Effect of electrical stimulation on the fate of
sulfamethoxazole and tetracycline with their corresponding
resistance genes in three-dimensional biofilm-electrode reactors

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ABSTRACT

Three-dimensional biofilm-electrode reactors (3D-BERs), which possess a large effective area to drive the reductive degradation of contaminants, have recently attracted attention for wastewater treatment. There have been few studies of the potential and risks of the application of this system on the removal of antibiotics. Here four 3D-BERs were designed to initially assess the potential for electrical stimulation to remove sulfamethoxazole (SMX), tetracycline (TC) and chemical oxygen demand, and to study the fate of the corresponding antibiotic resistance genes. The results indicated that the 3D-BER could significantly reduce antibiotic concentrations in wastewater, achieving removal rates of 88.9–93.5% and 89.3–95.6% for SMX and TC, respectively. The concentrations of target genes (*sulI*, *sulII*, *sulIII*, *sulIV*, *tetA*, *tetC*, *tetO*, *tetQ*, and *tetW*) in a granular-activated carbon (GAC) cathode were higher than those in a GAC anode in the 3D-BR (reactor with biological sludge and no voltage) and 3D-BER. An obvious increasing trend in the relative abundances of all target genes was observed in the GAC. A low current density could not increase the development of *sul* and *tet* genes in the 3D-BER. The total resistance was in the following order: 3D-BER > 3D-BR > 3D-ER (reactor with 0.8V and without biological sludge). In addition, the dehydrogenase activity of the microorganisms in the 3D-BER was significantly higher than in the 3D-BR ($p < 0.05$). High-throughput sequencing revealed that the microbial communities and relative abundance at the phyla level were affected by current stimulation.

Key words

61 Three-dimensional biofilm-electrode reactors; Sulfamethoxazole; Tetracycline;

62 Antibiotic resistance genes; Microbial community

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82 1. Introduction

83 Biofilm-electrode reactors (BERs) (Sakakibara and Kuroda., 1993), as well as
84 electrochemical and biological reactors, involve the direct immobilization of
85 autotrophic denitrifying microorganisms on the surface of the cathode and the
86 production of hydrogen gas as an electron donor by the electrolysis of water (Feleke
87 et al., 1998). However, traditional BERs consume significant amounts of electrical
88 energy to produce CO₂ and H₂, and have a relatively low efficiency. To enhance
89 BER performance, it is possible to enlarge the effective electrochemical activity of
90 the three-dimensional (3D) electrode by providing a large effective area (Hao et al.,
91 2013). A 3D-BER has been investigated for the treatment of groundwater (Zhou et
92 al., 2007), landfill leachate (Nageswara Rao et al., 2009), groundwater containing
93 nitrate and organic pollutants (Zhou et al., 2009), Acid Orange 7 wastewater (Zhao
94 et al., 2010), and municipal wastewater (Hao et al., 2013).

95 Antibiotics are among the most commonly used and successful group of
96 pharmaceuticals applied in human and animal medicine (Bouki et al., 2013). China
97 is the global leader in antibiotic production. Approximately 210,000 t of antibiotics
98 are produced annually, of which 85% is utilized in animal agriculture and medicine
99 (Luo et al., 2011). However, the majority of the applied antibiotics are excreted
100 through feces and urine in an unchanged form because they are weakly absorbed
101 and do not completely metabolize in the gut of humans and animals (Huang et al.,
102 2015). Excreted antibiotics eventually enter river environments through a variety of

103 pathways, including point discharges from wastewater treatment plants (WWTPs),
104 animal feeding operations (AFOs), and fish hatcheries or nonpoint sources, such as
105 overland flow from fields where manure or biosolids have been applied (Storteboom
106 et al., 2010). The overuse and misuse of antibiotics has led to the occurrence and
107 spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs),
108 which raises additional concerns and poses a health risk to humans. In addition,
109 antibiotics promote ARG transfer between nonpathogenic and pathogenic bacteria
110 by vertical gene transfer (VGT) and horizontal gene transfer (HGT) (Huang et al.,
111 2015). Hence, treatment processes that are effective and inexpensive for the removal
112 of antibiotics, ARBs, and ARGs are required.

113 However, novel 3D-BER technology is still in its early developmental period,
114 and the potential advantages and risks of a 3D-BER for antibiotic removal are still a
115 cause of concern (Zhou et al., 2009; Hao et al., 2013). In this study, a 3D-BER was
116 used to treat artificial wastewater containing sulfamethoxazole (SMX) and
117 tetracycline (TC) for eight months. In addition, three *sul* genes (*sulI*, *sulII*, and *sulIII*)
118 and five *tet* genes (*tetA*, *tetC*, *tetO*, *tetQ*, and *tetW*) were chosen as representatives of
119 the antibiotic resistance genes due to their frequent reporting in livestock manure
120 and wastewater treatment lagoons (Liu et al., 2014; Wu et al., 2015). The main
121 objectives of the study were: (1) to analyze the effect of electrical stimulation in the
122 3D-BER on the removal efficiencies of chemical oxygen demand (COD), SMX, and
123 TC from synthetic domestic sewage, (2) to assess the influence of electrical
124 stimulation on the development of ARGs during the treatment process; and (3) to

125 study the potential impact of low current density on electrochemical characteristics,
126 dehydrogenase activity (DHA), and the microbial community.

127 **2. Materials and methods**

128 **2.1 Experimental design and configuration of 3D-BERs**

129 Experiments were conducted indoors in four 3D-BERs, as shown schematically
130 in Fig. 1. The 3D-BER was assembled using a polyacrylic plastic column (internal
131 diameter: 140 mm; height: 350 mm). The reactor was composed of four layers (from
132 upper to lower): (1) a 20-mm active carbon fiber layer/titanium mesh anode, (2) a
133 200-mm layer filled with a granular-activated carbon (GAC, diameter: 3–5 mm;
134 specific area: 500–900 m² g⁻¹), (3) a 20-mm active carbon fiber layer/titanium mesh
135 cathode, and (4) a 60-mm layer with gravel (diameter: 3–4 mm). The volume of the
136 whole container was 10.1 L, with an effective working volume (liquid volume) of
137 3.3 L.

138 **2.2 Inoculation, system start-up and experiments**

139 Anaerobic sludge (mixed liquor suspended solids (MLSS): 60 g L⁻¹) was
140 obtained from the South City Municipal Wastewater Treatment Plant of Nanjing,
141 China. The sludge was pretreated by mixing with GAC (sludge volume ratio of 2.5:1)
142 and introduced into a 3D-BR and 3D-BER for microbial inoculation. Inactivated
143 sludge (autoclaving: 121 °C, 15 min) was mixed with GAC (sludge volume ratio
144 2.5:1) and introduced into a 3D-R and 3D-ER. The nutrient solution contained

145 glucose (0.125 g L^{-1}), NH_4Cl (0.025 g L^{-1}), KH_2PO_4 (0.005 g L^{-1}), NaCl (0.1 g L^{-1}),
146 and 0.15 mL of a trace essential element solution (Cao et al., 2015).

147 The nutrient solution was fed continuously into the 3D-BERs using peristaltic
148 pumps (BT100-1L, Baoding Longer Precision Pump Co., Ltd., Baoding, China).
149 The concentrations of TC and SMZ in the influents were $200 \mu\text{g L}^{-1}$ for all 3D-BERs.
150 The hydraulic retention time was 40 h. All experiments were conducted in the dark
151 at $28 \pm 2 \text{ }^\circ\text{C}$. The experiment was conducted for eight months from April 2015 to
152 November 2015. The 3D-ER and 3D-BER were operated under a continuous
153 voltage of 0.8 V provided by a DC power supply (LP3003D; Shenzhen Lodestar S.T
154 Co., Ltd., Shenzhen, China) (Liu et al., 2015). The reactors were defined as follows:
155 3D-R, reactor without biological sludge and no voltage; 3D-ER, reactor without
156 biological sludge, under a voltage of 0.8 V; 3D-BR, reactor with biological sludge
157 and no voltage; 3D-BER, biofilm-electrode reactor under a voltage of 0.8 V. The
158 current density ranged from $31.5\text{--}46.3 \text{ mA m}^{-2}$.

159 **2.3 Quantification of antibiotics and COD removal**

160 Effluent samples at the anode and cathode were collected in triplicate during
161 three sampling periods in June, September, and November of 2015. The antibiotics
162 in the effluents were detected using an LC-MS system (LCQAD-60000, Thermo
163 Fisher Scientific, Waltham, MA, USA). Water samples were filtered through
164 $0.45\text{-}\mu\text{m}$ fiber filters and extracted using Oasis HLB (6 mL, Waters, Milford, MA,
165 USA) extraction cartridges. Antibiotics analysis in the samples was based on a

published method (Huang et al., 2015; Wu et al., 2015). The antibiotics concentrations were determined from external calibration curves; the determination coefficients (R^2) exceeded 0.996 (Table SM-1). The COD in the effluents were determined in May, June, July, August, and November of 2015, based on guidelines provided by the American Public Health Association (APHA, method 5220).

2.4. DNA extraction and ARG analysis

GAC samples from the anode and cathode were collected in triplicate during three sampling periods in June, September, and November of 2015. A sample of the original GAC was also obtained. Effluents were collected twice in November 2015. Genomic DNA was extracted using a Power Soil DNA isolation kit (MoBio, Carlsbad, CA, USA), following the manufacturer's instructions. Eight resistance genes (*sulI*, *sulII*, *sulIII*, *tetA*, *tetC*, *tetO*, *tetQ*, and *tetW*) and the 16S rRNA gene were quantified using a CFX Connect Real-Time PCR System (Bio-Rad, Shanghai, China) and an SYBR Green qPCR mix (Bio-Rad, Shanghai, China). Primer sequences targeting these genes and the PCR protocol were based on published papers (Liu et al., 2014; Huang et al., 2015; Wu et al., 2015) (Table SM-2). The determination coefficients (R^2) were higher than 0.990 (Table SM-3).

2.5 Characteristics of electrochemical impedance spectroscopy and DHA analysis

Electrochemical impedance spectroscopy (EIS: CHI 660D, Shanghai Chen Hua Instrument Co., Ltd, Shanghai, China) was conducted every hour after open circuit status was reached. The accumulated activated carbon electrode inside the middle of

187 the BER was chosen as the working electrode. Simultaneously, an electrode on the
188 top of the 3D-BER had dual roles as a counter and reference electrode. EIS was
189 conducted under the following conditions: AC potential of 10 mV rms and a
190 frequency decrease from 10^5 Hz to a final frequency of 0.1 Hz (Feng et al., 2010).
191 To fit data points, an equivalent circuit was constructed using ZSimpWin 2.1
192 software, according to a published method (Du et al., 2014). DHA was measured at
193 492 nm using a UV-9100 spectrophotometer (Lab Tech Ltd, Beijing, China) based
194 on a published method (Xia et al., 2015).

195 **2.6 DNA sequencing**

196 GAC samples were collected (cathode, middle layer, and anode and then mixed
197 in equal quantities) in triplicate from each installation in two sampling periods, June
198 and November of 2015. DNA extraction methods, a measurement of concentration,
199 and a purity test were conducted according to the procedures outlined in Section 2.4.
200 The V4-V5 region of the bacteria 16S ribosomal RNA gene were amplified by a
201 PCR using the primers 515F 5' -GTGCCAGCMGCCGCGG-3' and 907R
202 5'-CCGTCAATTCMTTTRAGTTT-3'. Purified amplicons were pooled in equimolar
203 and paired-end sequences (2×250) on an Illumina sequencing platform following
204 standard protocols (Bokulich et al., 2013).

205 **2.7 Statistical analysis**

206 All data were statistically analyzed with Microsoft Excel 2010. An unweighted
207 pair-group method with arithmetic mean (UPMGA) tree of relative abundance at the

208 phylum level were processed using QIIME v.1.7.0 (Chen et al., 2012). An
209 independent-sample t-test ($p < 0.05$) was performed to test comparisons of
210 experimental data between different groups.

211 **3. Results and discussion**

212 **3.1 Removal efficiencies of SMX and TC in the reactors**

213 The concentrations of SMX and TC in both the cathode and anode effluent
214 water of four reactors in three sampling periods are shown in Table 1. The
215 concentrations of SMX and TC in the cathode effluent water were much higher than
216 that in the anode effluent water. The anode effluent removal efficiencies ranged from
217 21.8 to 60.2%, 37.2 to 66.0%, 65.3 to 87.0%, and 88.9 to 93.5% for SMX in the
218 3D-R, 3D-ER, 3D-BR, and 3D-BER respectively. The anode removal rates for TC
219 ranged from 23.3 to 64.2%, 42.3 to 64.7%, 73.8 to 85.6%, and 89.3 to 95.6% in
220 3D-R, 3D-ER, 3D-BR, and 3D-BER respectively. The SMX and TC elimination rate
221 for both the final effluent and cathode were in the order of 3D-BER > 3D-BR >
222 3D-ER > 3D-R. SMX and TC could be efficiently removed in the 3D-BER.

223 Previous studies have demonstrated that 3D-BERs are excellent for nitrogen and dye
224 removal (Mousavi et al., 2012; Liu et al., 2015). However, the removal of trace
225 antibiotics from domestic sewage using 3D-BERs has received less attention. It was
226 noteworthy that high SMX and TC removal efficiencies (91–95%) were achieved
227 using the 3D-BER in this study.

228 Previous studies have revealed the different soil–water distribution coefficients

229 (log K_d) of TC and SMX, with TC having higher log K_d values (2.58–4.2) than
230 SMX (0.05–1.42) (Gong et al., 2012). In constructed wetlands, a high elimination
231 rate of oxytetracycline HCl was obtained compared with that of sulfamethazine (Liu
232 et al., 2013). In this study, the average antibiotic removal efficiencies were
233 maintained at a similar level. The absorbance spectrum of the antibiotics and the
234 quantum yield of photolysis in the water matrix were also considered to be
235 important factors affecting the photodegradation of antibiotics (Klavarioti et al.,
236 2009). In this study, photolytic degradation could be ignored because all reactors
237 operated in darkness.

238 Some recent studies have suggested that the TC removal mechanism in
239 WWTPs can mainly be attributed to sorption in the sludge rather than
240 biodegradation. Microbial communities readily utilize sulfonamides as energy,
241 carbon, and/or nitrogen sources for growth, because the functional amino group on
242 the aromatic ring of sulfonamides can be oxidized by autotrophic nitrifying bacteria
243 (Gao et al., 2012). Furthermore, with a low current density (200 mA m⁻²), a 3D-BER
244 was successfully developed to achieve the complete removal of
245 autohydrogenotrophic nitrogen (Tang et al., 2013). However, the low current density
246 could not produce the required amount of hydrogen (Tong et al., 2013). This is
247 probably because the electric potential in the 3D-BER prompted biofilm formation
248 and stimulated microbial activity.

249 3.2 The fate of typical *sul* and *tet* genes in reactors

250 To estimate the behavior of ARGs during the reactor treatment process, the
251 relative abundances of three *sul* and five *tet* genes were determined. The relative
252 abundances of ARGs are an indicator of the proportion of bacteria carrying ARGs
253 and also minimize DNA manipulation efficiencies. The relative abundances of *sul*
254 and *tet* genes in the effluent and GAC of reactors are shown in Fig. 2. The different
255 fates of *sul* and *tet* genes in the 3D-BR and 3D-BER were mainly caused by their
256 specific characteristics. In this study, *sul* and *tet* genes exhibited a relatively low and
257 stable abundance in effluent water compared with the GAC. This indicates that the
258 reactors performed well in inhibiting the development of ARGs compared with the
259 techniques reported in other studies (Huang et al., 2015). Some previous studies
260 found that the abundance of *sulIII* was usually slightly higher than that of *sulII* in
261 some samples, with *sulIII* generally connected to small non-conjugative or large
262 transmissible multi-resistant plasmids as indicators of HGT (Gao et al., 2012). The
263 data in this study also showed a similar correlation. The relative abundances of *sulIII*
264 were relatively high in the effluent water of 3D-BR and 3D-BER ($p < 0.05$).

265 Only *sulIII*, *tetC*, and *tetO* were observed in the initial GAC sample, but as with
266 SMX and TC in the reactors, the corresponding ARGs were detected in all samples.
267 Some previous studies have indicated that swine wastewater was a major source of
268 ARG pollution (Liu et al., 2014). From the comparison between different treatment
269 periods, a clear increasing trend was observed for all target genes in GAC after
270 treatment. These results indicated that *sul* and *tet* genes would have a higher vertical

271 and HGT in microorganisms in the 3D-BR and 3D-BER. In general, the target genes
272 in the GAC cathode were present in a higher concentration than in the GAC anode
273 in the 3D-BR and 3D-BER. Previous studies have indicated that most target genes
274 were insignificantly correlated with TC in the influent (Huang et al., 2015; Wu et al.,
275 2015). In this study, the SMX and TC in the cathode were present in higher
276 concentrations than in the anode in the 3D-BR and 3D-BER (Table 1). This implies
277 that the relative abundance of *sul* and *tet* genes in the reactors is mainly affected by
278 the antibiotic content. Homogeneous relative abundances of target genes were
279 obtained for the 3D-BR and 3-BER during the treatment processes. It was
280 noteworthy that the low current density did not increase the risk of the development
281 of *sul* and *tet* genes in the 3D-BER.

282

283 3.3 COD removal efficiency in reactors

284 Fig. 3 shows the average COD removal efficiency during the study period in all
285 reactors. The COD elimination efficiency for both the anode and cathode followed
286 the order of 3D-BER > 3D-BR > 3D-ER > 3D-R. The COD removal efficiencies of
287 the 3D-R and 3D-ER decreased from July 2015 onward, and reached a relatively
288 low level in November 2015. This phenomenon can be attributed to the decrease of
289 activated carbon adsorption. The 3D-BER remained stable at high COD removal
290 efficiencies (95.4–98.9%) in this study, with removal involving adsorption, water
291 electrolysis, microbial degradation, and electro-oxidation processes(Nageswara Rao

292 et al., 2009).

293 The 3D-BER had the highest efficiency of cathode COD removal (62.6–75.1%).

294 This phenomenon indicated that COD removal occurred in the cathode. Previous
295 results indicated that the maximum nitrate and COD removal emerged when the
296 current density was 200 mA m^{-2} (Tang et al., 2013). In this study, the COD removal
297 efficiencies remained stable at high levels and were unaffected by antibiotics in the
298 influent. The heterotrophic microorganisms involved in COD degradation are not
299 limited to specific bacteria, and the microbial community structure and enzyme
300 activity can be changed under the stress of antibiotics (Huang et al., 2015).

301

302 **3.4 Electrochemical characteristics of the reactors**

303 A Nyquist plot of the three installations is shown in Fig. 4 b. The resistance
304 distribution of the 3D-ER, 3D-BR, and 3D-BER is presented in Fig. 4 a. The ohm
305 resistance, which was derived from the same structure, was similar at $\sim 180 \Omega \text{ cm}^{-2}$.
306 With the same anodes structure in all installations, it was inferred that the narrow
307 range of electrode resistance, from $157.4\text{--}221.9 \Omega \text{ cm}^{-2}$, originated from the anodes.
308 As for the cathodes, the resistance of the 3D-ER was smaller than that of the 3D-BR
309 and 3D-BER. The total resistances were in the order of $3\text{D-BER} > 3\text{D-BR} > 3\text{D-ER}$.
310 The 3D-BR and 3D-BER were inoculated by activated sludge, whereas the 3D-ER
311 was fed with sterilized sludge. There was a relationship between resistance and
312 sludge in the installations. Large numbers of microorganisms, without extracellular
313 electron transfer, were present on the surface of the GAC electrode, and they

314 excreted extracellular polymer substances. These substances covered the electrode
315 and impeded the electron transfer between carbon granules and electrodes to
316 solution. Furthermore, it was found that the resistance of the 3D-BER was greater
317 than that of the 3D-BR. According to the results of other studies, an electrical
318 current stimulates organisms and accelerates cell proliferation (She et al., 2006),
319 which suggests that the appropriate current stimulation resulted in the increased
320 biomass in the 3D-BER.

321 **3.5 DHA of the microorganisms in the reactors**

322 The DHA was 1882.9 and 3101.5 $\mu\text{gTF (gTS.h)}^{-1}$ for the 3D-BR and 3D-BER,
323 respectively (Fig. SM-1). The DHA of the 3D-BER was significantly higher than
324 that of the 3D-BR ($p < 0.05$). Dehydrogenase can directly indicate the activity of
325 microorganisms, due to the oxidoreductases group transferring electrons between
326 donors and acceptors (Fang and Liu., 2002). Previous results have revealed that
327 anaerobic treatment is suitable for pharmaceutical industry wastewater, with SMX
328 concentrations of up to 40 mg L⁻¹ (Cetecioglu et al., 2015). This is not surprising
329 because TC and SMX had little effect on microbial activity at the lower
330 concentrations. It is likely that DHA of the microorganisms may be developed via
331 electrochemical activity in the 3D-BER.

332 **3.6 Analysis of the similarity of microbial populations in reactors**

333 The composition and relative abundance of phyla in the reactors is shown in
334 Fig. 5. The top 15 phyla were identified from the samples in this study.

335 *Proteobacteria* were the most dominant, with an average relative abundance in all
336 samples of 47.3%, followed by *Bacteroidetes* (18.6%), *Chloroflexi* (4.2%), *Chlorobi*
337 (3.7%), *Actinobacteria* (3.7%), *Verrucomicrobia* (3.7%), and *Firmicutes* (3.1%).
338 Unweighted pair group method with arithmetic mean (UPGMA) is widely used in
339 ecology for the classification of samples based on their pairwise similarities among
340 relevant descriptor variables(Hamady et al., 2010; Chen et al., 2012). The results
341 from five samples indicated a divergence in the composition and relative abundance
342 into three clusters. Cluster I comprised a sample of the original GAC, cluster II
343 comprised the June 3D-BR and November 3D-BR samples, and cluster III included
344 all of the remaining samples. Antibiotic is well-known for its antibacterial growth
345 against a broad spectrum of bacteria (Tarushi et al., 2010). For example,
346 enrofloxacin acts principally by inhibition of bacterial DNA gyrase thereby
347 preventing DNA supercoiling and synthesis (e Souza et al., 2002). In addition,
348 appropriate current stimulation resulted in the increased biomass(Fig. 4) and
349 dehydrogenase activity (Fig. SM-1).In this study, the results indicated that the
350 microbial communities and their relative abundance at the phyla level in the reactors
351 were affected by current stimulation and antibiotic.

352

353 **4. Conclusion**

354 In this study, the 3D-BER clearly demonstrated a strong performance for
355 wastewater containing SMX, TC, and COD, with an 88.9–93.5% removal efficiency

for SMX, 95% removal efficiency for TC, and 89.3–95.6% removal efficiency for
COD. The target genes (*sulI*, *sulII*, *sulIII*, *tetA*, *tetC*, *tetO*, *tetQ*, and *tetW*) in the
GAC cathode were present at a higher concentration than in the GAC anode of the
reactors. A clearly increasing trend in the relative abundances of all target genes in
the GAC was observed during the treatment processes. In addition, the development
of the *sul* and *tet* genes was not impacted by the low current density in the 3D-BER.
The total resistances were in the order of 3D-BER > 3D-BR > 3D-ER. The DHA of
the microorganisms in the 3D-BER was significantly higher than in the 3D-BR. The
data support the notion that a low current density stimulated the DHA of
microorganisms and accelerated cell proliferation in the 3D-BER. The top 15 phyla
were identified, and it was found that microbial communities and their relative
abundance at the phyla level were influenced by current stimulation. Future studies
should investigate ARB characteristics in both reactors and effluents.

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Table 1. Concentrations of SMX and TC (mean $\pm$ SD, n=3) in the cathode effluent water and anode effluent water of installations

	Concentrations of anode effluent (µg L ⁻¹)				Anode removal (%)		Concentrations cathode effluent (µg L ⁻¹)				Cathode removal (%)		Concentrations of anode effluent (µg L ⁻¹)				Anode removal (%)		Concentrations cathode effluent (µg L ⁻¹)				Cathode removal (%)		Concentrations of anode effluent (µg L ⁻¹)				Anode removal (%)	
	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC	SMX	TC
3D-R	79.6 ±5.4	71.5 ±6.5	60.2	64.2	180.3 ±9.5	177.6 ±11.3	9.8	11.2	141.2 ±11.7	146.5 ±13.2	29.7	26.8	185.2 ±15.0	179.5 ±13.6	7.4	10.3	156.7 ±19.6	153.4 ±18.3	21.8	23.3										
3D-ER	68.0 ±2.8	70.6 ±3.7	66.0	64.7	163.2 ±22.5	152.0 ±19.0	18.4	24.0	118.3 ±9.5	102.7 ±14.3	40.8	48.8	157.6 ± 15.5	144.4 ±13.7	21.3	27.8	126.2 ±12.0	115.4 ±10.3	37.2	42.3										
3D-BR	25.9 ±3.2	28.8 ±3.9	87.0	85.6	120.5 ±14.8	128.3 ±13.2	39.8	35.8	58.4 ±2.8	42.2 ±4.3	71.0	78.9	131.3 ±11.1	120.7 ±10.2	34.4	39.7	69.4 ±6.7	52.5 ±2.3	65.3	73.8										
3D-BER	13.0 ±0.7	8.7 ±1.1	93.5	95.6	98.0 ±6.5	106.6 ±9.8	51.0	46.7	17.1 ±2.4	10.5 ±3.8	91.5	94.7	110.4 ± 8.2	100.6 ±7.4	45.1	49.7	22.3 ±6.6	21.5 ±2.5	88.9	89.3										

3D-R, reactor without biological sludge and voltage; 3D-ER, reactor without biological sludge but under the voltage of 0.8 V; 3D-BR, reactor with biological sludge without the voltage of 0.8 V; 3D-BER, biofilm-electrode reactor under the voltage of 0.8 V.

1 **Figure Captions**

2 Fig. 1. Schematic diagram of the 3D-BER: (1 peristaltic pump; 2 DC regulated power
3 supply; 3 active carbon fiber/Ti mesh anode; 4 granular activated carbon; 5 active
4 carbon fiber/ Ti mesh cathode; 6 gravel; 7 cathode effluent; 8 anode effluent.)

5 Fig. 2. Quantities of eight resistance genes (*sulII*, *sulIII*, *sulIII*, *tetA*, *tetC* *tetO*, *tetQ* and
6 *tetW*) normalized to 16S rRNA genes in the Effluent, Cathode and Anode of 3D-ER
7 and 3D-BER, respectively.

8 Fig. 3. Comparison of COD removal (mean $\pm$ SD, n=3) among the four installations.
9 (a) Anode COD elimination efficiency. (b) Cathode COD elimination efficiency.

10 Fig. 4. Nyquist plot and resistance distribution of three installations. (a) The resistance
11 distribution, (b) Nyquist plot.

12 Fig. 5. Cluster analysis of relative abundance in phylum level. UPGMA tree on the
13 left and the relative phylum-level abundance map on the right.

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Fig. 1

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Fig. 2

Fig. 4

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Fig. 5

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