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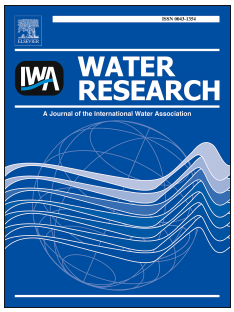

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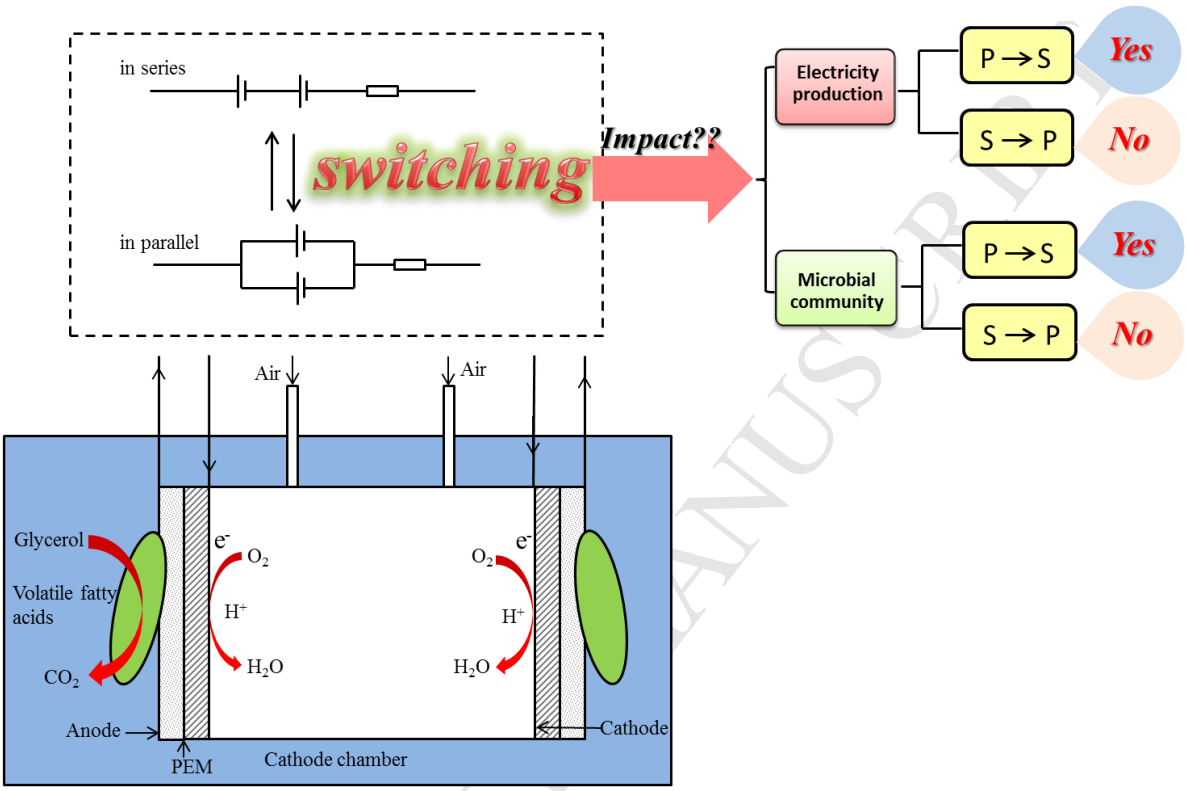

Self-stacked submersible MFC

Electricity generation and microbial community in response to short-term changes in stack connection of self-stacked submersible microbial fuel cell powered by glycerol

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Abstract

Stack connection (i.e., in series or parallel) of microbial fuel cell (MFC) is an efficient way to boost the power output for practical application. However, there is little information available on short-term changes in stack connection and its effect on the electricity generation and microbial community. In this study, a self-stacked submersible microbial fuel cell (SSMFC) powered by glycerol was tested to elucidate this important issue. In series connection, the maximum voltage output reached to 1.15 V, while maximum current density was 5.73 mA in parallel. In both connections, the maximum power density increased with the initial glycerol concentration. However, the glycerol degradation was even faster in parallel connection. When the SSMFC was shifted from series to parallel connection, the reactor reached to a stable power output without any lag phase. Meanwhile, the anodic microbial community compositions were nearly stable. Comparatively, after changing parallel to series connection, there was a lag period for the system to get stable again and the microbial community compositions became greatly different. This study is the first attempt to elucidate the influence of short-term changes in connection on the performance of MFC stack, and could provide insight to the practical utilization of MFC.

Keywords: Self-stacked submersible microbial fuel cell; Stack operation; Glycerol; Series connection; Parallel connection; Microbial community.

1. Introduction

Microbial fuel cell (MFC) is a bioelectrochemical device in which the chemical energy stored in organic matter was converted into electricity with the help of microorganisms as catalysts (Rabaey and Verstraete, 2005; Logan and Regan, 2006; Lovley 2008). MFC has gained increasing attention due to its unique advantages in wastewater treatment and energy production such as mild operational condition, less sludge production and high electric efficiency (Rabaey and Verstraete, 2005; Logan and Regan, 2006; Lovley 2008). In the last decade, great efforts with regard to architecture, microbiology, materials and applications have been made in the field to accelerate the practical application of the technology (Rabaey and Verstraete, 2005; Logan and Regan, 2006).

In practical utilization of MFC as power source, it is generally required a high voltage or current. However, the power output from a single MFC will remain limited since it cannot exceed a theoretical open circuit voltage (approx. 1.14 V if oxygen is used as electron acceptor) (Aelterman et al., 2006). Recently, it has been reported that when MFC units were stacked together, the power generation of MFC can be greatly boosted and is greatly dependent on the connection modes (Yazdi et al., 2015; Ye et al., 2014; An et al., 2014; Oh and Logan, 2007). The stack operations usually refer to parallel connection and series connection. When MFC units are stacked in series, an addictive increase in total voltage is produced, whereas high current was usually achieved in parallel connection (Dekker et al., 2009; Winfield et al., 2012; Ieropoulos et al., 2013). Theoretically, when two MFC units were connected in series, the total voltage in the circuit would be equal to the sum of separate voltage generation of two units, while the total current in parallel circuit was equal to the sum of electrons flowing in two units. Nevertheless, the actual power output could be affected by several factors. For instance, voltage reversal has been always observed both in series and parallel connection (Oh and Logan, 2007; Zhang and Angelidaki, 2012), which greatly deteriorate the stack performance. So far, most of studies focused on the system performance in

individual stack mode (Yazdi et al., 2015; Kim et al., 2013; An et al., 2016; An et al., 2015a, 2015b; Ledezma et al., 2013; Choi and Ahn, 2013; Zhuang et al., 2012). In field applications (e.g., alternately powering multiple devices with varied electric properties), high voltage or current output might be intermittently required, thus it is necessary to switch from the series connection to parallel connection, or vice versa. The change of connection mode could be essentially regarded as changing external resistance or adding external voltage on a conventional single MFC, though more complicated interaction may exist. It has been reported by several studies that the external resistance can significantly affect the anodic microbial communities (Rismani-Yazdi et al. 2011, Jung and Regan 2011). In addition, it was found that the anodic microbial communities were changed when reactors were shifted from MFC to microbial electrolysis cell (add external voltage) (Kiely et al. 2011). Thus, the changes in stack connection could affect both the reactor performance and anodic microbial communities. However, the effect of short-term changes in stack connection on the power output and microbial communities of MFC stack, which is of importance for better understanding the stack operation and for practical application, has never been explored. In addition to the connection mode, substrate is also important for the performance of MFC stack in terms of power generation and microbial community. Most of the investigations in previous MFC stack studies are based on simple substrate such as acetate (Ledezma et al., 2013; Ieropoulos et al., 2008; Wu et al., 2016). Glycerol, as a major byproduct of biodiesel production (Dounavis et al., 2015), has been widely used as substrate in various biological processes such as anaerobic digestion due to its easily degradable property (Dounavis et al., 2015; Fountoulakis and Manios, 2009; Siles Lopez et al., 2009; Zahedi et al., 2016; Zhang et al., 2015). Glycerol has been also utilized as substrate in bioelectrochemical systems including MFC (Feng et al., 2011; Sharma et al., 2011; Reiche and Kirkwood, 2012; Guimaraes and Linares, 2014). However, the feasibility of glycerol as substrate to power a MFC stack has never been demonstrated.

In this study, the electricity generation and microbial community and their responses to connection changes (series or parallel) were investigated using a self-stacked submersible MFC (SSMFC) powered by glycerol. The SSMFC has been previously demonstrated by our group as an innovative MFC stack for harvesting energy from sediment with reduced construction costs (Zhang and Angelidaki 2012). However, the application of SSMFC for wastewater treatment and its response to the connection change have never been explored. The degradation of glycerol in SSMFC stack was also analyzed to get a better understanding of the substrate utilization. The effect of initial concentration of glycerol on system performance was investigated as well. This study may offer instructive information for the practical application of MFC stacks in the future.

2. Material and methods

2.1. SSMFC construction and operation

SSMFC reactors were developed as previously described (Zhang and Angelidaki, 2012). As shown in Fig.1, the SSMFC consisted of one rectangular chamber (3 cm × 3 cm × 1 cm, 9 cm^3) made of nonconductive polycarbonate plates. The sandwich structured membrane electrode assembly (MEA) was placed on each side. The cathode was a 5% water proof carbon paper with one side covered of Pt catalysts (3 cm × 3 cm, 0.5mg/cm2 with 20% Pt, E-TEK division, USA). The membrane used as separator was a proton exchange membrane (Nafion 117, DuPont Co., USA). The anode was made of a non-wet-proofed plain carbon paper (3 cm × 3 cm, Toray carbon paper, E-TEK division, USA). The anode electrode, cathode electrode, and PEM were hot pressed together as an MEA (Min and Logan, 2004). In SSMFC, there was only one cathode chamber including two cell units. A plastic tube was connected to the cathode chamber for aeration (open to the air). Electrical connections and electrode pretreatment were done as previously (Zhang and Angelidaki, 2012).

Figure 1 is here

The SSMFC was submersed into an anaerobic reactor (working volume 500 ml, total volume of 1000 ml). For inoculation step, 500 ml of wastewater amended with 1000 mg/L glycerol was fed into the anaerobic reactor to enrich the anodic microorganism. After two weeks, the anaerobic reactor was refilled with 50 mM phosphorus buffer solution (PBS) containing1000 mg/L glycerol to ensure adequate organic matter supply and provide stable buffering capacity. The cathode chamber was open to the air through the plastic tube. The SSMFC was further operated for another two months to ensure a stable voltage generation before continuing the test. In order to investigate glycerol degradation in SSMFC, PBS with glycerol (1000 mg/L) was introduced into anaerobic reactor. The solution in the anaerobic reactor was refilled when the voltage was lower than 10 mV. To compare effects of different connection modes on whole system performance, the SSMFC was changed from series connection to parallel connection, while the other SSMFC was changed from parallel to series connection. To investigate the influence of different substrate concentration under different connection, the initial glycerol concentration varied from 100 mg/L to 2000 mg/L. All experiments were conducted in duplicate at room temperature (21± 3°C).

2.2. Analysis and calculations

Volatile fatty acids (VFAs) were measured by gas chromatography (Agilent 6890). Glycerol was determined by a HPLC equipped with ultraviolet (UV) and refractive index detectors (Agilent Technologies, Science Park Scion DTU, Horsholm, Denmark). A Vertisep^{TM} OA 8 µm column (7.80 × 300 mm) was used for the analysis. H_2SO_4 solution (4 mM) flowed through the column at a rate of 0.5 mL/min at 45 °C. The wave length in UV detector was set at 210 nm.

The voltage (V) across an external resistor (517 Ω, unless otherwise stated) was measured every 30 min using a digital multimeter (Model 2700, Keithley Instruments, Inc., Cleveland, OH, USA). Current (I) was calculated according to the Ohm's law, I=V/R, where V is the voltage and R is the resistance. Power density (P= IV/A) was calculated as previously described, with the power density

normalized by the projected surface area of anode (insert reference). In a polarization curve test, the external resistor was varied from 10 to 11,000 Ω to determine the max power density and internal resistance of SSMFC.

2.3. Microbial community

To explore the influence of different stack connections on anodic microbial communities, the biofilm attached on anode was sampled at the end of each batch as indicated in section 3.5 by scraping the electrode surface with a sterilized scalpel. For each biofilm sample, only a small area of the thick biofilm (less than 10% area in total) was scraped from 10 different parts on the electrode, in order to make the sample representative and without disturbing the system. Total DNA extraction, PCR-DGGE and 16 S rRNA analysis were done as previously described (Zhang et al., 2011a).

3. Results and discussion

3.1 Power generation of stack MFC in series and parallel connection

Stable power generation of SSMFC was observed after about three months of enrichment. Fig.2 shows the polarization curves of SSMFC operated in different connection mode. In series SSMFC connection, the open circuit voltage (OCV) of 1.15 V was observed, which was much higher than that (0.71 V) in parallel SSMFC mode. This demonstrated the additive voltage output when the SSMFC was connected in series, which was in a good agreement with the results previously described (An et al., 2014; Winfield et al., 2012; Kim et al., 2013; Sun et al., 2009; Wang and Han, 2009). The observed OCV of 1.15 V in series connected-SSMFC fed with glycerol is a slightly higher than the OCV (1.12 V) of same reactor fed with acetate (Zhang and Angelidaki, 2012), which indicated that glycerol was easily degradable as simple substrate such as acetate in SSMFC stack. It was also noticed that at lower current zone, the voltage in series connection decreased quickly, which could be caused by the activation overpotential (Zhang and Angelidaki, 2012).

Moreover, it was also noted that the higher maximum current (5.73 mA) was as expected to appear in parallel connection compared to the current of 2.90 mA in series connection. The maximum power density of series connection (488 mW/m^2) was slightly higher than that of parallel connection (450 mW/m^2). However, the maximum power density in series stack was observed at 2.20 mA (0.40 V), while it was observed at a relatively higher current of 2.99 mA (0.27 V). In a previous study (Aelterman et al., 2006), the OCV was 4.16 V (series) and 0.67 V (parallel) when six MFCs were connected in parallel and series respectively. The OCV achieved in this study was comparable to that obtained in their work considering that only two cell units were connected here. The maximum power density was much higher than that observed in their research, in which the maximum power densities were 308 mW/m^2 (series) and 263 mW/m^2 (parallel). Furthermore, based on the polarization curve, internal resistance of SSMFC was 296 Ω (series) and 130 Ω (parallel), respectively. The lower internal resistance in parallel could be due to the increased surface area for electron flow (Yazdi et al., 2015).

Figure 2 is here

The results indicated that parallel connection of SSMFC can lead to high current while series connection can boost the voltage output. Ye et al. (2014) also observed the similar trend in their MFC stack consisted of four cell units. Winfield (Winfield et al., 2012) has mentioned that shunt losses, which took place via fluidic or electrical connections in the series, most likely resulted in the superiority of current in parallel over that in series. Besides, according to Aelterman et al. (2006), the microbial community decreased in diversity during stack operation in series. Since the microbial community determined the organic degradation, it could influence the electricity generation and electron flow in the circuit. Thus, the different microbial community may also be a reason for the different electricity output in series and parallel connections.

Figure 3 is here

3.2 Effect of series and parallel connection on substrate degradation in SSMFC

From the beginning, glycerol with the concentration of 1000 mg/L was used as the sole substrate. In order to get a better understanding of substrate degradation during the operation, the glycerol concentration and VFA composition in series and parallel connections were tested along the operation time (Fig.3). As shown in Fig.3, in series connection, glycerol concentration decreased from 1000 mg/L to 0 mg/L after 48 h operation, while it only took about 24 hours in parallel connection. The faster the degradation of glycerol, the more electrons produced. Compared to the degradation of glycerol in series connection, it was much faster in parallel connection (approx. 2 times higher), which was also in accordance with the higher current appeared in parallel connection (approx. 2 times higher) (Fig. 2). Higher current often indicates production of more electrons from oxidation of organic matter. It has been reported that the higher current densities contributed to a more rapid chemical oxygen demand (COD) removal in MFC stacks (Winfield et al., 2012). Thus, the parallel connection of the SSMFC accelerated the electron flows and in return promoted the substrate degradation, which could explain the relatively higher glycerol degradation. Though only two cell units were utilized, the organic matter removal rate observed in this study was comparable or even faster than that of previously reported MFC stacks powered by pure chemicals (e.g., acetate) (An et al., 2016; Wu et al., 2016). The fast glycerol degradation could be attribute to the compact configuration of SSMFC which reduced the internal resistant. Wu et al. (2016) reported that in parallel connection of three MFCs power by sodium acetate, the COD concentration could sharply decrease from 1200 mg/L to 700 mg/L within 3 hours. In a five-MFCs stack reported by Zhuang et al.(2012), the COD concentration in series stack and parallel stack was degraded from 5845 to 1190 and 948 mg/L, resulting in the removal efficiency of 79.6% and 83.8%, respectively. The relatively higher COD removal part reason may be that the batch operation adopted in this study allowed enough time for organic matter to be oxidized by bacteria. No significant degradation of glycerol

213 was observed in the anaerobic reactor without anodic biofilm (data was not shown), which excluded
214 the contribution of suspended biofilm to the substrate degradation. In addition, similar or even
215 smaller ratio between anode surface (or volume of MFC) and anaerobic reactor has been widely
216 adopted in previous studies (Zhang and Angelidaki 2015, Heidrich et al. 2014). Thus, the size of
217 anodic biofilm in this study could be adequate for substrate degradation. Other operational factors
218 may also influence the substrate degradation, but the differences observed in here were mainly
219 caused by connection, as all other conditions/factors were kept at the same level.
220 Although the glycerol was fully degraded after 48 hours in series connection, the further
221 degradation of intermediates such as acetate and propionate still took more time (approx. 121 h).
222 Comparatively, the utilization of degradation intermediates in parallel connection is more faster (89
223 h). In MFCs, beside stack connection, the microbial communities of the enriched anodic biofilm
224 could also determine the features of electron transfer. If the microbial communities of biofilm
225 changed, the electrochemical properties such as electrical conductivity and redox potential would
226 also change as well. These parameters would also in turn influence the substrate degradation. Thus,
227 in addition to the stack connection, the different organic degradation rates between series and
228 parallel connection might also be related to the different microbial communities on anode (evidence
229 shown in section 3.5).

230 **3.3 Effect of glycerol concentration on system performance**

231 In order to investigate how the initial glycerol concentration affected the maximum power density
232 in different stack modes, the SSMFC was operated at an external resistance that equal to internal
233 resistance (296 Ω for series connection and 130 Ω for parallel connection). As shown in Fig.4, the
234 maximum power density in both series connection and parallel connection notably increased with
235 the glycerol concentration. For instance, when the glycerol concentration increased from 100 to
236 2000 mg/L, the maximum power density increased from 21 to 511 mW/m^2 in series, while it

increased from 63 to 473 mW/m^2 in parallel connection. The similar trend was also observed in previous study, in which the maximum power density of serially stacked MFCs also increased with the increasing of influent COD (Wu et al., 2016). The results indicated that substrate availability was important to the power generation regardless of the way of stack connection.

Figure 4 is here

It was noted that the maximum power density was higher in series than that in parallel connection, which was different from the previous observation (Winfield et al., 2012). In previous study, the maximum power density increased with the increasing of acetate concentration both in series and parallel. The different results observed here was mainly owing to the different reactor configuration which would affect the system performance. Due to diverse microbial composition in different anode biofilms, the open circuit voltage and the internal resistance may be different among MFCs. When two cells are connected in parallel, even a small variation between them may result in adverse interactions (e.g., one cell was discharging while another was in charging). Thus, the unsuitable parallel connection may lower the power output, which was also observed in previous studies (Sun et al., 2009). In addition, voltage reversal could also happen in parallel operation, which could also cause lower power density. Thus, the cause of relatively lower power density in parallel mode was likely to be due to a combination of several reasons such as voltage reversal, internal resistance and microbial community.

3.4 Effect of short-term changes in stack connection on system performance

In practical applications, higher current is wished when chemical reduction in cathode chamber is the goal, whereas it is higher voltage when the application is for power supply. In order to investigate how the system electricity generation was affected by the switch of stack mode, one set of SSMFC was switched from series to parallel connection, and the other identical set of SSMFC inversely was changed from parallel to series connection. Every connection mode was operated for

around 100 h. Fig.5 shows the voltage variation across external resistance (1000 Ω) during the whole operation. Both set of SSMFCs were operated in single cell mode fed with glycerol. It's very fast to achieve the stable voltage (0.42 V) and maintained for about 150 hs. Due to the substrate depletion, the voltage decreased close to 0.01 V after 168 h. The voltage was improved in series connection and parallel connection mode. It was consistent with previous report that the series and parallel connection of MFCs could increase the voltage output contrast to the single cell (An et al., 2014). The voltage in series connection (during two consecutive batches S1 and S2) theoretically should be double of single cell according to Ohm's law. Nevertheless, the real voltage output during S1 and S2 was 0.60 V, which was a little lower than the theoretical value. It could be due to the overpotential on the electrode (Zhong et al., 2011).

Figure 5 is here

When SSMFC was changed from series (S2) to parallel (P1) connection, it was very fast to achieve the stable voltage output. However, when SSMFC was changed from parallel (P2) to series (S1) connection, there was a lag period (shown in S1 of Fig.5b). When SSMFC was connected in series in second batch, the voltage increased immediately and maintained a stable value around 0.60 V. This could be due to the adaption of anodic microbial community to the new connection mode. It has been reported that the microbial community might change due to the high current and possible voltage reversal during parallel connection (Aelterman et al., 2006). It has also been found that parallel operation may deteriorate the performance of one or more cell units in the circuit due to the varied open circuit and internal resistant among the cell units in the stack (Sun et al., 2009). Thus, the anodic biofilm might be negatively affected by the parallel operation and thus recovery period was required. Our previous research has also demonstrated that the internal resistance was the key factor determining the voltage reversal in stacked MFC systems (Zhang et al., 2011b). In this work,

284 when the SSMFC was switched from parallel to series, the corresponding internal resistance was
285 changed from 130 to 296 Ω, which might cause voltage reversal in batch S1..
286 Therefore, for the practical application especially when the change in stack connection is required,
287 it should be paid attention to the adaption time of microbial community when switching from
288 parallel to series connection.

289 **3.5 Microbial communities**

290 Microbial communities established before and after switching to new connection mode were
291 analyzed by PCR-DGGE and 16S rRNA sequencing. The biofilm on anode were sampled at the end
292 of each batch and the DGGE profiles were summarized in Fig.6. Based on the migration distance,
293 intensities and similarities between the lanes on the DGGE gel, the banding patterns of biofilm on
294 the two anodes were same during the single cell operation. After stacking two MFC units into series
295 connection, the patterns of the bands for both anodes were still same but they were greatly different
296 from that in single cell mode (from S_{11}). The similarities between lanes (I_1 and S_{11}, I_2 and S_{21}) were
297 lower than 50%, and some new bands appeared (e.g., bands 3, 6, 7 and 9). It is clear that in the stack
298 operation, some electrochemically active bacteria might have been enriched. When changing series
299 into parallel connection, the banding patterns of biofilm remained unchanged, suggesting the stable
300 microbial community compositions. This could explain why the electricity production was not
301 negatively affected after switching series to parallel connection (as shown in Fig.5). Additionally, it
302 is also noted that in the second batch of parallel operation, the banding patterns of anodic biofilm
303 (P_{12} and P_{22}) started to differentiate between anode 1 and anode 2. The intensities of some bands
304 became stronger (e.g., bands 5 and 11), while some bands became weaker and even disappeared
305 (e.g., bands 6 and 7).

306 **Figure 6 is here**

In Fig.6b, the patterns of bands also changed greatly after changing the MFC single cell into parallel connection. The similarities between four lanes (I_1 and P_{11}, I_2 and P_{21}) were lower than 50% and some new bands appeared (e.g., bands 3, 6, 7 and 9), suggesting the dominant species changed during the shift from single cell to parallel stack operation. With operation time increasing, in the second batch, the microbial community compositions started to be different on the anodes of two cell units. The similarities between band P_{12} and P_{22} were lower than 30%, indicating the microbial communities were affected by the parallel connection. On anode 2 (band P_{22}), some bands disappeared (e.g., bands 2, 4, 5, 6, 7, 13 and 9), while some new bands appeared (e.g., bands 10, 11 and 12). When it was changed from parallel to series mode, obvious change in the banding patterns was observed. The similarities between two lines (S_{11} and S_{21}) were lower than 15%, suggesting the influence of microbial communities by parallel operation still continued even after switching into series connection. The changes in microbial community were in consistence with the adaptation time appeared in voltage generation during the first batch of series connection, as shown in Fig.5b. However, with series operation continuing, the banding patterns between anode 1 and anode 2 tended to be similar. The similarities between two lines (S_{12} and P_{22}) were higher than 90%, suggesting the microorganisms were recovered in the second batch of series operation.

Table 1 is here

In order to provide greater insight into the microbial ecology and diversity, bacterial 16S rRNA gene libraries were examined (Table 1). The microbial community in the biofilm of single MFC unit was dominated by *Betaproteobacteria* (33.3% of sequenced bands), *Alphaproteobacteria* (33.3%), followed by *Thermomonas* (16.7%) and *Flavobacteriia* (16.7%). After stacking the units into series connection (Fig.6a), the microbial community became more diverse, and the biofilm was dominated by *Betaproteobacteria* (28.6%) and *Alphaproteobacteria* (28.6%), followed by *Deltaproteobacteria* (14.3%), *Thermomonas* (14.3%) and *Flavobacteriia* (14.3%). Aelterman

(Aelterman et al. 2006) reported that microbial community became more diverse in the stack configuration. After switching series to parallel connection, the diversity of microbial community didn't change significantly, and the biofilm was dominated by *Betaproteobacteria* (36.3%), followed by *Alphaproteobacteria* (27.3%), *Deltaproteobacteria* (18.2%), *Thermomonas* (9.1%) and *Flavobacteriia* (9.1%). In the case of switching from single cell to parallel connection (Fig.6b), the microbial community changed greatly and the dominant bacteria in biofilm was *Alphaproteobacteria* (33.3%), followed by *Deltaproteobacteria* (22.2%), *Betaproteobacteria* (22.2%), *Thermomonas* (11.1%) and *Flavobacteriia* (11.1%), which actually stayed the 85% similarities with the microbial population in series connection in Fig.6a. In other words, whether stacking the MFC units into series or parallel, the dominant microbial community which might possess electrochemical activity almost remained the same composition in both conditions. After switching the parallel connection to series connection, the microbial community in the first batch of series connection showed a great change and the biofilm was dominated by *Betaproteobacteria* (25%), *Deltaproteobacteria* (25%) and *Alphaproteobacteria* (25%), followed by *Thermomonas* (12.5%) and *Flavobacteriia* (12.5%). However, in the second batch, the microbial communities tend to become stable and the biofilm was dominated by *Betaproteobacteria* (36.3%), followed by *Alphaproteobacteria* (27.3%), *Deltaproteobacteria* (18.2%), *Thermomonas* (9.1%) and *Flavobacteriia* (9.1%), further indicating the microbial communities could be recovered with longer running time.

Stack MFCs configuration greatly affected the composition of microbial community. Sequence related to *Deltaproteobacteria* was detected after stacking the MFC units. This specie recovered from band 6 and 7 appeared to be phylogenetically related (95% and 89% similarity) to the genus *Geobacter*. Previous studies have confirmed that *Geobacter sulfurreducens* and *Geobacter metallireducens* had conductive pili which was very helpful to transfer electrons to electrode

355 directly (Bond and Lovley, 2003; Richter et al., 2008; Gregory et al., 2004; Eaktasang et al., 2016).
356 The appearance of *Geobacter* after stacking MFCs demonstrated the enrichment of
357 electrochemically active microorganisms. The bands in series connection and parallel connection of
358 Fig.6a showed almost the same composition, suggesting the microbial communities remained stable
359 during this stack switching. Comparatively, in Fig.6b, when it was changed from parallel to series
360 connection, especially in the first batch of series operation, bands showed a great difference. The
361 intensities of band 5 and 6 became weaker, suggesting *Geobacter sulfurreducens* became minor
362 microorganisms during the parallel connection. As previously reported (Sun et al., 2009), the
363 parallel operation could deteriorate the electrochemically active bacteria due to the different voltage
364 output from each cell unit. The sequence of band 3 showed 96% similarity to the *Acidovorax ebreus*
365 which was isolated from anaerobic iron-oxidizing bacterium. It has been confirmed that ferric could
366 be used as the electron acceptor in MFC anode (Feng et al., 2016; Tran et al., 2015; Nguyen et al.,
367 2015). The gene sequence from band 9 showed 92% similarity to the *Rhodopseudomonas sp.*
368 *AAP120* which was identified on anodes in MFCs (Sanchez-Herrera et al., 2014; Park et al., 2014;
369 Teng et al., 2010). The intensities of band 6 and 7 became weaker in series connection, explaining
370 the adaptation time appearing in the voltage generation after switching parallel into series
371 connection as shown in Fig.5b.

372 **3.6 Significance and outlook**

373 Stack operation is an important approach for the practical utilization of the electric energy generated
374 by MFC. To the best of our knowledge, this work for the first time demonstrated how the MFC
375 stack responded to the short-term changes in the stack connection in the view of system
376 performance and microbiology. It was proved in this study that the changes in stack connection can
377 affect the power generation and anodic microbial community, especially when the connection was
378 changed from parallel to series. The scientific outcomes may provide new knowledge in the area of

379 microbial electrochemistry, push forward the future research on practical application of MFC stacks,
380 and assist future development of cost-effective MFC stacks for complex substrate degradation and
381 production of renewable energy.

382 Though promising, more efforts should be made before industrial application of SSMFC stacks.
383 First of all, further investigation of the long-term impact result from connection changes is required.
384 Secondly, pilot or large-scale SSMFC stack should be developed and tested in order to accelerate
385 the commercialization of the technology. Thirdly, the effect of connection on the SSMFC stack
386 should be further tested in different subsurface environments such as sediment or groundwater, in
387 order to broad the application fields. Lastly, any strategy (e.g., better control of the biofilm) that can
388 avoid the adverse effect from connection changes in SSMFC should be pursued.

389 **4. Conclusions**

390 This study, for the first time, demonstrated how the SSMFC performance fed with glycerol was
391 affected by the connection changes during stack operation. The system performance, in terms of
392 power generation, current, substrate degradation and microbial community, had different response
393 to the change of connections. Glycerol as substrate degraded much faster in parallel than that in
394 series connection. It was also found that maximum power density increased with the increasing of
395 glycerol concentration both in series and parallel connection, whereas the maximum power density
396 in series was a little higher than that in parallel connection all the time. During the switching from
397 one connection mode to the other, the voltage output and microbial communities were changed
398 differently. Adaptation time for microorganisms in the case of switching parallel to series
399 connection was needed. When SSMFC was connected in series, followed by parallel stack,
400 microbial communities remained stable. Comparatively, microbial communities were greatly
401 affected by the parallel connection when SSMFC was operated in parallel stack first. Elucidating
402 the response of system performance to different stack connection modes will assist in the practical

application of SSMFC in the future and also give another new way to get profitable values (electricity) from glycerol.

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544 Table 1. DGGE 16S rRNA gene band identifications

Band	Phylum	Class[a]	GenBank closest match (Accession no.)	Identity (%)[b]	Isolation source
1	*Proteobacteria*	*Thermomonas*	uncultured Xanthomonadales bacterium SHBZ679 (EU639124)	94	Thermophilic microbial fuel cell
2	*Bacteroidetes"*	*Flavobacteriia*	Flavobacterium sp. PAMU-2.98 (AB118230)	97	Soils
3	*Proteobacteria*	*Betaproteobacteria*	Acidovorax ebreus TPSY (NC011992)	96	Anaerobic iron-oxidizing bacterium
4	*Proteobacteria*	*Betaproteobacteria*	Alcaligenes sp. PAOSE174 (AY994313)	97	Activated sludge
5	*Proteobacteria*	*Betaproteobacteria*	uncultured beta proteobacterium; l.34.p. (AY887015)	99	Fresh water
6	*Proteobacteria*	*Deltaproteobacteria*	Geobacter sulfurreducens PCA chromosome (NC002939)	96	Subsurface environments
7	*Proteobacteria*	*Deltaproteobacteria*	Geobacter metallireducens GS-15 (NC007517)	89	Aquatic/subsurface environments
8	*Proteobacteria*	*Alphaproteobacteria*	Devosia sp. LC5 contig7 (NZ_JNNO01000045)	98	Deep within Lechuguilla Cave
9	*Proteobacteria*	*Alphaproteobacteria*	Rhodopseudomonas sp. AAP120 AAP120_Contigs_108 (NZ_LJIC01000108)	92	Freshwater lake
10	*Proteobacteria*	*Betaproteobacteria*	Curvibacter sp. PAE-UM (NZ_KQ483358)	98	River sediment
11	*Proteobacteria*	*Alphaproteobacteria*	Nitratireductor indicus C115 contig44 (NZ_AMSI01000044)	98	Deep seawater
12	*Bacteroidetes*	*Flavobacteriia*	Sediminibacter sp. Hel_I_10 (NZJHZX01000001)	83	Seawater
13	*Proteobacteria*	*Alphaproteobacteria*	hizobium sp. YS-1r CONTIG.23 (NZ_JPYQ01000020)	94	Decaying Wood

545 [a] The phylotypes were assigned to phyla based on Ribosomal Database Project II taxonomy
546 classifications.
547 [b] The values represent the similarities between the associated DGGE band sequence and the closest-
548 match sequence from GenBank.

549 **Figure captions**

550 Fig.1 Schematic of the SSMFC.

551 Fig.2 Polarization curve in parallel (a) and series stack (b).

552 Fig.3 Substrate degradation as function of time in series (a) and parallel (b) stack.

553 Fig.4 Maximum power density as a function of initial glycerol concentration under different stack
554 modes.

555 Fig.5 The response of voltage output to short-term changes in stack connection. S1 and S2: first and
556 second batch of series connection; P1 and P2: first and second batch of parallel connection.

557 Fig.6 Bacterial community profiles revealed by DGGE. (a) Microbial community at the end of each
558 batch shown in Fig.5a. (b) Microbial community at the end of each batch shown in Fig.5b. I:
559 individual unit; S: series stack; P: parallel stack. I_1: MFC unit 1; I_2: MFC unit 2; S_{11}: unit 1 in series
560 stack in first batch; S_{21}: unit 2 in series stack in first batch; S_{12}: unit 1 in series in second batch; S_{22}:
561 unit 2 in series in second batch; P_{11}: unit 1 in parallel stack in first batch; P_{21}: unit 2 in parallel stack
562 in first batch; P_{12}: unit 1 in parallel stack in second batch; P_{22}: unit 2 in parallel stack in second
563 batch.

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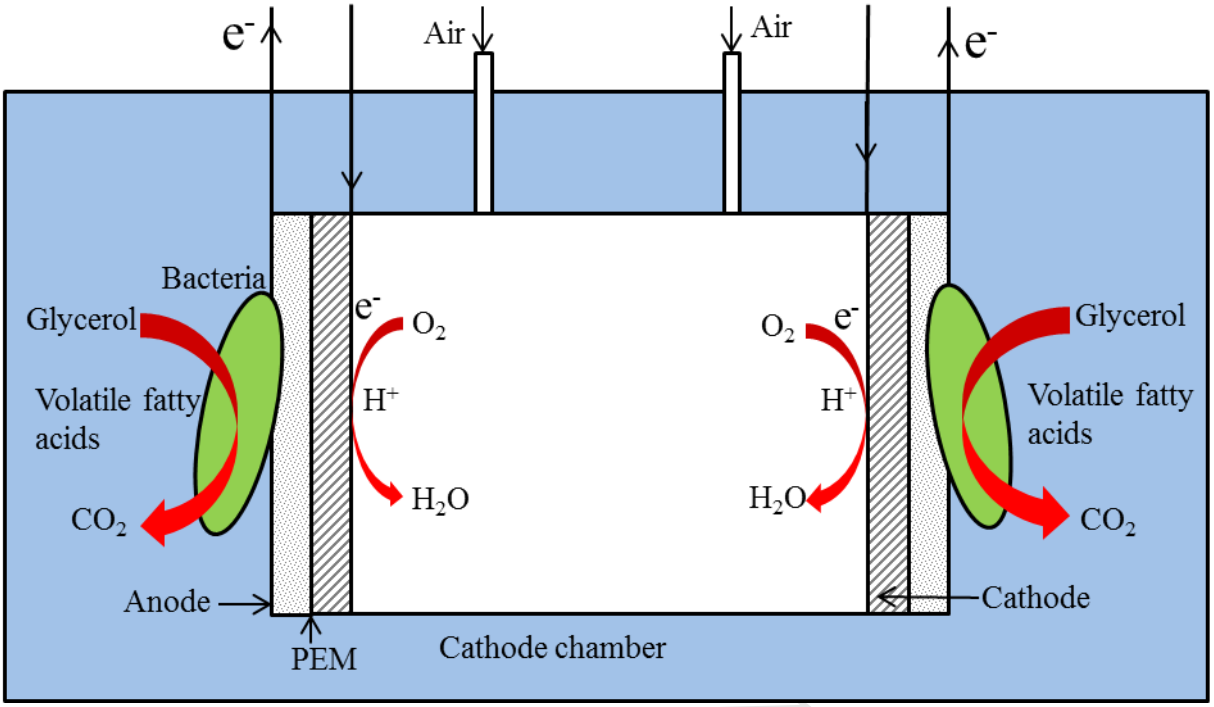

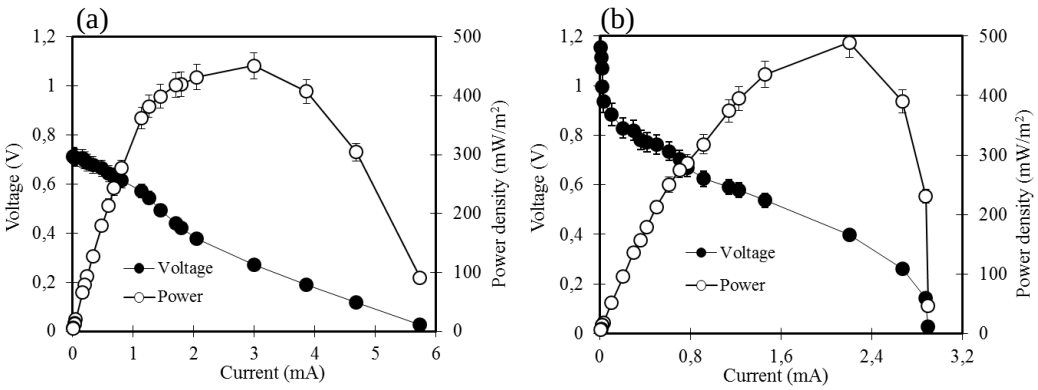

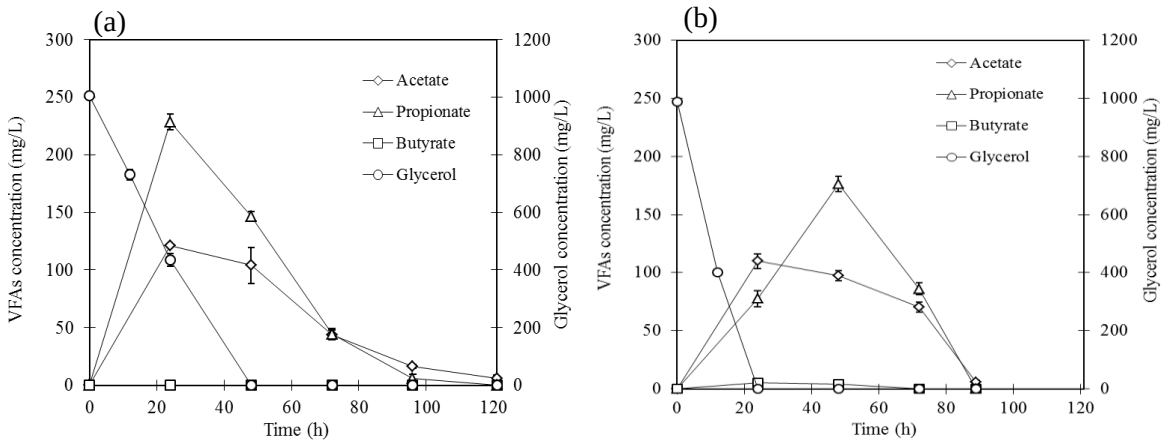

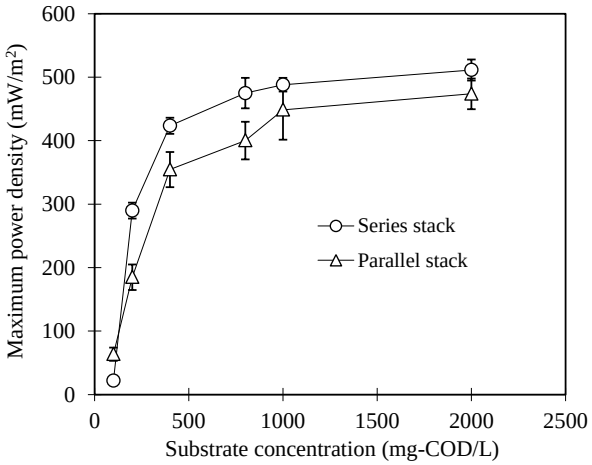

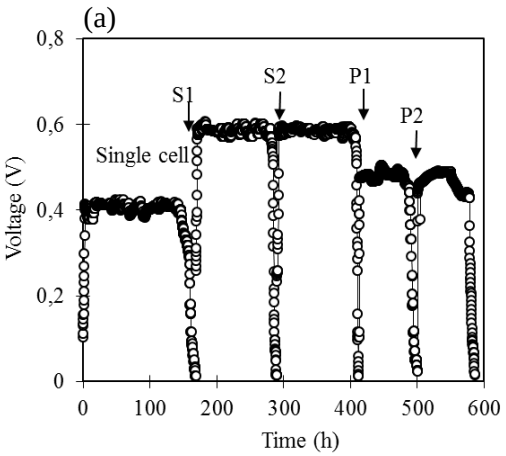

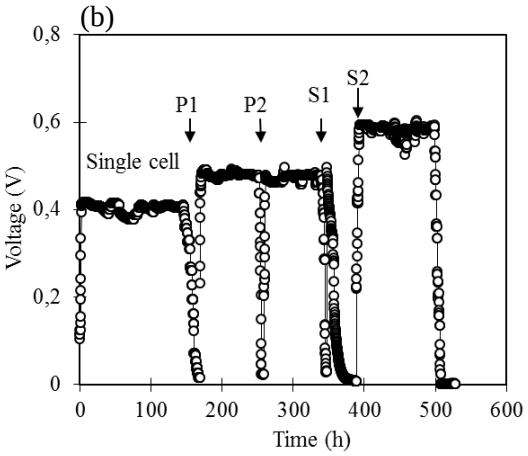

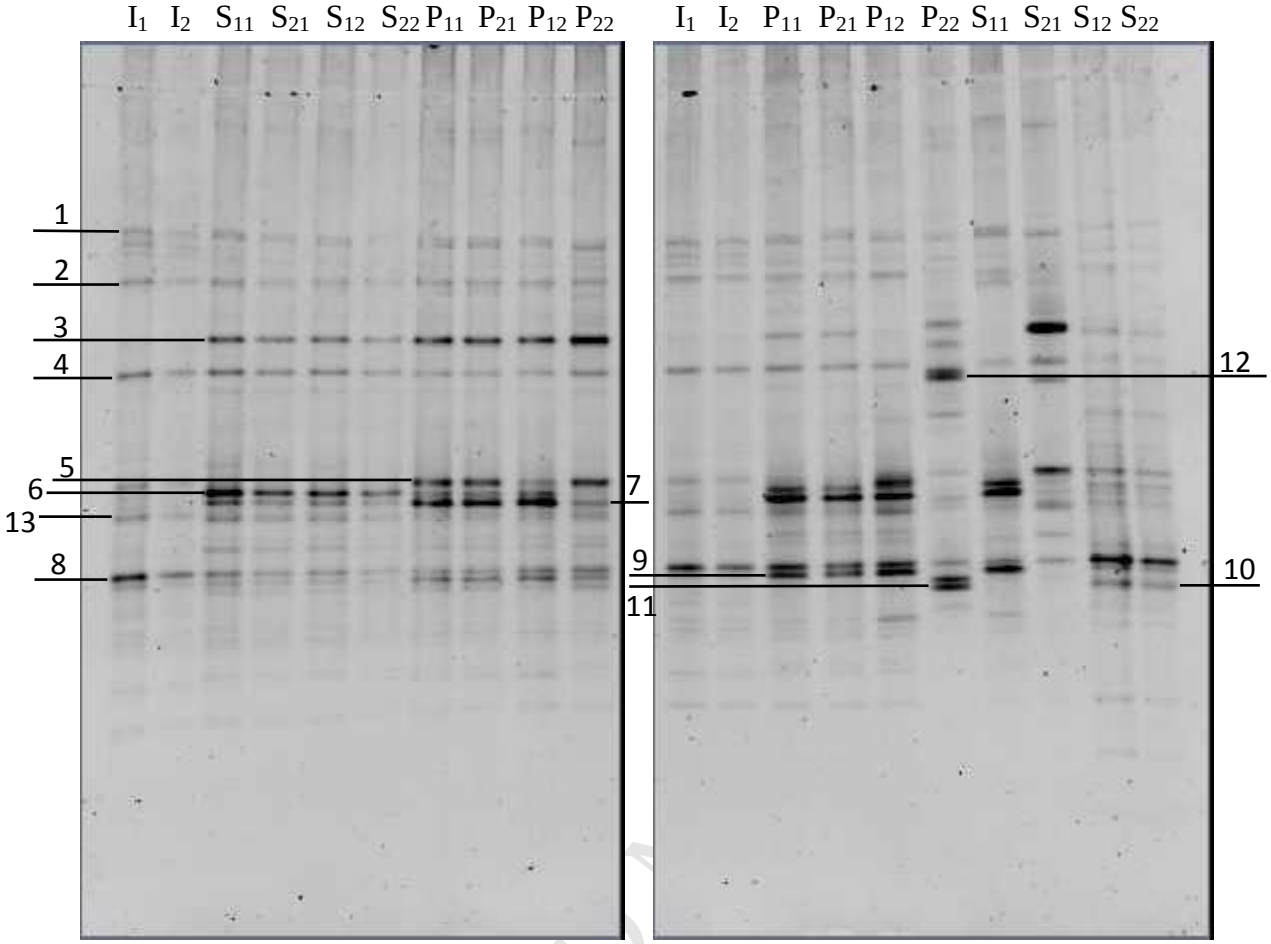

(a) (b)

- Self-stacked submersible microbial fuel cell powered by glycerol.
- Electricity production responding to short-term changes in stack connection.
- Adaption time needed when switching from parallel to series connection.
- Microbial community dependent on the way of changing stack connection.
- Microbial community was negatively affected by parallel connection.