DTU Library

Exoelectrogenic anaerobic granular sludge for simultaneous electricity generation and wastewater treatment

Zhao, Nannan; Treu, Laura; Angelidaki, Irini; Zhang, Yifeng

Energy and the Environment

Exoelectrogenic anaerobic granular sludge for simultaneous electricity generation and wastewater treatment

Nannan Zhao, Laura Treu, Irini Angelidaki, and Yifeng Zhang

Just Accepted

Exoelectrogenic anaerobic granular sludge

for simultaneous electricity generation and

wastewater treatment

Nannan Zhao[1,2], Laura Treu[1], Irini Angelidaki[1], Yifeng Zhang[1]*

[1]Department of Environmental Engineering, Technical University of Denmark, DK-2800

Lyngby, Denmark.

[2]College of Life Sciences, Zhejiang Sci-Tech University, 310018 Hangzhou, PR China.

8

9

10

11

12

13

14

15

16

*Corresponding author, Tel.: +45 45251410; fax: +45 45932850.

E-mail address: yifz@env.dtu.dk; yifzmfc@gmail.com (Yifeng Zhang)

1

17   **Abstract**

18   Thick and electroactive biofilm is the key for successful development of microbial

19   electrochemical technologies and systems (METs). In this study, intact anaerobic granular

20   sludge (AGS), which are spherical and dense microbial associations, was successfully

21   demonstrated as novel and efficient biocatalysts in METs such as microbial fuel cell

22   (MFC). Three different strategies were explored to shift the microbial composition of AGS

23   from methanogenic into exoelectrogenic microbes, including varying external resistance,

24   organic loading, and manipulating anode potential. Among other strategies, only with

25   positive anode potential, AGS was successfully shifted from methanogenic to

26   exoelectrogenic conditions, as indicated by the significantly high current response (10.32

27   A/m^2) and 100% removal of organic carbon from wastewater. Moreover, AGS bioanode

28   showed no significant decrease in current generation and organic removal at pH 5,

29   indicating good tolerance of AGS to acidic conditions. Finally, 16S rRNA sequencing

30   revealed the enrichment of exoelectrogens and inhibition of methanogens in the microbial

31   community of AGS after anode potential control. This study provides a proof-in-concept

32   of extracting electrical energy from organic wastes by exoelectrogenic AGS along with

33   simultaneous wastewater treatment, and meanwhile opens up a new paradigm to create

34   efficient and cost-effective exoelectrogenic biocatalyst for boosting the industrial

35   application of METs.

36   **Keywords**: Anaerobic granular sludge; Exoelectrogenic biocatalyst; Electric energy; 16S

37   rRNA analysis; Wastewater treatment.

38

Introduction

Growing concerns over the intensive energy consumption for conventional wastewater treatment technologies has boosted interest in the development of energy-neutral treatment technologies[1]. Microbial electrochemical technologies and systems (METs) has shown promising potential in several applications spanning from renewable electricity production to biochemical and bioproducts production by using the electrons derived from waste organic matters by bacteria to perform dedicated reduction reaction[2-4]. Though promising, MFC technologies are still encountering a long-standing challenge to develop thick and efficient electroactive biofilm on the anode electrode. On the one hand, the limited biomass content and retention in biofilm would lead to the low capacity for organic carbon removal compared to conventional biotechnologies, and thus, extra post-treatment processes are always required[5, 6], which would greatly increase the operational and maintenance cost. On the other hand, MFC reactors which rely on thin anodic biofilm can't produce substantial quantities of power to offset the practical energy demands for wastewater treatment[7, 8]. Thus, the conventional ways of fabricating electroactive biocatalysts (as biofilm) on the anode do limit the wide application of MFC technology for wastewater treatment and energy generation[6, 9].

In the past decades, anaerobic granular sludge (AGS), as aggregates of microorganism, is popular among anaerobic biocatalysts for simultaneous bioenergy production (i.e., biogas through anaerobic digestion) and wastewater treatment, due to its high organic removal capacity and good tolerance to extreme conditions (e.g., toxic compounds and acidic shocks)[10-12]. In a previous MFC study[13], homogeneous bacterial suspension, derived from grinded AGS using a mortar and pestle followed by filtration (0.25-mm pore size sieve),

62   has even been demonstrated as efficient inoculum for cultivating anodic biofilm. Thus,

63   AGS could be a potential habitat of exoelectrogenic bacteria, in addition to methanogens.

64   More recently, it has been found that the whole microbial aggregates can be electroactive

65   if direct interspecies electron transfer occurs among the diverse microbial consortia[14, 15].

66   Considering the essential properties of AGS with dense microbes, special channel

67   morphology and potential conductivity, it is reasonable to hypothesize that intact AGS

68   could function as an effective biocatalyst for an MFC if electroactive bacteria are enriched

69   inside of granule. To date, intact AGS has never been tested as electroactive biocatalyst in

70   the field of METs. Integration of intact AGS into anode could address the key challenge of

71   MFC and greatly boost its capacity for electricity generation and wastewater treatment.

72   Such combination could further strength the advantages of MFC over conventional

73   anaerobic treatment processes, in addition to the inherent merits of mild operating

74   conditions, high removal and energy efficiency for low strength wastewater, and easy use

75   and transport of end product (electricity in this case)[16, 17].

76   In this context, switching intact AGS from methanogenic to exoelectrogenic is a key to

77   achieve a successful integration. Thus, in this study, intact AGS was for the first time

78   manipulated and explored as biocatalyst in MFC for wastewater treatment and

79   bioelectricity generation. Several strategies to transform the intact AGS from

80   methanogenic to exoelectrogenic, were employed, and the outcomes were evaluated in

81   terms of organic removal, current response, and coulombic efficiency. Finally, the

82   microbial dynamics during manipulation of anode potential and microbial composition at

83   different sites of anode electrode were analyzed. To the best of our knowledge, it is the first

84   time to investigate the feasibility of tailoring intact granular sludge as biocatalyst for

85   bioelectricity generation, which offers new insights in development of viable and

86   sustainable technology for cost-effective and efficient wastewater treatment.

87   **Materials and methods**

88   **MFC set up**

89   An MFC, made of nonconductive polycarbonate plates was constructed. The anode and

90   cathode chambers with the same dimension size ($4 \times 5 \times 5$ cm) were separated by a cation

91   exchange membrane (CEM, CMI 7000, Membrane international, NJ). Rubbers and screws

92   were used to tighten the reactor to avoid leakage. The anode electrode was made of a carbon

93   fiber brush wound into two twisted titanium wires (5.0 cm diameter, 5.0 cm length, Mill-

94   Rose, USA), which were heated at 450 °C for 15 minutes before use as reported

95   previously[18]. A reference electrode of Ag/AgCl electrode (+0.197 V vs SHE) was placed

96   ~ 0.3 cm close to the anode for accurate control of anode potential. The anodic chamber

97   was filled with 80 g wet AGS, which was collected from a mesophilic upflow anaerobic

98   sludge blanket reactor fed with potato wastewater (Colsen, Netherland). A stainless-steel

99   mesh was used to avoid the washing out of AGS and possible blocking issues. The total

100   volume of anode chamber was 100 ml, while the working volume was 50 ml. An external

101   recirculation bottle (filled with 500 ml synthetic wastewater) was connected to anode

102   chamber with a recirculation flow rate of 50 ml/min. To maintain a sufficient mixing, the

103   recirculation bottle was stirred at 400 rpm. A titanium woven wire mesh (4×4 cm, 0.15 mm

104   aperture, William Gregor Limited, London) coated with 0.5 mg/cm^2 Pt was used as cathode

105   electrode. In closed circuit, the anode and cathode electrodes were connected through an

106   external resistance (1000 Ω, unless otherwise stated). During anode potential control by

5

107   potentiostat (Ivium-n-Stat, Ivium Technologies, Eindhoven, The Netherlands), three-

108   electrode cell mode was adopted; anode as working electrode, cathode as counter electrode

109   and Ag/AgCl as reference electrode.

110   **Inoculation and operational strategies**

111   AGS was directly used as the inoculum for MFC start-up. The synthetic wastewater

112   contained (in g/L of distilled water): sodium acetate, 1 (unless otherwise stated); NH_4Cl,

113   0.31; $NaH_2PO_4 \cdot H_2O$, 2.69; Na_2HPO_4, 4.33; KCl, 0.13; 12.5 ml mineral solution and 12.5

114   ml vitamin solution as described before[19]. The final pH of synthetic wastewater always

115   kept 7.0 ± 0.2. The anode chamber and external bottle was filled with the aforementioned

116   synthetic wastewater, reaching a total volume of 550 ml. The cathode chamber was filled

117   with 100 ml ferricyanide solution (50 mM, pH 7) to exclude the instability of cathodic

118   reaction. The catholyte was 50 mM phosphate buffer solution containing 50 mM

119   ferricyanide.

120   Multiple reactors including duplicate set-ups have been operated for the tests according to

121   different purposes. Three strategies were employed successively in the same reactor. As

122   summarized in Table S1 in supporting information, strategy 1 referred to the effect of

123   external resistance on MFC performance under closed circuit. During strategy 1 operation,

124   the sodium acetate concentration was controlled at 1500 mg/L. Thereafter, the influence of

125   different organic loading (1000, 1500 and 3000 mg/L) on system performance was

126   evaluated in strategy 2, during which the resistance was selected as 10 Ω. Subsequently, in

127   strategy 3, MFC was connected to the potentiostat and chronoamperometry measurement

128   was used to control anode potential at +20 mV (VS Ag/AgCl). During strategy 3, the

6

129   sodium acetate level was 1000 mg/L. Sequentially, to evaluate the persistence of positive

130   effect by controlling anode potential, MFC was connected in a closed circuit with 10 Ω

131   resistance, and fed with 1000 mg/L sodium acetate. Afterwards, the AGS were removed

132   out of the anode chamber to evaluate the functions of AGS, denoted as Control 1. The

133   cultivated AGS were transferred into another identical MFC with a completely new anode

134   to explore the current generation of the removed AGS, denoted as Control 2. The

135   transferring process was performed in an anaerobic box. The operational parameters (1000

136   mg/L sodium acetate and 10 Ω resistance) were employed for Control 1 and Control 2. At

137   the end, to evaluate the robust resistance to low pH conditions, same AGS were placed

138   back to the anode chamber and operated under different initial wastewater pH varying from

139   5 to 7. For comparison of AGS powered MFC with conventional MFC inoculated with

140   domestic wastewater, one set of MFC reactors with same configuration was constructed

141   (Control 3) and inoculated with domestic wastewater obtained from primary clarifier at

142   Lundtofte Wastewater Treatment Plant (Lyngby, Denmark). For all the reactors, at the

143   beginning of each batch, anode chamber and recirculation bottle was flushed with pure N_2

144   for 10 minutes to keep anaerobic conditions.

145   **Analytical methods and calculations**

146   During strategy 1 and 2, the voltage across an external resistance was recorded by a digital

147   multimeter (model 2700, Keithley Instruments, Inc.; Cleveland, OH) every 30 minutes.

148   Current was calculated according to ohm's law ($I=U/R$). Current density was normalized

149   by the projected cathode area (16 cm^2). During strategy 3, the current response was

150   recorded by the potentiostat every 1 min. Coulombic efficiency (CE) was calculated as

7

151    $CE = \dfrac{Ct}{Cth} \times 100\%$, where C_t was the total coulombs calculated by integrating current

152   response with time, calculated as $C_t = \int Idt$, C_{th} was the theoretical amount of coulombs

153   based on the COD degradation, calculated as $C_{th} = \dfrac{Fb\Delta COD}{M}$, where F is Faraday's

154   constant (96485 C mol^{-1} e$^-$), b is 4 referring to the transferred electrons per mole of oxygen,

155   M is 32 representing the molecular weights of oxygen, ΔCOD is the removed COD amounts

156   (unit gram)[20].

157   Total chemical oxygen demand (TCOD) was measured according to the standard method

158   (APHA, 1999). COD removal rates were fitted assuming a first-order kinetic reaction with

159   respect to substrate concentrations, and calculated according to the following equation:

160
$$Ln\frac{COD_t}{COD_0} = -kt \quad \text{Eq (1)}$$

161   where COD_0 is the initial COD concentration, COD_t is the COD concentration at time t,

162   and k is the first-order kinetic rate coefficient. The coefficient k at varied pH was calculated

163   and compared in section 3.2, in order to distinguish the optimal pH regarding to the fastest

164   carbon utilization.

165   Acetate was measured via a GC with FID detection (Agilent 6890). The sample pH was

166   immediately measured by using A PHM 210 pH meter (Radiometer). Produced gas was

167   collected by connecting a gasbag to the headspace of recirculation bottle. The volume was

168   measured by a 100 ml syringe. CO_2 and CH_4 were analyzed by a GC-TCD fitted with

169   paralleled column of 1.1 m × 3/16 'Molsieve 137 and 0.7 m × 1/4' with H_2 as the carrier

170   gas (MGC 82−12, Microlab A/S, Denmark), and H_2 was determined by a GC-TCD fitted

8

171   with a 4.5 m × 3 mms-m stainless column packed with Molsieve SA (10/80), as previously

172   described[21].

173   Mastersizer 2000 (Malvern Instruments, UK) was employed to measure the particle size

174   distribution of the raw AGS and cultivated AGS after strategy 3. Scanning electron

175   microscopy (SEM - FEI Quanta 200 ESEM FEG equipped with energy dispersion

176   spectroscopy, EDS - Oxford) was used for the observation of AGS morphology. For

177   morphological characterization, the raw AGS and AGS after strategy 3 were sampled,

178   washed with phosphorus buffer solution (50 mM, pH 7) and fixed by soaking into 4%

179   formaldehyde for 24 hours at 4 °C. Subsequently, the samples were washed by gradient

180   25%, 50%, 75%, 90%, 95%, and 100% ethanol/distilled water solutions successively.

181   Afterwards, samples were freeze-dried for overnight to get the powder specimens. The

182   specimens were coated with a gold thin layer (Quorum sputter coater, UK) and observed

183   by SEM-EDS at 3.0 kV.

184   **Microbial community analysis**

185   To characterize changes in microbial community before and after operation, the raw

186   granules, and the enriched granules and anodic biofilm after strategy 3 were all collected

187   by using sterilized scalpel or spoon as previously described[22]. Granules were sampled at

188   either in the direct vicinity, or further away from the anode. All the samples were collected

189   in triplicate except the biofilm which was sampled in duplicate. Total DNA extraction was

190   performed using PowerSoil DNA Isolation Kit (MoBio PowerSoil, Carlsbad, CA, USA).

191   Total genomic DNA amplification using universal primers 515F/806R was conducted on

192 V4 hypervariable region of 16S rRNA gene, and amplicons were sequenced by Illumina

193 MiSeq desktop sequencer (Ramaciotti Centre for Genomics, Kensington, Australia).

194 Raw data was deposited in the Sequence Read Archive database

195 (https://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA485399. OTU

196 clustering and taxonomy identification were performed using microbial genomics module

197 plug of CLC Workbench software (V.8.0.2, QIAGEN) as previously described[23]. OTU was

198 chosen to represent the Alpha diversity, while Principal Component Analysis (PCA)

199 performed by STAMP software[24] was selected to represent Beta diversity. The taxonomical

200 assignments of the selected interesting OTUs (relative abundance over 0.5%) was

201 performed including a manual comparison of CLC results with 16S ribosomal RNA

202 sequences (Bacteria and Archaea) database at the National Center for Biotechnology

203 Information (NCBI) by using BLAST[23]. Microbial relative abundance and folds change

204 were visualized in heat maps using Multi experiment viewer software (MeV 4.9.0).

205 Statistics regarding to the significant differences in microbial communities were identified

206 by t-test in STAMP software.

207 **Results and discussion**

208 **Different strategies to enhance the electroactivity of AGS for bioelectricity generation**

209 **and wastewater treatment**

210 *Impact of external resistance*

211 Figure 1 is here.

10

212　　The strategy of varying external resistance was first applied to the MFC reactor inoculated

213　　with AGS. The current density, as representative of electricity generation, showed a

214　　different behavior with different resistances (Figure 1A). When external resistance was

215　　changed from 1000 to 10 Ω, 14 fold increase of maximum current density (from 0.41 to

216　　5.84 A/m^2) was observed at the same acetate level (1500 mg/L). The trend of current

217　　generation at different external resistances was consistent with previous studies[25, 26].

218　　During the same period (Figure 1B), The COD removal was greatly improved (from 67%

219　　to 87%) when MFC was switched from 1000 to 10 Ω. The higher COD removal rate at 10

220　　Ω indicated that the substrate oxidation rate was enhanced when subjected to lower

221　　resistance. Regarding to the biogas production rate and methane yield, it was noticeable

222　　that the methane production was significantly increased at 10 Ω (Figure 1C). The result

223　　showed that the increase of COD removal after changing resistance to 10 Ω was partly due

224　　to the anaerobic methanation. The results were different from the previous studies that

225　　methanogens activity was inhibited at lower resistance[27-29]. In this study, the AGS was

226　　originally cultivated for biomethanation which was different from previous MFC studies.

227　　In addition, decreasing external resistance could be an effective way to enrich

228　　exoelectrogens, but it may also facilitate interspecies electron transfer between

229　　exoelectrogens and methanogens as reported previously[30]. There was no significant

230　　difference in anodic potential (around -500 mV) and pH (approx. pH 7) during the

231　　operation with two different external resistances (data not shown). The influence of pH in

232　　the methanogenic activity could be neglected. Thus, strategy 1 referred to changing

233　　resistance was not an effective way to inhibit methanogenic activity. Considering the

234　　enhanced electricity production, R-10 Ω was selected for the following experiments.

11

235 *Impact of substrate concentration*

236 According to the previous study[31], the methanogens activity could be manipulated by

237 organic loading. Therefore, as strategy 2, the acetate concentrations ranging over 1000,

238 1500 and 3000 mg/L was applied consecutively. As shown in Figure 1A, the current

239 response significantly decreased when the acetate concentration increased from 1000 to

240 1500 or 3000 mg/L. It suggested the exoelectrogens weren't activated by elevated organic

241 loading. The electricity production was inhibited by increasing organic loading, as the

242 current density at 1000 mg/L was the highest among all conditions. From the COD removal

243 performance, it was clearly observed that COD removal rate was greatly enhanced with

244 elevated acetate concentrations. The average COD removal rate for 1000, 1500 and 3000

245 mg/L was 70.99, 110.59 and 360.60 mg/L/d, respectively. The COD removal was probably

246 contributed by (1) acetate oxidation by exoelectrogens; (2) acetate oxidation by

247 methanogens; (3) acetate oxidation by aerobic microbes. On one hand, from the

248 aforementioned current response (Figure 1A), it was clearly observed that current density

249 didn't increase dramatically with increasing of organic loading, which indicated that the

250 contribution of exoelectrogens to acetate oxidation wasn't enhanced with improved carbon

251 loading. On the other hand, due to the anolyte was flashed with nitrogen gas before starting

252 each test, the contribution by aerobic oxidation could be limited. Thus, the only possible

253 reason would be due to the activity of methanogens. To confirm our speculation, the biogas

254 production rate and methane yield were further analysed. As depicted in Figure 1C, the

255 higher acetate concentration, the faster methane production rate was observed, suggesting

256 the active methanogenesis process at elevated acetate concentration. Also, the methane

257 yield increased accordingly, which indicated the unsuccessful inhibition of methanogens

12

258 activity by improving organic loading. It was noticeable that in all cases, methane contents

259 always kept almost ten times higher than carbon dioxide. This could be due to its high

260 solubility of CO_2. Recirculation of liquid was applied in the anode, which may promote the

261 dissolution of CO_2. Overall, the above results demonstrated that the acetate concentration

262 of 1000 mg/L was good to obtain a superior electricity generation, and the substrate

263 concentration was not the contributing for turning methanogenic AGS into electrogenic.

264 ***Impact of anodic potential***

265 Figure 2 is here.

266 In addition to external resistance and organic loading, the anode potential has been reported

267 to impact microbial community structure and electrochemical performance[29, 32]. Therefore,

268 controlling anode potential at +20 mV (VS Ag/AgCl) as the third strategy was employed.

269 Clearly, during the period of anode potential control, the acetate was degraded rapidly in 5

270 days (Figure 2B). In fact, after 3 days, the acetate concentration already decreased from

271 800 to 33 mg/L, resulting in 96% removal. Correspondingly, the peak current density

272 increased significantly to 10.32 A/m² (Figure 2A). The high current response with fast

273 acetate degradation indicated that anode potential motivated the exoelectrogenic reactions

274 other than methanogenic. On the one hand, positive anode potential meant more energy to

275 support electroactive bacterial growth. It was reported that at relatively higher anode

276 potential, exoelectrogens can theoretically gain more energy for their growth and

277 maintenance[33, 34], according to:

278
$$\Delta G^{0'} = -nF(Eanode - E^{0'}_{donor})$$

279   where $\Delta G^{0'}$ is the Gibbs free energy change at standard conditions (pH 7 and 25°C), n is

280   the number of electrons transferred, F is Faraday's constant (96485 C mol^{-1} e^{-}), E_{anode} is

281   the anode potential, $E^{0'}_{donor}$ is the standard biological redox potential of electron donor. On

282   the other hand, positive anode potential may affect the electron transfer kinetics and attract

283   bacteria to move towards the electrode to form a thick biofilm[35]. Therefore, when the anode

284   potential was increased from -550 mV (measured anode potential at closed-circuit) to +20

285   mV, both of the carbon removal and current generation were greatly enhanced. To confirm

286   the inhibited methanogenic activity at high anode potential, biogas production rate and

287   methane yield were analysed. As shown in Figure 2C, methane yield almost decreased to

288   0, which indicated that the methanogenic activity was fully suppressed at high anode

289   potential. Furthermore, it was reported that the amounts of proteins (i.e. OmcA), which are

290   responsible for extracellular electron transfer, increased with elevating anodic potential[35].

291   More direct electron transfer-related protein at positive potential helped to stimulate an

292   electroactive-biofilm formation[35].

293   To examine the persistence of this strategy for electroactive bacteria enrichment, the

294   reactor was subsequently switched to MFC mode (without potential control) again. It was

295   shown that the peak current density increased from 3.30 (before potential control) to 6.41

296   A/m^2 (after potential control) when it was fed with 1000 mg/L acetate (Figure 2A). The

297   acetate removal efficiency increased from 50% (9 days) to 100% (8 days), indicating

298   effectiveness of anode potential control on enrichment of exoelectrogens. The pH was quite

299   stable (around pH 7 during each batch run, data not shown) before and after potential

300   control. The contribution of capacitive effect to the high current generation after potential

301   control could be neglected, since the maximum stable current generation lasted for a few

14

302   hours while discharging behaviour is normally around few seconds to minutes. To further

303   explore the contribution of AGS in electricity generation and carbon removal, the

304   performance of both MFC after removing AGS from anode chamber (Control 1) and new

305   MFC anode with removed AGS (Control 2) were investigated, respectively. In control 1,

306   the peak current density immediately decreased from 6.59 to 0.52 A/m^2, suggesting that

307   the AGS was partially involved in electron transfer. Accordingly, the acetate concentration

308   decreased from 731 to 166 mg/L after 7 days, resulting in 77% acetate removal. Since no

309   methane was produced in control 1, the contribution of methanogens to acetate removal

310   could be excluded. The current response and carbon removal observed in control 1 could

311   be due to small amount of residual AGS on anode since it is impossible to remove all AGS

312   from the anode. As shown in Figure S1, a completely new anode with cultivated AGS

313   produced a maximum current density of 1.11 A/m^2 after 1 day, which was higher than

314   control 1. But it didn't recover to the level observed before moving. The results of two

315   controls indicated that both AGS and formed biofilm on electrode played a

316   vital/cooperative role for the current generation. Even the AGS was exoelectrogenic, the

317   last step of electron transfer from bulk solution to solid electrode may still require an

318   electroactive biofilm as electron conduit.

319   To short conclude, the above results indicated that anode potential controlled at +20 mV is

320   effective to facilitate electroactive species growth and electron transfer in AGS.

321   Methanogens are well known strict anaerobes i.e. they require very low potential to grow

322   (<-300 mV). Therefore, exoelectrogens could dominate and got exclusively the chance to

323   use acetate as substrate.

15

324   **Acid resistance**

325   Figure 3 is here.

326   In the previous studies, the most common inoculum for MFC electroactive biofilm

327   enrichment was domestic wastewater, which was either attached on anode or suspended in

328   liquid[36, 37]. Comparatively, the AGS with diverse microbes and intrinsic granular structure

329   was used as inoculum in our work. It was previously reported that wastewater pH would

330   significantly affect MFC performance[29]. Thus, in this section, the effect of pH shock on the

331   electrogenic capacity of the enriched AGS anode was investigated. Figure S2 depicts

332   acetate removal rates which showed good agreement with the current output at different

333   pH ranging over 5 to 7 (Figure S2). The maximum current density at each condition was

334   shown in Figure 3. The highest maximum current density (5.21 A/m^2) was observed at pH

335   7 in AGS-MFC. When pH was decreased from 7 to 5, the ability of electron production

336   was significantly deteriorated for both reactors. It was reported that exoelectrogens

337   couldn't survive in the acidic environment when pH was lower than 5.5[38]. Although both

338   of reactors were negatively affected by the acid pH, AGS-MFC showed a relatively

339   stronger resistance to low pH compared to typical MFC inoculated with wastewater.

340   Assuming first-order kinetics, the rate coefficient was calculated according to Eq. (1), as

341   displayed in Figure 3. In both reactors, the rate coefficient showed a similar trend to pH

342   variations. The highest rate of 0.35 d^{-1} was obtained at pH 7 in AGS-MFC. In AGS-MFC,

343   when pH was decreased from 7 to 5, the rate coefficient decreased correspondingly from

344   0.35 to 0.20 d^{-1}, indicating diminished substrate degradation at acidic environment.

345   Similarly, for control MFC, the rate coefficient decreased from 0.20 to 0.12 d^{-1}. The rate

346   coefficient at pH 5 in AGS-MFC was even close to the value of control reactor at pH 7,

16

347   meaning a superior performance of acetate oxidative reaction in AGS-MFC even at

348   unfavourable pH conditions. Clearly, neutral pH conditions proved to be the optimal

349   environment for the exoelectrogenic bacteria. AGS inoculated MFC would have better

350   resistance considering that the biofilm from AGS might become even thicker during long-

351   term operation.

352   **Morphological characteristics and elemental composition of AGS**

353   Figure 4 is here.

354   The morphological image of single AGS taken after strategy 3 was depicted in Figure 4.

355   As shown in Figure 4A, an AGS has a spherical rough surface and macroporous carbon

356   architecture. A zoomed in image of the surface is shown in Figure 4B, in which the entire

357   surface of AGS was covered with compact rod-shaped bacterial cells. The porous structure

358   and rough surface would be beneficial for microbial growth and biofilm formation[39]. In

359   addition to the excellent porous structure, granules exhibit good mechanical strength for

360   microbes to resist the changes of surrounded environments (such as extreme pH or organic

361   loading shock) compared to the conventional flocs or biofilm[12, 40]. High-resolution of SEM

362   images of AGS channels (Figure 4C) demonstrated deep channels of ca. 1 um diameter,

363   with rod-shaped bacteria aligned on the channel sides. It revealed that the bacterial cells

364   were densely adhered not only to AGS surface, but also interior sections of AGS, indicating

365   the porous structure of AGS permitted sufficient substrate exchange from outside to inside

366   to support internal biofilm growth[41]. All of these attractive properties (the porous structure,

367   rough surface and dense microbes) enabled AGS as an ideal inoculum candidate for MFC

368   exoelectrogens enrichment.

17

369   According to the EDS results (Figure 4D), the raw AGS contained high levels of carbon

370   (53%) and oxygen (32%), and small amounts of minerals such as silicon, calcium and other

371   traditional metals including iron. These minerals were reported as the main skeleton of

372   granular structure, and may be involved in the electrical double layer formation of AGS as

373   reported before[42, 43]. After strategy 3, the cultivated AGS contained higher amounts of

374   carbon, which could be assigned to the increasing biomass contents. The low deviation

375   suggested a homogeneous mineralized granular structure. To get further information of the

376   granule size, Mastersizer 2000 was used to measure the particle size distribution (Figure

377   4E). It was found the mean diameter based on the volume weighted was significantly

378   increased from 122 (raw AGS) to 760 μm (cultivated AGS). It means over 50% granules

379   had the diameter of 760 μm after anode potential control. The bigger size demonstrated

380   that granulation of AGS was enhanced after being shifted from methanogenic to

381   electrogenic condition[44]. This is also consistent with the higher energy gain of bacteria at

382   higher anodic potential which would inevitably result in higher cell biomass.

383   **The influence of anode potential manipulation in microbial community dynamics**

384   After the selection and comparison of three strategies, strategy 3 (anode potential) was

385   demonstrated to be the most effective to inhibit methane production and improve current

386   generation. To gain an insight into microbial communities residing in granules and in

387   biofilm of carbon brush, 16S rRNA gene analysis was employed.

388   According to alpha diversity results shown in Figure S3 (Supporting Information), an

389   increase in microbial diversity (represented as OTU) regardless of sampling position was

390   observed after potential control. The results indicate that a more diverse microbiome was

391   enriched after a positive anode potential. Beta diversity shown in Figure S4A demonstrated

392   a distinct microbial dynamic change before and after anode potential control. A dramatic

393   change was found between raw granules and enriched granules/biofilms after strategy 3

394   based on the principal percentage (PC1 and PC2) (Figure S4A). When taking further

395   analysis of PC3, AGS taken from different positions (close and far from anode) were also

396   different from each other in microbial community compositions. Same distinct difference

397   was observed between granules and anodic biofilm. The above results were in agreement

398   with previous findings that the anode potential significantly affected the microbiome

399   clustering in anode[34].

400   Figure 5 is here.

401   High throughput 16S RNA amplicon sequencing was used to analyze the microbial

402   dynamics in AGS and attached anodic biofilm, and the relative abundance of taxa over 0.5%

403   is illustrated in Figure 5A. The vast majority of raw AGS microbial community was

404   composed of 90% bacteria (based on the average relative abundance). *Bacteroidetes* (23%),

405   *Firmicutes* (23%), *Proteobacteria* (12%), followed by *Synergistetes* (9%), and others

406   (23%), were the most dominant phyla (Figure S5). The microbial composition in raw AGS

407   was in agreement of common mesophilic AGS as reported before[45]. *Bacteroidetes*,

408   *Firmicutes* and *Proteobacteria* has always been detected in MFC, which were supposed to

409   be responsible for electricity generation[32, 46]. Thus, the raw AGS probably already

410   contained electroactive bacteria in the innate microbial community, which could enable its

411   utilization as biocatalyst in an MFC.

412   To get an insight into how the microbial community composition in MFC, the changes

413   between the raw and enriched AGS after manipulating anode potential were compared. The

19

414   results are shown in Figure 5B. As illustrated, in a cluster of taxa (Figure 5B, Group 1),

415   increasing significantly in relative abundance after manipulating anode potential was

416   mainly composed by exoelectrogenic bacteria. For example, *Synergistaceae* spp. (5 and 6)

417   increased from 0.2% to over 10% of relative abundance in both the granules and biofilm,

418   indicating that the proliferation of species belonging to *Synergistaceae* was due to the

419   improved anode potential. The family *Synergistaceae* was often found in MFC anode[47]. It

420   was noticeable that the strain of *Arcobacter butzleri* spp. (16 and 8), known as

421   exoelectrogens[48], appeared after the potential control (accounting for 6.5% of relative

422   abundance in the biofilm sample), strongly supported the enrichment of exoelectrogens.

423   Also, *Desulfurmonadales* spp. appeared after improving anode potential, suggesting a

424   strong correlation to the potential change. The *Desulfurmonadales* spp. (22 and 65) showed

425   a 97% similarity to *Pelobacter propionicus* and *Geobacter chapellei*. *Pelobacter*

426   *propionicus* was known as propionate producer from acetate, while no propionate was

427   detected during the experiment. Therefore, the high similarity of the strain was very likely

428   affiliated to *Geobacter chapellei*, which was reported as Fe(III) reducer[49]. Since iron-

429   reducing bacteria are known to use electrode as electron acceptor, we deduce that

430   *Desulfurmonadales* spp. represented by *Geobacter chapellei* may also have been involved

431   in direct electron transfer to anode[35]. Furthermore, the strain affiliated to the family

432   *Marinilabiliaceae* also increased in abundance, which has been previously found in MFC

433   bioanode[50]. Interestingly, another known species *Methanobacterium beijingense* was

434   dominant after improving anode potential. *M. beijingense* was known as hydrogenotrophic

435   methanogens using H_2/CO_2[51]. However, since no methane was detected, the species might

436   contribute mainly to maintain the granular structure by acting as the nucleation center, as

20

437   described elsewhere[52]. It has to be mentioned that although the enrichment of

438   exoelectrogens was demonstrated at positive anodic potential, the microbial community

439   was different from the previous findings. The predominance of *Geobacter* species was

440   typically reported for the acetate-fed MFCs[53, 54], while in this study, the microbial

441   community was more diverse with relatively fewer numbers of *Geobacter*. It was mainly

442   due to the different inoculum sources[53, 55]. In this study, methanogenic AGS was used as

443   the inoculum, while the domestic wastewater was often reported as the inoculum when

444   *Geobacter* was the most abundant in the acetate-fed exoelectrogenic biofilms.

445   Comparatively, a cluster of taxa decreased significantly in relative abundance, which

446   demonstrated that the transition from low anode potential (-550 mV) to high anode

447   potential (+20 mV) created a hostile habitat to these taxa. More specifically, *Mesotoga*

448   *infera*, which was involved in the conversion of acetate to H_2/CO_2[56], decreased from 9%

449   close to 0%, indicating that this pathway was negatively affected by increasing anode

450   potential. This was further supported by the undetectable H_2 throughout the whole

451   experiment. Similarly, *Methanosaeta concilii*, known as acetoclastic methanogens that has

452   ability of interspecies electron transfer with *Geobacter* species for CO_2 reduction into CH_4,

453   was diminished from 2.89% to 0.15%. This significant decrease indicated its inability to

454   survive at high anode potential +20 mV. It has been reported that methanogens require a

455   reductive environment where potential should be less than -527 mV (vs SHE) for its

456   growth[57]. That simply explained the inhibition of methanogens at +20 mV. In a more recent

457   work[58], long-term open circuit was found preferable for the growth of methanogens in the

458   cathode of acetogenic microbial electrosynthesis process, which implies the important role

459   of circuit potential on the microbial communities on the electrode.

21

460   Furthermore, regarding the competition between exoelectrogens and methanogens, it has

461   been proposed that a special structure of tightly packed aromatic amino acids enabled a

462   long-range electron transport between *Geobacter* and *Methanosaeta*[15]. In the

463   methanogenic aggregates, the known role of *Geobacter* species is converting acetate to

464   CO_2 with electrons generating. Through the metallic-like conductive pili, electrons are

465   released and flow to *Methanosaeta* for CO_2 reduction. The final electron sink is methane.

466   The direct interspecies electron transfer way is broken down at positive anode potential

467   since the *Methanosaeta* is not able to survive/active at high potentials[59]. Thus, when

468   inserting a conductive electrode in the aggregates, the realised electrons from *Geobacter*

469   metabolism would flow to the electrode instead of being involved in the methane

470   production. In the exoelectrogenic condition, the solid electrode substitutes the

471   *Methanosaeta* as the electron acceptor.

472   Figure 6 is here.

473   In order to elucidate the difference between the microbial community composition in

474   anodic biofilm (taken from carbon brush), enriched AGS closed to anode electrode and

475   enriched AGS far from anode, significant analysis based on the overall taxa were

476   performed (Figure 6A and B). Distinct consortia were formed in enriched AGS samples

477   and anodic biofilm. Compared to the anodic biofilm, a significant increase in relative

478   abundance of 9 bacterial taxa was observed in the enriched AGS close to carbon brush.

479   Particularly, well-known electrogenic bacteria such as *Marinilabiliaceae* spp.,

480   *Anaerobineaceae* spp., and *Desulfovibrionales* spp. were found significantly increased in

481   the AGS close to carbon brush. Besides, the significantly high abundance of *Synergistaceae*

482   sp., which was previously demonstrated to be potentially electrogenic, was in accordance

22

483   with the electricity generation of Control 1 (MFC after removing granules). Comparatively,

484   no significant difference was observed between AGS far from carbon brush and anodic

485   biofilm, except one strain.

486   To get an additional insight into the difference between two AGS samples (one taken close

487   to carbon brush and the other taken far from carbon brush), the statistical analysis was

488   performed as well (Figure 6C). Clearly, 10 bacterial taxa were observed in significantly

489   higher relative abundance in AGS close to carbon brush compared to the AGS far from

490   carbon brush. The most significant increase was found in *Arcobacter butzleri*, which was

491   characterized to be capable of transfer electrons from acetate to the electrode[60]. Therefore,

492   the above results strongly implied the AGS close to carbon brush might play more

493   important role in the electricity generation than the AGS far from carbon brush.

494   **Implications**

495   This study demonstrated the proof concept of using intact AGS as biocatalyst in an MFC

496   for simultaneous carbon removal and electricity generation. Compared to the conventional

497   biocatalyst (e.g., domestic wastewater), the AGS has several merits. Firstly, the large

498   surface area of AGS enabled a substantial electrogenic bacterial growth. Secondly, the

499   MFC inoculated with AGS generated much higher current compared to the conventional

500   MFC at same level of substrate. Lastly, the coulombic efficiency improved from 13.62%

501   (before potential control, 1g/L, 10 Ω) to 33.82% (with potential control, 1 g/L) as indicated

502   in Table S2. Although small improvement, the coulombic losses from methanogenic

503   process were diminished. The relatively low value (34%) might be due to other process

504   such as the cathodic oxygen diffusion or competition from other biological species[61, 62].

505　　Meanwhile, it must be pointed out that though AGS contributed the major part of current

506　　generation, the biofilm derived from AGS on the surface of anode electrode is also crucial,

507　　as it might play a role of conduit for electron transfer between bulk AGS and electrode.

508　　The results indicate that it is possible to boost the current generation of MFC by employing

509　　AGS as biocatalyst, but a thin biofilm between ASG and electrode is still needed and may

510　　play an important role to efficiently harvest the energy generated by AGS. The special

511　　conductive property between AGS and the electrode may open up many other intriguing

512　　applications. For instance, the exoelectrogenic AGS could be used as the bed electrodes in

513　　METland (wetland plus MET) and other fluidized bed reactor systems[63].

514　　Furthermore, more efforts should be made to further boost the application of AGS for

515　　energy recovery and simultaneous wastewater treatment. For instance, the mechanisms of

516　　electron transfer among granules should be explored to get better understanding of the

517　　system. In that case, how to accelerate the long distance of electron transfer in bacterial

518　　community could be identified and addressed well. Another interesting focus could be the

519　　studying of layer bacterial distribution in the granules and their involvements in the

520　　electron transfer. Further work should also focus on the continuous operation mode and

521　　reactor configuration, for example up flow MFC to optimize the settlement of granules for

522　　the future potential up scaling, or utilizing gas diffusion air cathode to bringing MFC closer

523　　to practical applications[64].

524　　**Supporting Information**

525　　Table S1, Figure S1, Figure S2, Figure S3, Figure S4, and Figure S5 as noted in the text.

526　　This material is available free of charge via the Internet at http://pubs.acs.org/

24

527 **Author Contributions**

528 YZ, IA and NZ designed the experiments, NZ and LT carried out the research. The

529 manuscript was written through contributions of all authors. YZ is responsible for

530 correspondence.

540 **References**

541 1.   McCarty, P. L.; Bae, J.; Kim, J., Domestic wastewater treatment as a net energy

542 producer--can this be achieved? *Environ Sci Technol* **2011**, 45, (17), 7100-7106.

543 2.   Harnisch, F.; Urban, C., Electrobiorefineries: Unlocking the Synergy of

544 Electrochemical and Microbial Conversions. *Angew.Chem-International Edition* **2018**, 57,

545 (32), 10016-10023.

25

546   3.   Liu, H.; Ramnarayanan, R.; Logan, B. E., Production of electricity during

547   wastewater treatment using a single chamber microbial fuel cell. *Environ Sci Technol* **2004**,

548   38, (7), 2281-2285.

549   4.   Min, B.; Logan, B. E., Continuous electricity generation from domestic wastewater

550   and organic substrates in a flat plate microbial fuel cell. *Environ Sci Technol* **2004**, 38,

551   (21), 5809-5814.

552   5.   Ren, L.; Ahn, Y.; Logan, B. E., A Two-Stage Microbial Fuel Cell and Anaerobic

553   Fluidized Bed Membrane Bioreactor (MFC-AFMBR) System for Effective Domestic

554   Wastewater Treatment. *Environ Sci Technol* **2014**, 48, (7), 4199-4206.

555   6.   Wang, Y. K.; Sheng, G. P.; Li, W. W.; Huang, Y. X.; Yu, Y. Y.; Zeng, R. J.; Yu,

556   H. Q., Development of a novel bioelectrochemical membrane reactor for wastewater

557   treatment. *Environ Sci Technol* **2011**, 45, (21), 9256-9261.

558   7.   Lovley, D. R., Bug juice: harvesting electricity with microorganisms. *Nat Rev*

559   *Microbiol* **2006**, 4, (7), 497-508.

560   8.   Zhang, Y.; Angelidaki, I., Microbial Electrochemical Systems and Technologies: It

561   Is Time To Report the Capital Costs. *Environ Sci Technol* **2016**, 50, (11), 5432-5433.

562   9.   An, J.; Li, N.; Wan, L.; Zhou, L.; Du, Q.; Li, T.; Wang, X., Electric field induced

563   salt precipitation into activated carbon air-cathode causes power decay in microbial fuel

564   cells. *Water Res* **2017**, 123, 369-377.

565   10.   Kosaric, N.; Blaszczyk, R.; Orphan, L.; Valladares, J., The characteristics of

566   granukes from upflow anaerobic sludge blanket reactors. *Water Res* **1990**, 24, (12), 1473-

567   1477.

568   11.   Lettinga, G.; Pol, L. W. H., UASB-process design for various types of wastewaters.

569   *Water Sci Technol* **1991**, 24, (8), 87-107.

570   12.   Schmidt, J. E.; Ahring, B. K., Granular sludge formation in upflow anaerobic sledge

571   blanket (UASB) reactors. *Biotechnol Bioeng* **1996**, 49, (3), 229-246.

572   13.   He, Z.; Minteer, S. D.; Angenent, L. T., Electricity generation from artificial

573   wastewater using an upflow microbial fuel cell. *Environ Sci Technol* **2005**, 39, (14), 5262-

574   5267.

575   14.   Morita, M.; Malvankar, N. S.; Franks, A. E.; Summers, Z. M.; Giloteaux, L.; Rotaru,

576   A. E.; Rotaru, C.; Lovley, D. R., Potential for Direct Interspecies Electron Transfer in

577   Methanogenic Wastewater Digester Aggregates. *Mbio* **2011**, 2, (4), 1-8.

578   15.   Lovley, D. R., Happy together: microbial communities that hook up to swap

579   electrons. *ISME J* **2017**, 11, (2), 327-336.

580   16.   Gell, K., Review of Small Scale, Community Biogas in the Industrialized World.

581   Wageningen University, Netherlands: Community Composting Network. **2008**.

582   17.   Chong, S.; Sen, T. K.; Kayaalp, A.; Ang, H. M., The performance enhancements of

583   upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment--a state-

584   of-the-art review. *Water Res* **2012**, 46, (11), 3434-3470.

585   18.   Zhang, Y.; Angelidaki, I., Recovery of ammonia and sulfate from waste streams

586   and bioenergy production via bipolar bioelectrodialysis. *Water Res* **2015**, 85, 177-184.

587   19.   Zhao, N.; Li, X.; Jin, X.; Angelidaki, I.; Zhang, Y., Integrated electrochemical-

588   biological process as an alternative mean for ammonia monitoring during anaerobic

589   digestion of organic wastes. *Chemosphere* **2018**, 195, 735-741.

590   20.   Zhang, Y.; Noori, J. S.; Angelidaki, I., Simultaneous organic carbon, nutrients

591   removal and energy production in a photomicrobial fuel cell (PFC). *Energy Environ. Sci*

592   **2011**, 4, (10), 4340-4346.

593   21.   Jin, X.; Zhang, Y.; Li, X.; Zhao, N.; Angelidaki, I., Microbial Electrolytic Capture,

594   Separation and Regeneration of CO2 for Biogas Upgrading. *Environ Sci Technol* **2017**, 51,

595   (16), 9371-9378.

596   22.   Zhao, N.; Angelidaki, I.; Zhang, Y., Electricity generation and microbial

597   community in response to short-term changes in stack connection of self-stacked

598   submersible microbial fuel cell powered by glycerol. *Water Res* **2017**, 109, 367-374.

599   23.   Zhao, N.; Jiang, Y.; Alvarado-Morales, M.; Treu, L.; Angelidaki, I.; Zhang, Y.,

600   Electricity generation and microbial communities in microbial fuel cell powered by

601   macroalgal biomass. *Bioelectrochemistry* **2018**, 123, 145-149.

602   24.   Parks, D. H.; Tyson, G. W.; Hugenholtz, P.; Beiko, R. G., STAMP: statistical

603   analysis of taxonomic and functional profiles. *Bioinformatics* **2014**, 30, (21), 3123-3124.

604   25.   Jadhav, G. S.; Ghangrekar, M. M., Performance of microbial fuel cell subjected to

605   variation in pH, temperature, external load and substrate concentration. *Bioresour Technol*

606   **2009**, 100, (2), 717-723.

607   26.   Ren, Z.; Yan, H.; Wang, W.; Mench, M. M.; Regan, J. M., Characterization of

608   Microbial Fuel Cells at Microbially and Electrochemically Meaningful Time scales.

609   *Environ Sci Technol* **2011**, 45, (6), 2435-2441.

610   27.   Song, T.-S.; Yan, Z.-S.; Zhao, Z.-W.; Jiang, H.-L., Removal of organic matter in

611   freshwater sediment by microbial fuel cells at various external resistances. *J Chem Technol*

612   *Biotechnol* **2010**, 1489-1493.

613   28.   Jung, S.; Regan, J. M., Influence of external resistance on electrogenesis,

614   methanogenesis, and anode prokaryotic communities in microbial fuel cells. *Appl Environ*

615   *Microbiol* **2011**, 77, (2), 564-571.

616   29.   Butti, S. K.; Velvizhi, G.; Sulonen, M. L. K.; Haavisto, J. M.; Oguz Koroglu, E.;

617   Yusuf Cetinkaya, A.; Singh, S.; Arya, D.; Annie Modestra, J.; Vamsi Krishna, K.; Verma,

618   A.; Ozkaya, B.; Lakaniemi, A.-M.; Puhakka, J. A.; Venkata Mohan, S., Microbial

619   electrochemical technologies with the perspective of harnessing bioenergy: Maneuvering

620   towards upscaling. *Renew Sust Energ Rev* **2016**, 53, 462-476.

621   30.   Rotaru, A.-E.; Shrestha, P. M.; Liu, F.; Shrestha, M.; Shrestha, D.; Embree, M.;

622   Zengler, K.; Wardman, C.; Nevin, K. P.; Lovley, D. R., A new model for electron flow

623   during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the

624   reduction of carbon dioxide to methane. *Energy Environ. Sci* **2014**, 7, (1), 408-415.

625   31.   Sleutels, T. H.; Molenaar, S. D.; Heijne, A. T.; Buisman, C. J., Low Substrate

626   Loading Limits Methanogenesis and Leads to High Coulombic Efficiency in

627   Bioelectrochemical Systems. *Microorganisms* **2016**, 4, (1), 1-11.

628   32.   Logan, B. E., Exoelectrogenic bacteria that power microbial fuel cells. *Nat Rev*

629   *Microbiol* **2009**, 7, (5), 375-381.

630   33.   Schroder, U., Anodic electron transfer mechanisms in microbial fuel cells and their

631   energy efficiency. *Phys Chem Chem Phys* **2007**, 9, (21), 2619-2629.

632   34.   Hari, A. R.; Katuri, K. P.; Logan, B. E.; Saikaly, P. E., Set anode potentials affect

633   the electron fluxes and microbial community structure in propionate-fed microbial

634   electrolysis cells. *Sci Rep* **2016**, 6, 38690.

635   35.   Carmona-Martinez, A. A.; Harnisch, F.; Kuhlicke, U.; Neu, T. R.; Schroder, U.,

636   Electron transfer and biofilm formation of Shewanella putrefaciens as function of anode

637   potential. *Bioelectrochemistry* **2013**, 93, 23-29.

638   36.   Logan, B. E.; Regan, J. M., Electricity-producing bacterial communities in

639   microbial fuel cells. . *Trends Microbiol* **2006**, 14, (12), 512-518.

640   37.   Ren, Z.; Steinberg, L. M.; Regan, J. M., Electricity production and microbial

641   biofilm characterization in cellulose-fed microbial fuel cells. *Water Sci Technol* **2008**, 58,

642   (3), 617.

643   38.   Margaria, V.; Tommasi, T.; Pentassuglia, S.; Agostino, V.; Sacco, A.; Armato, C.;

644   Chiodoni, A.; Schilirò, T.; Quaglio, M., Effects of pH variations on anodic marine consortia

645   in a dual chamber microbial fuel cell. *Int J Hydrog Energy* **2017**, 42, (3), 1820-1829.

646   39.   Pevere, A.; Guibaud, G.; Goin, E.; van Hullebusch, E.; Lens, P., Effects of physico-

647   chemical factors on the viscosity evolution of anaerobic granular sludge. *Biochem Eng J*

648   **2009**, 43, (3), 231-238.

649   40.   Quarmby, J.; Forster, C. F., A comparative study of the internal architecture of

650   anaerobic granular sludges. *J Chem Technol Biotechnol* **1995**, 63, (1), 60-68.

651   41.   Diaz, E. E.; Stams, A. J.; Amils, R.; Sanz, J. L., Phenotypic properties and microbial

652   diversity of methanogenic granules from a full-scale upflow anaerobic sludge bed reactor

653   treating brewery wastewater. *Appl Environ Microbiol* **2006**, 72, (7), 4942-4949.

654 42. Kuznar, Z. A.; Elimelech, M., Adhesion kinetics of viable Cryptosporidium parvum

655 oocysts to quartz surfaces. *Environ Sci Technol* **2004**, 38, (24), 6839-6845.

656 43. De Kerchove, A. J.; Elimelech, M., Calcium and magnesium cations enhance the

657 adhesion of motile and nonmotile Pseadomonas aeruginosa on alginate films. *Langmuir*

658 **2008**, 24, (7), 3392-3399.

659 44. Harris, H. W.; El-Naggar, M. Y.; Bretschger, O.; Ward, M. J.; Romine, M. F.;

660 Obraztsova, A. Y.; Nealson, K. H., Electrokinesis is a microbial behavior that requires

661 extracellular electron transport. *Proc Natl Acad Sci USA* **2010**, 107, (1), 326-331.

662 45. Zhu, X.; Treu, L.; Kougias, P. G.; Campanaro, S.; Angelidaki, I., Characterization

663 of the planktonic microbiome in upflow anaerobic sludge blanket reactors during

664 adaptation of mesophilic methanogenic granules to thermophilic operational conditions.

665 *Anaerobe* **2017**, 46, 69-77.

666 46. Zhang, Y.; Wang, Y.; Angelidaki, I., Alternate switching between microbial fuel

667 cell and microbial electrolysis cell operation as a new method to control H_2O_2 level in

668 Bioelectro-Fenton system. . *J Power Sources* **2015**, 291, 108-116.

669 47. Borole, A. P.; Hamilton, C. Y.; Vishnivetskaya, T. A., Enhancement in current

670 density and energy conversion efficiency of 3-dimensional MFC anodes using pre-enriched

671 consortium and continuous supply of electron donors. *Bioresour Technol* **2011**, 102, (8),

672 5098-5104.

673 48. Fedorovich, V.; Knighton, M. C.; Pagaling, E.; Ward, F. B.; Free, A.; Goryanin, I.,

674 Novel Electrochemically Active Bacterium Phylogenetically Related to Arcobacter

675 butzleri, Isolated from a Microbial Fuel Cell. *Appl. Environ. Microbiol.* **2009**, 75, (23),

676 7326-7334.

677 49.   Liu, L.; Tsyganova, O.; Lee, D.-J.; Chang, J.-S.; Wang, A.; Ren, N., Double-

678 chamber microbial fuel cells started up under room and low temperatures. *Int J Hydrog*

679 *Energy* **2013**, 38, (35), 15574-15579.

680 50.   Sotres, A.; Tey, L.; Bonmati, A.; Vinas, M., Microbial community dynamics in

681 continuous microbial fuel cells fed with synthetic wastewater and pig slurry.

682 *Bioelectrochemistry* **2016**, 111, 70-82.

683 51.   Ma, K.; Liu, X. L.; Dong, X. Z., Methanobacterium beijingense sp nov., a. novel

684 methanogen isolated from anaerobic digesters. *Int J Syst Evol Microbiol* **2005**, 55, 325-329.

685 52.   Gagliano, M. C.; Ismail, S. B.; Stams, A. J. M.; Plugge, C. M.; Temmink, H.; Van

686 Lier, J. B., Biofilm formation and granule properties in anaerobic digestion at high salinity.

687 *Water Res* **2017**, 121, 61-71.

688 53.   Kiely, P. D.; Regan, J. M.; Logan, B. E., The electric picnic: synergistic

689 requirements for exoelectrogenic microbial communities. *Curr Opin Biotechnol* **2011**, 22,

690 (3), 378-385.

691 54.   Logan, B. E.; Rossi, R.; Ragab, A.; Saikaly, P. E., Electroactive microorganisms in

692 bioelectrochemical systems. *Nat Rev Microbiol* **2019**, 17, (5), 307-319.

693 55.   Chae, K. J.; Choi, M. J.; Lee, J. W.; Kim, K. Y.; Kim, I. S., Effect of different

694 substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel

695 cells. *Bioresour Technol* **2009**, 100, (14), 3518-3525.

696   56.   Ahlert, S.; Zimmermann, R.; Ebling, J.; Konig, H., Analysis of propionate-

697   degrading consortia from agricultural biogas plants. *Microbiologyopen* **2016**, 5, (6), 1027-

698   1037.

699   57.   Lange, M.; Ahring, B. K., A comprehensive study into the molecular methodology

700   and molecular biology of methanogenic Archaea. *Fems Microbiol Rev* **2001**, 25, (5), 553-

701   571.

702   58.   Mateos, R.; Escapa, A.; San-Martín, M. I.; Wever, H. D.; Sotres, A.; Pant, D., Long-

703   term open circuit microbial electrosynthesis system promotes methanogenesis. *J Eng Chem*

704   **2020**, 41, 3-6.

705   59.   Jadhav, D. A.; Chendake, A. D.; Schievano, A.; Pant, D., Suppressing methanogens

706   and enriching electrogens in bioelectrochemical systems. *Bioresour Technol* **2019**, 277,

707   148-156.

708   60.   Toh, H.; Sharma, V. K.; Oshima, K.; Kondo, S.; Hattori, M.; Ward, F. B.; Free, A.;

709   Taylor, T. D., Complete genome sequences of Arcobacter butzleri ED-1 and Arcobacter

710   sp. strain L, both isolated from a microbial fuel cell. *J Bacteriol* **2011**, 193, (22), 6411-

711   6412.

712   61.   Ahn, Y.; Logan, B. E., Domestic wastewater treatment using multi-electrode

713   continuous flow MFCs with a separator electrode assembly design. *Appl Microbiol*

714   *Biotechnol* **2013**, 97, (1), 409-416.

715   62.   Kim, K. Y.; Yang, W.; Logan, B. E., Impact of electrode configurations on retention

716   time and domestic wastewater treatment efficiency using microbial fuel cells. *Water Res*

717   **2015**, 80, 41-46.

718   63.   Ramírez-Vargas, C.; Prado, A.; Arias, C.; Carvalho, P.; Esteve-Núñez, A.; Brix,

719   H., Microbial Electrochemical Technologies for Wastewater Treatment: Principles and

720   Evolution from Microbial Fuel Cells to Bioelectrochemical-Based Constructed Wetlands.

721   *Water* **2018**, 10, (9).

722   64.   Rossi, R.; Jones, D.; Myung, J.; Zikmund, E.; Yang, W.; Gallego, Y. A.; Pant, D.;

723   Evans, P. J.; Page, M. A.; Cropek, D. M.; Logan, B. E., Evaluating a multi-panel air cathode

724   through electrochemical and biotic tests. *Water Res* **2019**, 148, 51-59.

725

34

726   **List of figure captions**

727   Figure 1 Current density (A), TCOD removal (B) and biogas production rate and methane

728   yield (C) over time at different external resistance and different acetate concentrations. Red

729   line and green line refer to the performance of R-10 ohm, 1500 mg/L in Strategy 1 and

730   Strategy 2, respectively.

731   Figure 2 Current density (A), acetate concentrations (B) and average biogas production rate

732   and methane yield (C) with time in different reactors. Control 1: MFC with only carbon

733   brush (after potential control and moving granules out).

734   Figure 3 The maximum current density and COD removal rate coefficient at varied pH

735   conditions. AGS-MFC: MFC after potential control; Control 3: MFC inoculated with

736   domestic wastewater.

737   Figure 4 SEM image of the surface structure of single GAS after anodic potential

738   control:(A) an intact granule; (B) high-resolution of SEM image of granular surface

739   showing the massive microbial colonization; (C) showing the rod-shape microbes aligned

740   on the side of deep channels. (D) Energy-dispersive X-ray (EDS) results of AGS before

741   and after strategy 3. (E) Particle size distribution of raw AGS and cultivated AGS after

742   strategy 3.

743   Figure 5 Microbial community compositions in raw AGS (G1), and enriched AGS after

744   anode potential control and close to carbon brush (G2), enriched AGS far from carbon

745   brush (G3), and biofilm on carbon brush (Biofilm). Relative abundance (%) and folds

746   change were reported in (A) and (B), respectively. Group 1: the taxa increased in relative

35

747   abundance after anode potential control. Group 2: the taxa decreased in relative abundance

748   after anode potential control.

749   Figure 6 OTUs that changed significantly ($p < 0.05$) in the comparison between G2

750   (enriched AGS taken from close to carbon brush after strategy 3) and Biofilm (A), between

751   G3 (enriched AGS taken far from carbon brush after strategy 3) and Biofilm (B), and
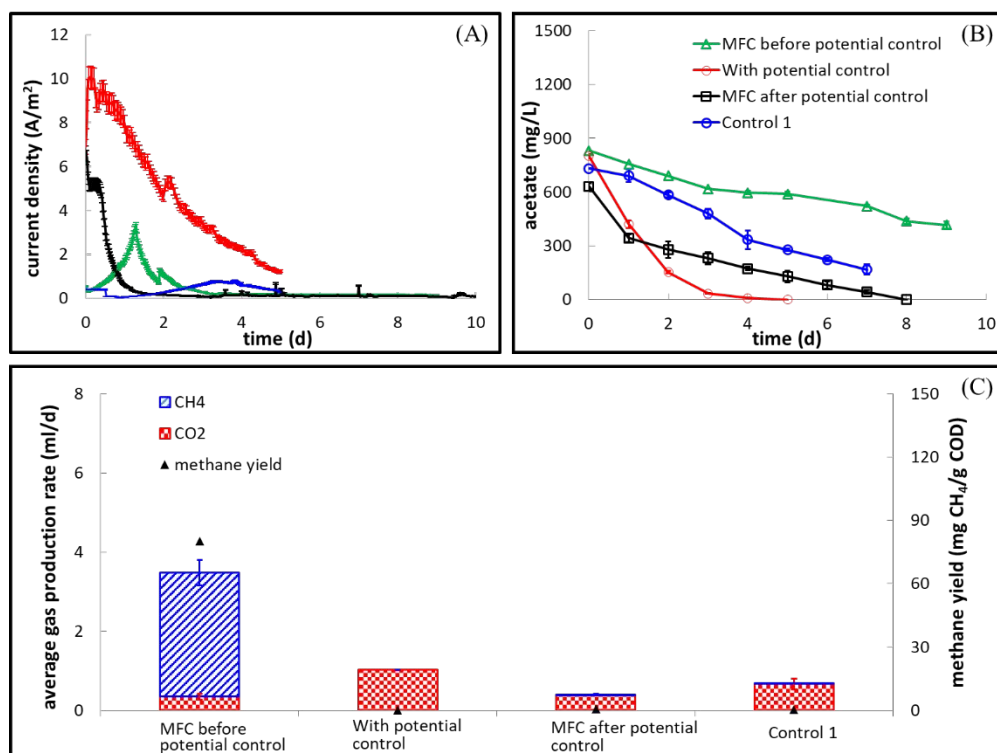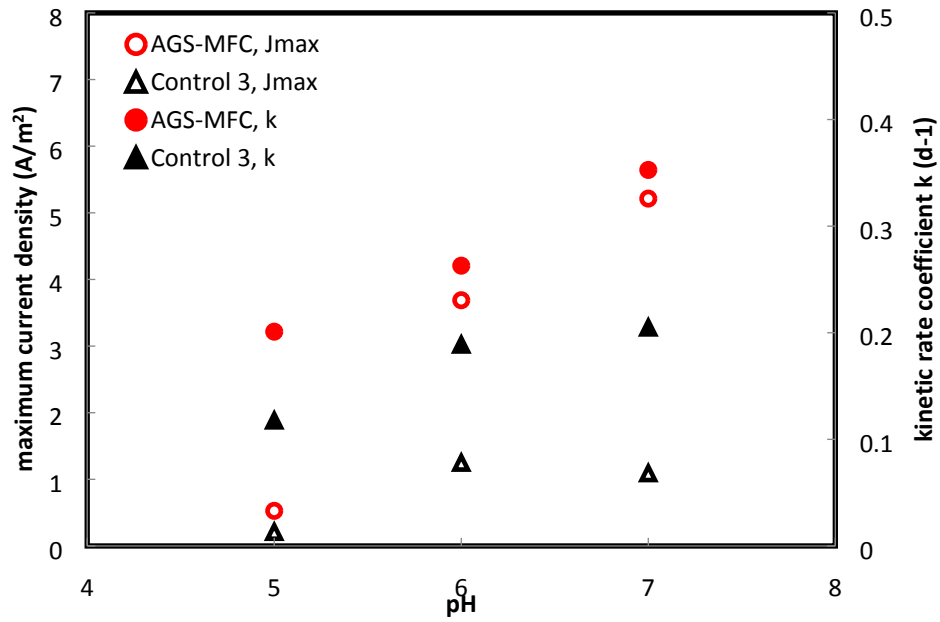
752   between G3 and G2 (C), respectively.

753

36
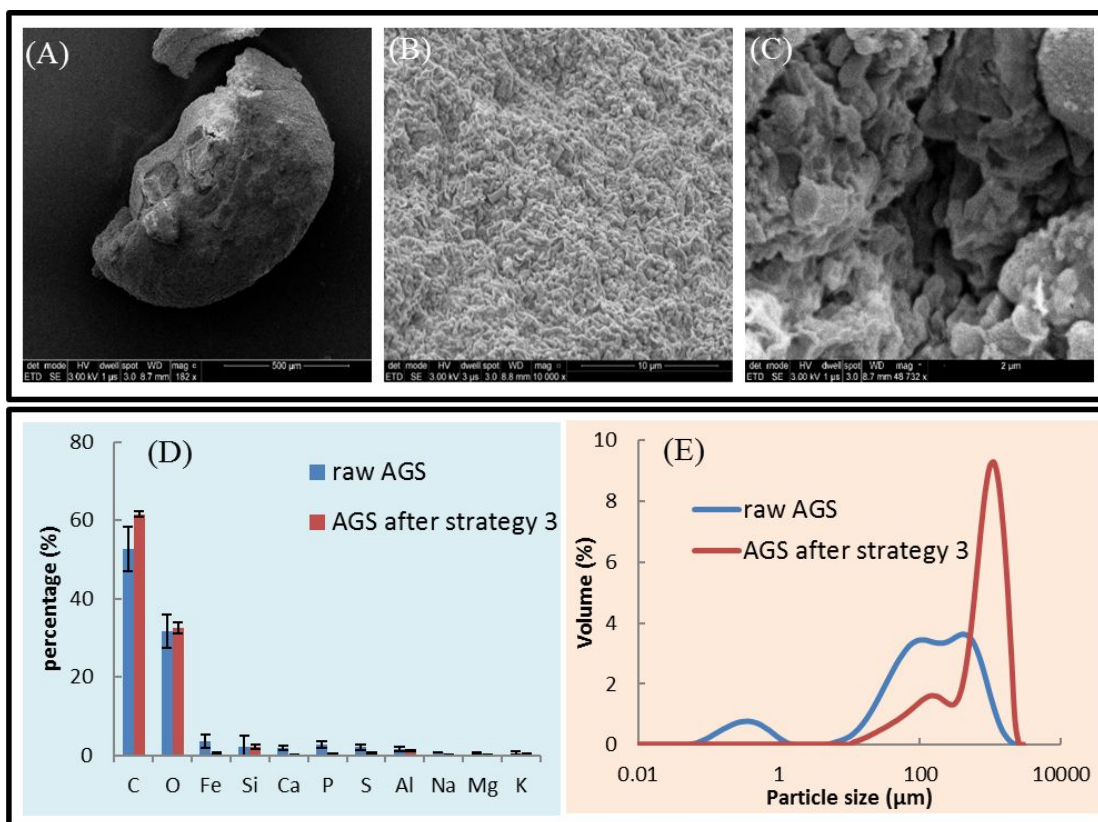
754

Figure 1

755

756

757

758

Figure 2

759

760

Figure 3

761

762

763

764

765

766   **Figure 4**

767

(A) **(B)**

Figure 5

(A)

770

(B)

771

(C)

Biofilm   G2   G3

772

Figure 6

773

774

775

42

776   **TOC Art**

777

778