anode communities [41].  
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6 212 Moreover, metal oxides possess superior electronic, optical, and dielectric properties  
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8 213 and had been employed for surface modifications of anodes in MFCs for high power  
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10 214 output [36]. Hence, the present results indicated that carbon-fiber-felt with TiO<sub>2</sub> and  
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12 215 Fe<sub>2</sub>O<sub>3</sub> nanosheets vertically oriented on the surface as anode materials could facilitate  
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14 216 adhesion, growth and activity of bacteria for enhancement of MFC performance.  
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18 217 The electrochemical behaviors of the modified anodes were also characterized.  
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20 218 More obvious redox peaks and much higher redox peak currents than untreated  
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22 219 carbon-fiber-felt anode were observed in CV curves, especially with TiO<sub>2</sub> nanosheets  
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24 220 (Fig. 3a). This indicated that electrodes with metal oxides nanosheets vertically  
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26 221 oriented on the surface of carbon-fiber-felt had better electron transfer properties than  
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28 222 an untreated electrode [42]. EIS was used to characterize the electrode surface and to  
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30 223 evaluate the kinetics of the electrochemical reaction and dramatically decrease of  
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32 224 internal resistances for MFCs with modified anodes were observed in Fig. 3b. Charge  
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34 225 transfer resistances derived from the MFC-TiO<sub>2</sub> and MFC-Fe<sub>2</sub>O<sub>3</sub> were also  
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36 226 remarkably smaller than that of the untreated electrode, consistent with the CV  
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38 227 behavior and showing the advantage of the proposed modification in electron  
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40 228 transfers.  
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### 48 229 **3.3 Sulfide and organics removals in the MFCs**

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51 230 Concentrations of sulfide and generated sulfate as well as TOC were monitored  
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53 231 initially and after 48 h operation (Fig. 4). Both sulfide and TOC declined after the  
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55 232 operation, demonstrating the feasibility of MFC technology for the removal of sulfide  
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4 233 and organic carbon simultaneously. Similar as the power outputs, sulfide removal  
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6 234 efficiencies of MFC-TiO<sub>2</sub> (94.4 ± 1.5%) and MFC-Fe<sub>2</sub>O<sub>3</sub> (91.6 ± 1.7%) were higher  
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8 235 than that of MFC-CF (88.5 ± 1.9%), with the removal efficiency obtained from  
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10 236 MFC-CF comparable with results from dual chamber MFCs that 84.7% of 100 mg L<sup>-1</sup>  
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12 237 sulfide in the influent was removed within 72 h [4] and higher than removal efficiency  
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14 238 of 60% with 80 mg L<sup>-1</sup> initial sulfide concentration in 72 h operation [26].  
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16 239 Nevertheless, sulfide removal efficiencies obtained in this study were relatively lower  
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18 240 than those achieved by Cai et al. [43], where above 99% of added sulfide were  
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20 241 removed, as our used cathode electron acceptor (K<sub>3</sub>[Fe(CN)<sub>6</sub>]) possesses lower redox  
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22 242 potential than their employed KMnO<sub>4</sub> [24]. The TOC removal efficiencies also  
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24 243 exhibited the similar principles, with MFC-TiO<sub>2</sub> (56.9 ± 1.8%) and MFC-Fe<sub>2</sub>O<sub>3</sub> (55.2  
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26 244 ± 1.4%) higher than that of MFC-CF (31.7 ± 1.7%). The removal efficiencies of  
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28 245 sulfide were superior to the TOC indicated that sulfide and organics acted as  
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30 246 co-electron donors with competitive relationship and sulfide was easier to be oxidized  
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32 247 than organic matter because of its lower redox potential [44,45]. Moreover, the  
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34 248 calculated CE based on COD of the MFC-TiO<sub>2</sub> (13.2 ± 1.7%), MFC-Fe<sub>2</sub>O<sub>3</sub> (12.2 ±  
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36 249 1.6%) and MFC-CF (11.4 ± 1.8%) was comparable with similar systems as previously  
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38 250 reported [4]. This implied that carbon-fiber-felt with these two kinds of metal oxide  
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40 251 (TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub>) nanosheets vertically oriented on its surface as promising anode  
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42 252 material could enhance both sulfide and organics removals in MFCs.  
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52 253 Oxidation products of sulfide in the MFCs were investigated. After 48 h  
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54 254 operation, concentrations of generated sulfate in the exhausted anolyte were 3.26 ±  
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4 255  $0.97 \text{ mg L}^{-1}$  in MFC-TiO<sub>2</sub> and  $2.74 \pm 0.89 \text{ mg L}^{-1}$  in MFC-Fe<sub>2</sub>O<sub>3</sub>, respectively (Fig. 4),  
5  
6 256 with other soluble species (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, SO<sub>3</sub><sup>2-</sup>) undetected in all MFCs, suggesting that most  
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8 257 oxidation products were insoluble. In fact, many obvious solid particles covered on  
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10 258 the anode surfaces after operation (Figure S1, Supporting Information). The particles  
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12 259 were examined using EDX and it was found that the main component was elemental  
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14 260 sulfur (Fig. 1d), which was the expected oxidation product as it was a non-corrosive  
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16 261 solid and was easy to remove from aqueous solutions [4]. The present results  
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18 262 suggested that elemental sulfur was the main product for sulfide removal when the  
19  
20 263 electrochemical and biological oxidations were performed in MFCs, also proved by  
21  
22 264 previous studies [4,46]. Recovery and quantification of generated elemental sulfurs  
23  
24 265 were difficult as they mixed with biofilms. The relatively lower sulfate concentration  
25  
26 266 and higher peak of elemental sulfur indicated that the modified carbon-fiber-felt  
27  
28 267 electrodes were more amenable to biofilm growth, enabling more sulfur to be  
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35 268 generated.

### 38 269 **3.4 Identification of the involved microbes**

40  
41 270 The monitored voltage outputs and sulfide removals were relatively stable during  
42  
43 271 the whole experiment. After 3 months operation, it was found that the biomass  
44  
45 272 densities of the MFC-TiO<sub>2</sub> ( $26.7 \mu\text{g cm}^{-1}$ ) and MFC-Fe<sub>2</sub>O<sub>3</sub> ( $22.1 \mu\text{g cm}^{-1}$ ) were higher  
46  
47 273 than that of MFC-CF ( $16.2 \mu\text{g cm}^{-1}$ ), suggesting more microbes adhering on the  
48  
49 274 modified anodes (Figure S1, Supporting Information). Total numbers of operational  
50  
51 275 taxonomy units (OTUs) estimated by Chao and Ace estimators with infinite sampling  
52  
53 276 in MFC-TiO<sub>2</sub> and MFC-Fe<sub>2</sub>O<sub>3</sub> were much larger than that in MFC-CF (Table 1),  
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4 277 indicating that MFC-TiO<sub>2</sub> and MFC-Fe<sub>2</sub>O<sub>3</sub> possessed greater richness of microbial  
5  
6 278 diversity than MFC-CF. Both Simpson and Shannon diversity index provide not only  
7  
8 279 the simply species richness (i.e., the number of species present) but how the  
9  
10 280 abundance of each species is distributed (the evenness of the species) among all the  
11  
12 281 species in the community. The increase of Shannon index and decrease of Simpson  
13  
14 282 index of MFCs with modified anodes compared with raw one implied the bacterial  
15  
16 283 communities in MFC-TiO<sub>2</sub> and MFC-Fe<sub>2</sub>O<sub>3</sub> were more diverse than those in the  
17  
18 284 MFC-CF due to the stimulated growth bacteria with the added metal oxides [36].  
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23 285 Besides, visible light-excited photoelectrons from metal oxide could stimulate the  
24  
25 286 growth of chemoautotrophic and heterotrophic bacteria [37].  
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28 287 16S rRNA gene sequence and taxonomy analyses for the microbes in the three  
29  
30 288 MFCs were performed at phylum, class and genus levels in order to understand the  
31  
32 289 role of bacteria in enhanced sulfide oxidation and bioelectricity generation (Table 2).  
33  
34 290 Electrochemically activated bacteria that were conducive to bioelectricity generation  
35  
36 291 were enriched in the MFCs, especially with modified anodes. Bacteroidetes, the most  
37  
38 292 frequently appeared species in the anode biofilms of MFCs with electrochemical  
39  
40 293 activity as reported by Ha et al. [46] were enriched in MFC-TiO<sub>2</sub> (4.94%) and  
41  
42 294 MFC-Fe<sub>2</sub>O<sub>3</sub> (1.45%) than those in MFC-CF (1.22%). Moreover, with the  
43  
44 295 enhancement of MFCs' functions of electricity generation and sulfide removal, plenty  
45  
46 296 of bacteria with electrochemical activity were domesticated, such as the  
47  
48 297 Deltaproteobacteria species, especially in the MFCs with modified anode. This  
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50 298 implied that the design with TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> nanosheets vertically oriented on the  
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4 299 surface of carbon-fiber-felt could accumulate more electrochemically activated  
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6 300 bacteria to generate bioelectricity than the untreated anode.  
7  
8 301 Sulfur related bacteria responsible for sulfide oxidation and sulfate reduction were  
9  
10 302 also detected. *Thiobacillus* in Alphaproteobacteria, a famous sulfur-oxidizing  
11  
12 303 bacterium that can oxidize sulfur to sulfate was enriched in MFC-TiO<sub>2</sub> [43,47].  
13  
14 304 *Spirochaeta* of Spirochaetes requiring sulfide in the growth medium and oxidizing it  
15  
16 305 non-enzymically to elemental sulfur was found in MFC-Fe<sub>2</sub>O<sub>3</sub> [48]. Sulfate-reducing  
17  
18 306 bacteria were also greatly enhanced in the MFCs with the proposed anodes, such as  
19  
20 307 *Desulfovibrio* genus of Deltaproteobacteria, which could reduce sulfate as well as  
21  
22 308 other sulfur species such as sulfite and thiosulfate [4,23]. These species worked  
23  
24 309 together to realized higher sulfide removals in the improved MFCs.  
25  
26 310 *Pseudoxanthomonas* of Gammaproteobacteria with an abundance of 4.63% in  
27  
28 311 MFC-CF could be responsible for sulfide removals in this reactor as this species can  
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30 312 oxidize sulfide to sulfate [49]. More species of sulfur related bacteria were found in  
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32 313 the three MFCs than previously reported by Sun et al. [50] who employed sulfide as  
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34 314 the sole electron donor in the MFCs; the greater variety and numbers of bacteria  
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36 315 probably occurred due to the complex substrate (glucose and sulfide) employed in the  
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38 316 present study.

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40 317 Less anaerobic fermentative bacteria without electrochemical activity appeared in  
41  
42 318 the MFCs with modified anodes. Firmicutes accounted for the largest portion of  
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44 319 bacteria found in anaerobic sludge [51] decreased more greatly in MFC-TiO<sub>2</sub> and  
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46 320 MFC-Fe<sub>2</sub>O<sub>3</sub> than in MFC-CF. *Rhodobacter* sp. (belonging to Alphaproteobacteria)

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4 321 promoting anaerobic fermentation [52] also exhibited the similar principles. These  
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6 322 indicated that anaerobic fermentation process competing with bioelectricity  
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8 323 generation was further weakened when MFCs equipped with carbon-fiber-felt anodes  
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10 324 with TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> nanosheets vertically oriented on their surfaces.

#### 13 325 **4. Conclusions**

16 326 The surfaces of carbon-fiber-felt were successfully modified by layers of  
17  
18 327 vertically oriented TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> nanosheets respectively and acted as anodes in  
19  
20 328 MFCs. Higher maximum power outputs of MFC-TiO<sub>2</sub> (607.75 mW m<sup>-2</sup>) and  
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22 329 MFC-Fe<sub>2</sub>O<sub>3</sub> (537.63 mW m<sup>-2</sup>) were obtained, compared with the MFC with untreated  
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24 330 carbon-fiber-felt (396.05 mW m<sup>-2</sup>), and the sulfide and TOC removal efficiencies also  
25  
26 331 increased. These results could be contributed to more active sites for microbial  
27  
28 332 adhesion with increasing biomass densities by the modified anodes. Increases of  
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30 333 microbial diversities were also observed by high-throughput 16S rRNA gene  
31  
32 334 sequencing analysis and specific functional species were found, such as the enhanced  
33  
34 335 Bacteroidetes responsible for bioelectricity generation with *Thiobacillus* and  
35  
36 336 *Spirochaeta* dominating sulfide removal, with less anaerobic fermentative bacteria  
37  
38 337 Firmicutes.

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4 501 **Figure Captions.**  
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6 502 **Fig. 1.** SEM images and EDX spectral intensities. (a) with TiO<sub>2</sub> nanosheets, (b) with  
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8 Fe<sub>2</sub>O<sub>3</sub> nanosheets, (c) bare carbon-fiber-felt, (d) corresponding EDX spectral  
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10 intensities.  
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14 505 **Fig. 2.** (a) Polarization curves and power outputs as well as (b) anode potentials of  
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16 MFCs with three kinds of anodes.  
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19 507 **Fig. 3.** (a) CV and (b) Nyquist plot of EIS data for the anodes of three MFCs.  
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21 508 **Fig. 4.** Changes of sulfide and TOC during 48 h operation as well as the generated  
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23 sulfate in the anode chambers of three MFCs. The black legend referred to left vertical  
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25 axis while the red legend referred to the right vertical axis.  
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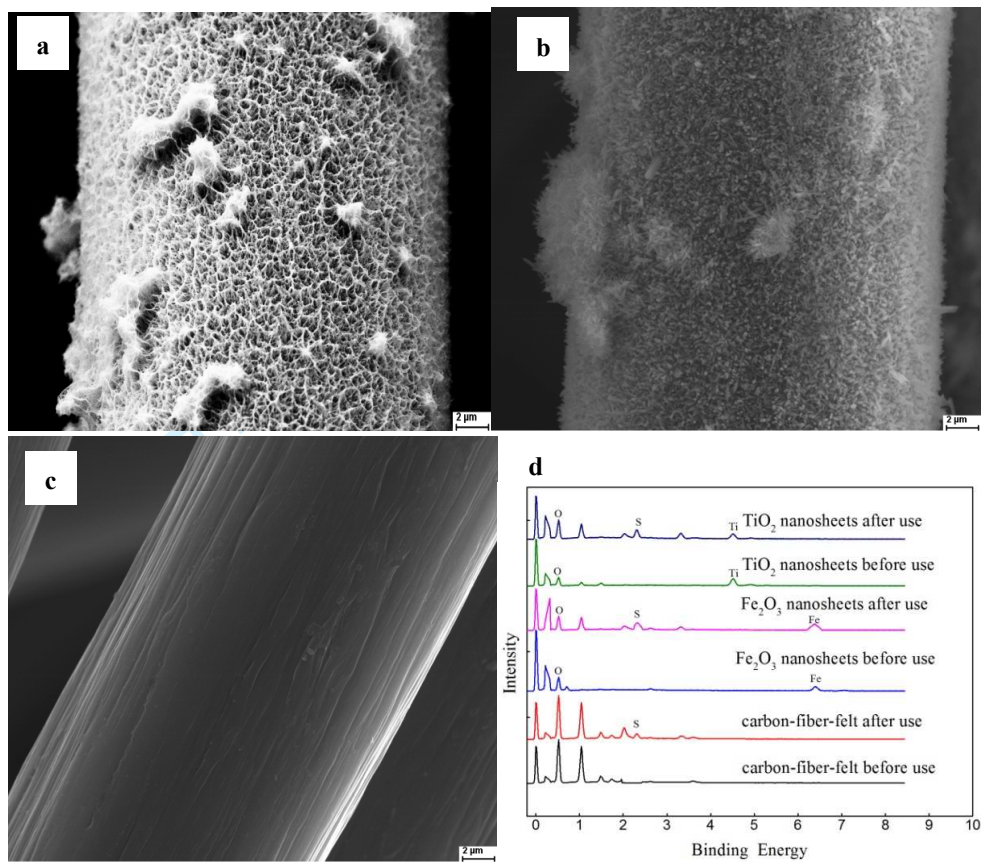
512 **Table 1.** Alpha-diversity of three MFCs employed in this study.

Type	Ace	Chao	Shannon	Simpson	Coverage
MFC-TiO <sub>2</sub>	768	760	4.33	0.031	0.996
MFC-Fe <sub>2</sub> O <sub>3</sub>	505	493	2.69	0.192	0.996
MFC-CF	394	375	2.45	0.240	0.995

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**Table 2.** Percentages of sequences identified to different phylogenies in the MFCs with three different anodes.

Phylum	Class	Genus	MFC-TiO <sub>2</sub> (%)	MFC-Fe <sub>2</sub> O <sub>3</sub> (%)	MFC-CF (%)	Phylum	Class	Genus	MF C-Ti O <sub>2</sub> (%)	MFC-Fe <sub>2</sub> O <sub>3</sub> (%)	MFC-CF (%)
Acidobacteria	Acidobacteria	norank	0.12	0.17	0.32	Nitrospirae	Nitrospira	norank	0.21	0.01	0.28
	Holophagae	norank	5.30	5.55	0.00			<i>Gemmata</i>	0.73	0.68	0.00
	Actinobacteria	uncultured	0.40	0.15	0.46			Planctomycetes	Planctomycetacia	<i>Pirellula</i>	0.96
Thermoleophilia	<i>Gaiella</i>	0.30	0.17	0.44	<i>Planctomyces</i>	1.81	1.75			0.00	
Armatimonadetes	norank	norank	0.07	0.08	0.07			uncultured	0.47	0.20	1.03
Bacteroidetes	Bacteroidia		4.41	1.03	0.71			norank	0.18	0.14	0.30
	Sphingobacteriia	norank	0.42	0.03	0.00	Alphaproteobacteria	<i>Rhodobacter</i>	0.04	0.02	14.8	
	Sphingobacteriia		0.00	0.22	0.48		<i>Delftia</i>	0.78	1.47	1.41	
vadinHA17	norank	0.11	0.17	0.03	<i>Thiobacillus</i>		1.21	0.01	0.00		
Candidate division BRC1	norank	norank	1.94	0.46	0.25	Proteobacteria		<i>Desulfovibrio</i>	12.97	6.86	4.30
Chloroflexi	Anaerolineae	<i>Leptolinea</i>	0.88	0.12	0.00		Deltaproteobacteria	norank	21.94	47.44	28.6
	Caldilineae	uncultured	0.98	0.43	0.42		Gammaproteobacteria	<i>Pseudoxanthomonas</i>	0.90	0.00	4.63
Firmicutes	Clostridia	<i>Anaerofustis</i>	0.01	0.00	0.17	Spirochaetae	Spirochaetes	<i>Spirochaeta</i>	0.10	0.05	0.11
		<i>Incertain_Sedis</i>	0.94	0.05	37.88	Synergistetes	Synergistia	uncultured	3.75	0.09	0.02
	Negativicutes	norank	37.26	31.73	0.03	Others			0.81	0.50	1.95

**Figure 1**

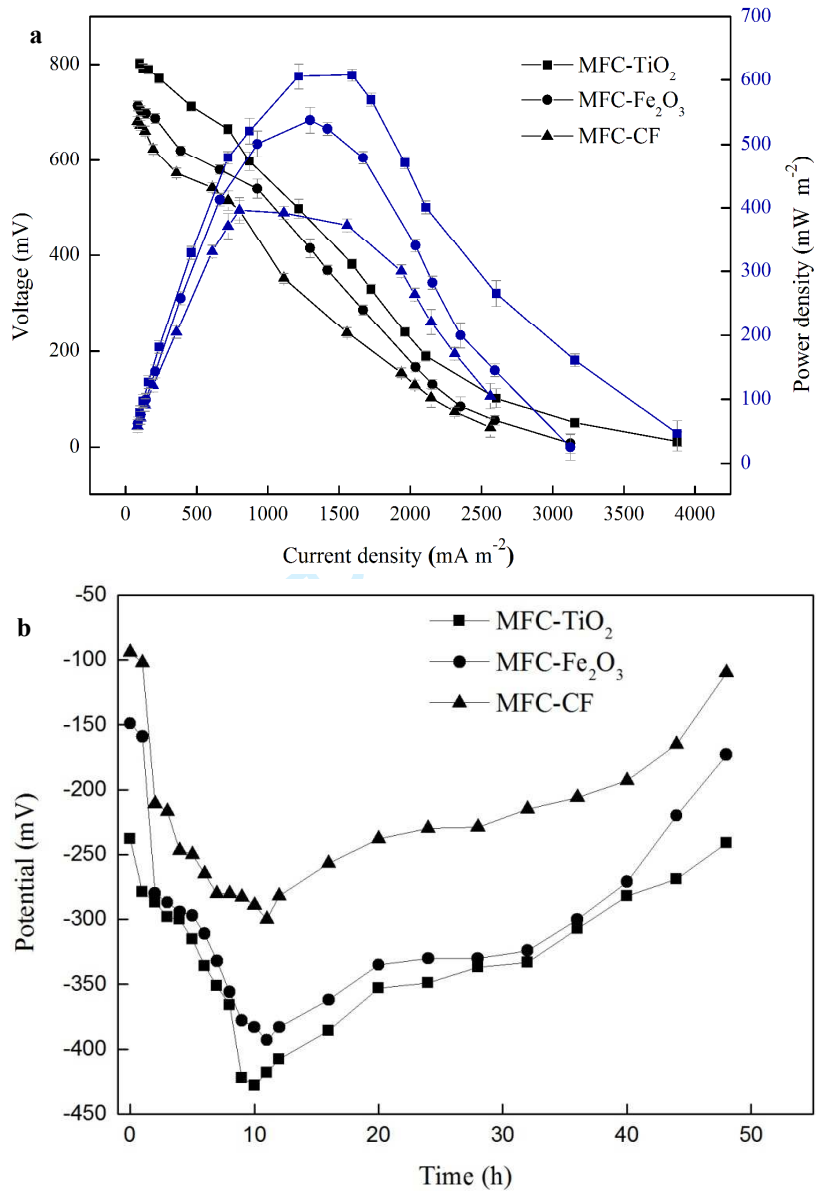
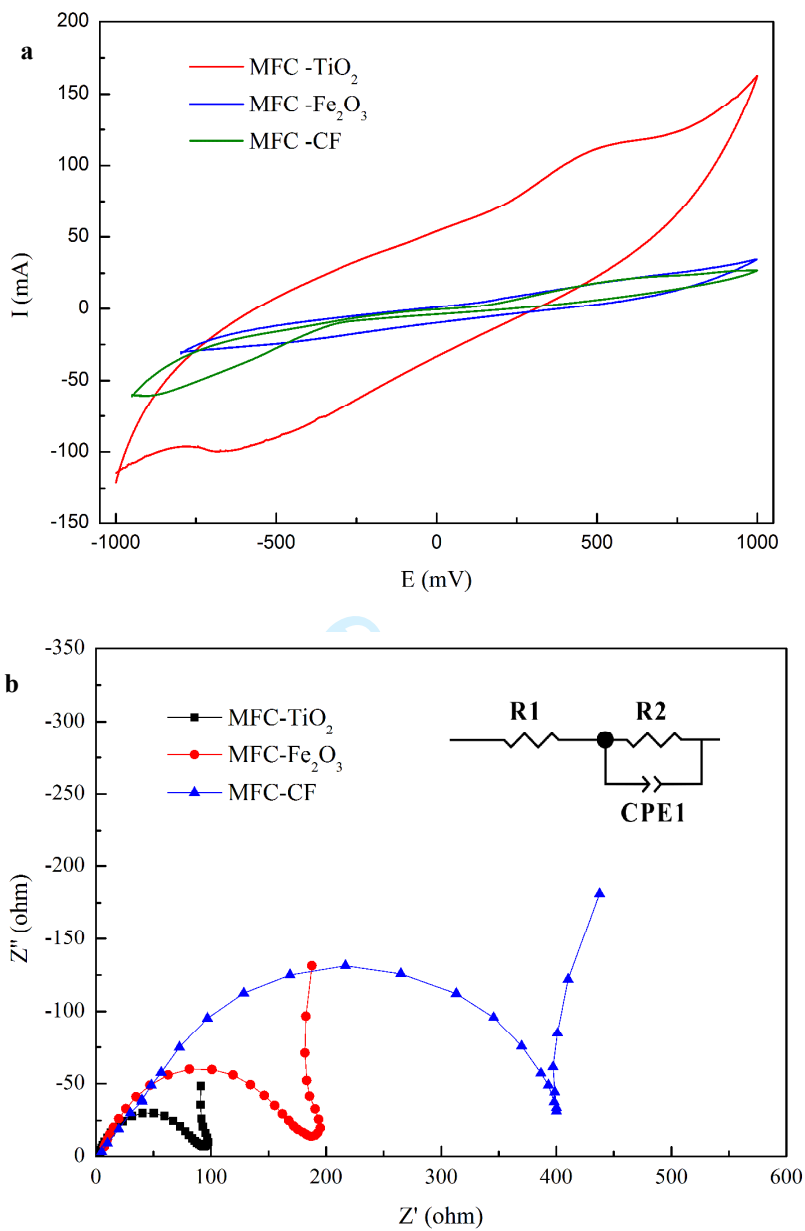
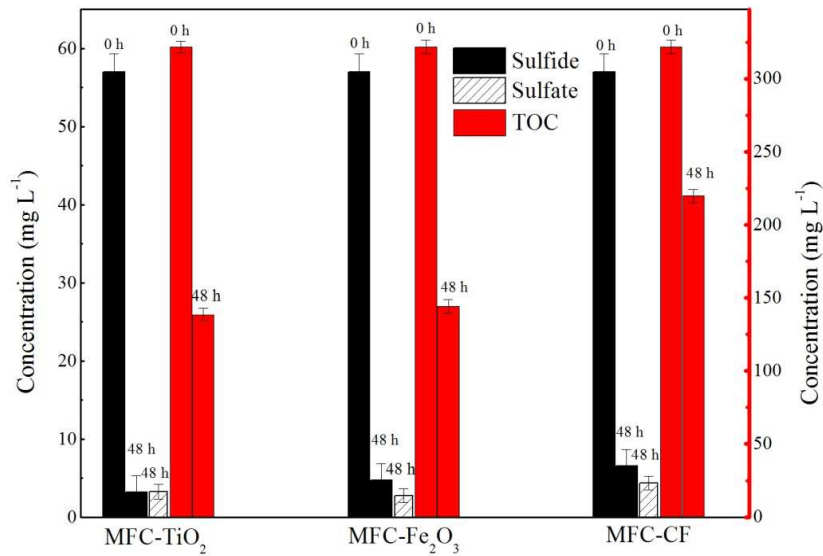


Figure 2



**Figure 3**



**Figure 4**

## Supporting Information

### **Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets**

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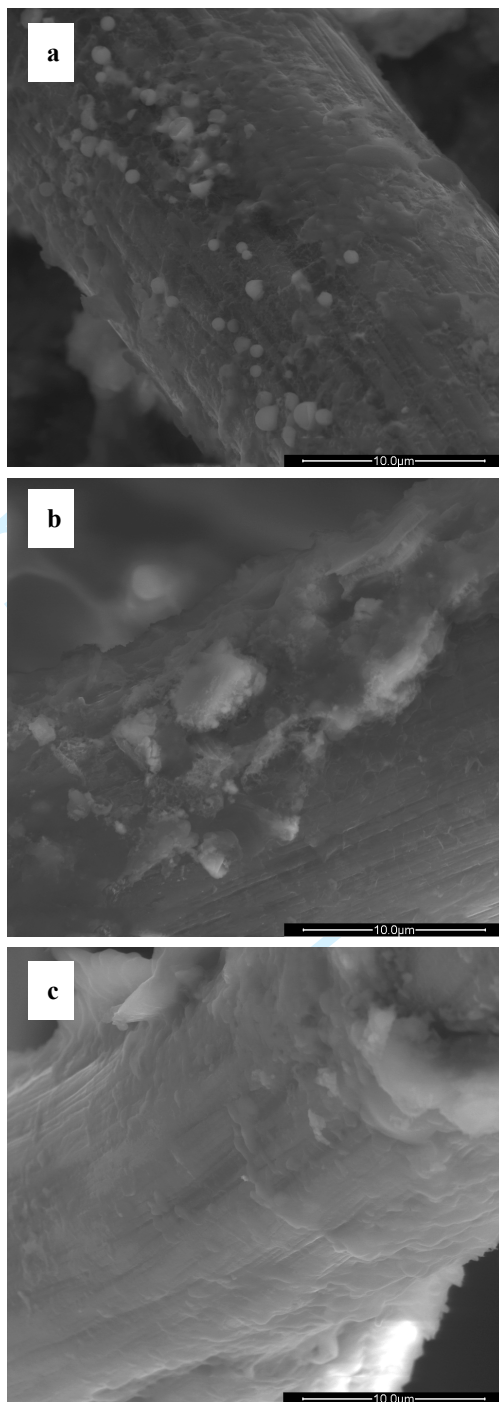


Figure S1. SEM images of anodes in the three MFCs after the whole experiment. (a) MFC-TiO<sub>2</sub>; (b) MFC-Fe<sub>2</sub>O<sub>3</sub>; (c) MFC-CF.