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Factors Affecting the Performance of Single-Chamber Soil Microbial Fuel Cells for Power Generation*[1]

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ABSTRACT

There is limited information about the factors that affect the power generation of single-chamber microbial fuel cells (MFCs) using soil organic matter as a fuel source. We examined the effect of soil and water depths, and temperature on the performance of soil MFCs with anode being embedded in the flooded soil and cathode in the overlaying water. Results showed that the MFC with 5 cm deep soil and 3 cm overlaying water exhibited the highest open circuit voltage of 562 mV and a power density of 0.72 mW m^{-2}. The ohmic resistance increased with more soil and water. The polarization resistance of cathode increased with more soil while that of anode increased with more water. During the 30 d operation, the cell voltage positively correlated with temperature and reached a maximum of 162 mV with a 500 Ω external load. After the operation, the bacterial 16S rRNA gene from the soil and anode was sequenced. The bacteria in the soil were more diverse than those adhere to the anode where the bacteria were mainly affiliated to *Escherichia coli* and *Deltaproteobacteria*. In summary, the two bacterial groups may generate electricity and the electrical properties were affected by temperature and the depth of soil and water.

Key Words: electrogenic bacteria, impedance, soil depth, soil organic matter, voltage

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INTRODUCTION

Microbial fuel cells (MFCs) are devices that convert chemical energy directly into electricity. In an MFC, electrogenic bacteria degrade organic compounds under anaerobic condition and transfer electrons to anode. The electrons then flow through a conducting wire to cathode where the electron acceptors are reduced. The electrical current can be generated during the process. Materials with a large population of microorganisms and high content of organic matter have been used to generate power in MFCs, including marine sediment (Bond *et al.*, 2002; Scott *et al.*, 2008), sewage sludge (Zhang *et al.*, 2012), garden compost (Parot *et al.*, 2008), industrial/domestic wastewater (Rabaey and Verstraete, 2005) and animal waste (Yokoyama *et al.*, 2006). Soil generally has a bacterial population of approximately 10^9 cells g^{-1} (Whitman *et al.*, 1998) and organic matter content of within 100 mg g^{-1} (Bot and Benites, 2005), in spite of the variation between different soil types, *e.g.*, in organic soil, the abundance of bacteria and organic matter can be much higher (Troeh and Thompson, 2005). The presence of bacteria and organic matter endows soil with the potential to be a vast resource of electrical energy.

Studies on soil MFCs exhibited various directions. Ishii *et al.* (2008a) found that methane emission from soil, which was filled in the anode chamber, was suppressed after running an MFC. The reason could be that the soil organic carbon was reduced to generate electrical power rather than methane. Another study showed that by running two chambered MFCs for 10 d with phenol contaminated soil in the anode chamber, 90% of phenol was removed from soil, compared with 13% in non-MFC control (Huang *et al.*, 2011). Power generation was studied by inoculating rice paddy field

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soil with cellulose as the energy resource in a two-chamber MFC (Ishii *et al.*, 2008b). However, soil MFC without the carbon addition may generate power by using its own organic matter as a fuel. Moreover, the cost of a single-chamber MFC is much lower than that of a two-chamber one. To keep O_2 away from anode, single-chamber MFCs such as sediment-MFCs need a thick layer of soil or sediment, leading to a high internal resistance (Deng *et al.*, 2012), thus the performance of single-chamber soil MFCs deserves studies. In addition, several factors were reported to have a major impact on the performance of MFCs. Internal resistance of MFCs increased with distance between electrodes (Jang *et al.*, 2004); dissolved oxygen impaired the anaerobic conditions at anode and decreased power output (Kim *et al.*, 2007); temperature was a major factor that seriously affected microbial activity and thus the electrogenic activity (Min *et al.*, 2008). As a result, a study on the performance and the influencing factors of soil MFCs may help improve the efficiency of power output and soil remediation.

Electrogenic bacteria enrich on anode during the generation of current. Their composition differs between inoculums, although *Geobacter* and many other *Deltaproteobacteria* are well-known electrogenic bacteria and are often detected in anode biofilms (Franks *et al.*, 2010; Logan, 2009). The sequencing method based on 16S rRNA gene was intensively used to understand the presence of electrogenic bacteria. Liu *et al.* (2011) found that *Betaproteobacteria*, *Acetoanaerobium noterae* and *Chlorobium* sp. dominated the anode biofilm when MFCs were fed with domestic wastewater. Ishii *et al.* (2008b) found that *Clostridiales*, *Chloroflexi*, *Rhizobiales* and *Methanobacterium* dominated the anode biofilm in MFCs inoculated with rice field soil and fed cellulose as fuels. Kaku *et al.* (2008) operated plant-MFCs in which soil organic carbon and root exudates served as energy resources, and found that *Natronocella acetinitrilica*, *Beijerinckiaceae bacterium* and *Rhizobiales bacterium* were dominant on anode. However, the fed cellulose or rhizosphere effects (Moorhead and Reddy, 1988) are selective for specific bacteria in soil. For MFCs using soil organic carbon as an energy resource, understanding the dominant bacteria in anode biofilm could help improve the performance of this kind of MFCs by optimizing the living conditions for these bacteria.

In this study, we hypothesized that the performance including cell voltage, power output and internal resistance were affected by electrode distance and temperature. To testify the hypothesis, MFCs were set up with a series of soil and water depths, and correlations between temperature and cell voltage were studied. Bacteria from soil and from anode were cloned and sequenced based on 16S rRNA gene. The aims of this study were to understand i) the effect of soil and water depths, and temperature on the performance of soil MFCs; and ii) the electrogenic bacteria from soil.

MATERIALS AND METHODS

Soil sampling

Soil was collected in Jimei District, Xiamen City, China (118°02′ N, 24°37′ E). The climate is subtropical and wet with a mean annual precipitation of 1 200 mm and mean annual temperature of 21 °C. Soil sampling was carried out in October 2011. Soil was collected from an arid farmland at a depth of 0–20 cm. After sampling, soil was gently separated by hand and passed through a 2 mm mesh.

Soil chemical analysis

After thorough mixing, a part of the soil was air-dried for physiochemical analysis. Soil texture was determined using the pipette method (Gee and Bauder, 1986). Soil organic matter was measured using the $K_2Cr_2O_7$ method (Sims and Haby, 1971). NO_3^- and NH_4^+ were measured using ion chromatography (Mou *et al.*, 1993). Soil pH was measured at a soil-water ratio of 1:2.5 (w:v). The soil physiochemical properties were as follows: texture, clay loam; organic matter, 27 mg kg^{-1}; NO_3^-, 50.2 mg kg^{-1}; NH_4^+, 2.9 mg kg^{-1}; and pH, 6.5.

MFC set-up

Six MFC reactors were constructed in Oroglas each with a dimension of 50 cm × 30 cm × 30 cm (length × width × height) (Fig. 1). In each reactor, the anode and cathode were carbon felt with an area of 0.150 m^2 (50 cm × 30 cm) and 0.045 m^2 (30 cm × 15 cm), respectively. The thickness of the carbon felt was 0.5 cm. The anode was positioned close to the bottom of the reactor and embedded with soil while the cathode was fixed at the overlaying water surface. The water was added gently to the soil and the MFCs were operated after the overlaying water was clear. The anode and cathode was connected with a 1 000 Ω external load using titanium wire. To study the relationship between electrical properties and depths of soil and overlaying water, five reactors were assigned to the following treatments: i) 3 cm soil with 3 cm water (3S+3W); ii) 5 cm soil with 3 cm water (5S+3W); iii) 7 cm soil with 3 cm water (7S+3W); iv) 5 cm soil with 6 cm water (5S+6W) and v) 5 cm soil with 9 cm water (5S+9W). Here the soil

depth refers to the distance between anode surface and soil surface. The water depth refers to the distance between soil-water interface and water surface. To determine whether power was originated from microbial process or chemical process, an MFC reactor with soil sterilized by chloroform fumigation method (Wolf *et al.*, 1989) was used as control treatment.

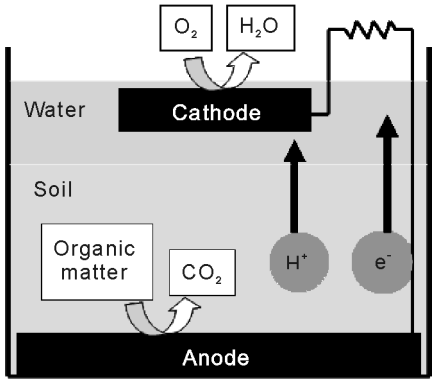

Fig. 1 Configuration of a microbial fuel cell using soil as energy resource.

Electrical properties and dissolved oxygen

Electrical properties of the MFCs treated with different depths of soil and water were determined after the voltage was stable at a constant temperature of 26 °C. Maximum power was assessed *via* polarization curves, which were obtained by varying external resistance (open circuit, 30 000, 10 000, 5 000, 2 000, 1 000, 500, 200, 100, 50, 20 and 10 Ω). Each resistance was connected for 20 min and the voltage was recorded using a data acquisition module. Impedance spectroscopy was determined by using Autolab potentiostat (Eco Chemie BV, the Netherlands) at a sinusoidal excitation potential of 10 mV and frequencies from 10^{-2} to 10^{5} Hz. Impedance for the MFCs was determined in a two-electrode mode with anode serving as working electrode and cathode as counter electrode. The impedance spectroscopy was measured three times for each MFC. The concentration of dissolved oxygen at the anode surface and at water surface was determined at three points with an interval of 20 cm in each MFC by using a hand-held dissolved oxygen instrument (YSI Inc., Yellow Spring, USA).

Dynamic performance of an MFC

The MFC that generated the highest voltage was connected with a 500 Ω external load and the voltage had been monitored for 30 d in a greenhouse. The voltage was recorded using a data acquisition module. The air temperature was recorded every 10 min using a Vaisala MAWS301 automatic weather station (Vaisala Inc., Finland).

Three rhizon samplers (Rhizosphere Research Products, Wageningen, Netherlands) each with a filter (mesh size 0.45 μm) were embedded in the soil and above the anode at an interval of 20 cm. Six milliliters of water was extracted in each rhizon sampler before and after the 30 d operation. The extract was subjected to pH and dissolved organic carbon (DOC) analyses with a pH meter and a TOC-V analyzer (Shimadzu Co., Japan), respectively.

DNA extraction

After the 30 d operation, a piece (1.5 cm × 1.5 cm) of the anode and 0.5 g soil above the anode was collected. The anode was rinsed with sterile deionized water before DNA extraction. The genomic DNA collected from the anode and soil was immediately extracted using the FastDNA® Spin kit for soil (Bio101 Inc., Carlsbad, USA) following the manufacturer's instructions. The purity and the quantity of the DNA were determined using a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, USA) at 230, 260 and 280 nm.

Cloning, sequencing and phylogenetic analysis

The DNA extracts from anode and soil served as the templates for polymerase chain reaction (PCR). The primer set 341F (5′-ACG GGG GGC CTA CGG GAG GCA GCA G-3′) and 534R (5′-ATT ACC GCG GCT GCT GG-3′) was used in the PCR amplification of 16S rRNA gene fragments (Muyzer *et al.*, 1993). The 50 μL reaction mixtures contained 1 μL of template DNA, 1 μL of each 1 μmol L^{-1} primer, 5 μL of 10 × buffer (Mg^{2+} plus), 4 μL of 10 mmol L^{-1} dNTPs mixture (2.5 mmol L^{-1} of each) and 2.5 units of *Taq* DNA polymerase (Sangon Biotech Co., Ltd., Shanghai, China). Thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles consisting of denaturation at 94 °C for 1 min, primer annealing at 52 °C for 40 s, and elongation at 72 °C for 40 s. The final elongation step was extended to 10 min. PCR products of the correct size were purified and then ligated to pMD18-T easy vector and transformed into *Escherichia coli* DH5α competent cells. The bacteria were transferred into Luria-Bertani (LB) agar plates containing ampicillin, X-Gal and isopropyl β-D-1-thiogalactopyranoside (IPTG). After incubation overnight at 37 °C, white colonies (putative positive clones) were picked as correct inserts. Two clone libraries each with 100 white clones were constructed for

soil and anode, respectively. The clones with correct insert size were used for sequencing analyses (Invitrogen Co., Ltd., Shanghai, China). Vector sequences were removed using DNAStar Lasergene 7.1. The gene fragment sequences were subjected to taxonomic assignments by comparing the sequences with the non-redundant nucleotide database on BlastX http://ncbi.nlm.nih.gov/blast).

Calculations and statistical analysis

The power (P, W) and power density (PD, W m^{-2}) were calculated with the following formula:

$$P = U^2/R \tag{1}$$

$$\mathrm{PD} = P/A \tag{2}$$

where U, R and A is the voltage (V), external load (Ω) and anode area (m^2), respectively. The quantity of electrons, Q (coulomb, C), produced from soil during the 30 d was evaluated with the following formula:

$$Q = \sum_{n=1}^{4212} \frac{(I_n + I_{n+1})}{2} \times 600 \tag{3}$$

where I is the current (A), n is the number of data that were recorded by a data acquisition module with a time interval of 600 s. There are a total of 4 212 data points.

The data of impedance spectra were fitted to an equivalent electrical circuit using the autolab impedance analysis software FRA 2 (Eco Chemie BV, the Netherlands). The total resistance (R) is defined as:

$$R = R_\Omega + R_\mathrm{P}^\mathrm{a} + R_\mathrm{P}^\mathrm{c} \tag{4}$$

where R_Ω is ohmic resistance, R_P^a and R_P^c is the polarization resistance at anode and cathode, respectively (Manohar *et al.*, 2008).

The significant correlation between cell voltage and air temperature was determined with the Pearson's correlation coefficient (r). The significance in changes of DOC concentration and soil pH before and 30 d after the MFC operation was determined at the level of $P < 0.05$ using independent-samples t-test. One-way ANOVA was used to determine the significant difference of dissolved oxygen between the MFCs at the level of $P < 0.05$ using Duncan's test. All statistical tests were performed using SPSS software (version 14.0).

RESULTS AND DISCUSSION

Impedance spectroscopy

Equivalent circuit model that comprised two resistance-constant phase element (R-CPE) parallel circuits and an ohmic resistance was utilized to fit the impedance data (Figs. 2 and 3). Constant phase element (CPE) was employed in simulation because the electrode surface was rough and thus the dispersion effect was strong. R_Ω, R_P^a and R_P^c explain major parts of internal resistance. The R_P^c value was much higher than R_P^a (Table I), indicating that the oxidation of organic matter at the anode was much easier than the reduction of oxygen at the cathode. The use of modified cathode could accelerate the oxygen reduction rate and increase the power output (Zhao *et al.*, 2005). There was a trend that R_Ω increased with the depths of soil and water or electrode distance. It was notable that R_P^a sharply increased from 67.6 to 526.5 Ω when the water depth increased from 3 to 9 cm. The possible reason could be that carbon substrates were diluted with water and the anodic reaction rate was lowered. The R_P^c value increased from 416.5 to 611.5 Ω when soil depth increased from 3 to 7 cm at a constant 3 cm water depth. As shown in Fig. 4, the dissolved oxygen on the cathode significantly decreased with the increase of

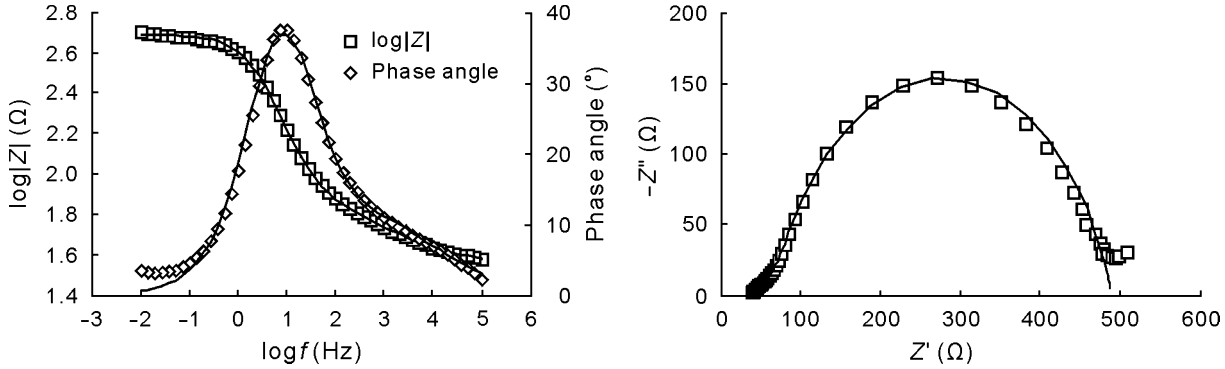

Fig. 2 Impedance spectra obtained in two electrode modes for a microbial fuel cell in the reactor treated with 3 cm soil and 3 cm overlaying water. $|Z|$ = impedance amplitude spectra; Z'' = imaginary impedance; Z' = real impedance; f = the single frequency of a monochromatic signal (Bard and Faulkner 2000; Barsoukov and Macdonald, 2005).

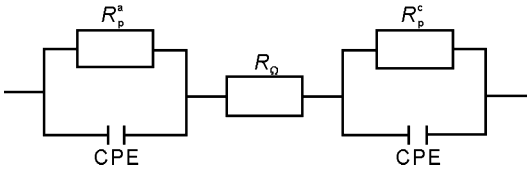

Fig. 3 Equivalent circuit for the analysis of impedance spectra data. R_P^a = the polarization resistance of anode; R_P^c = the polarization resistance of cathode; R_Ω = ohmic resistance; CPE = constant phase element.

TABLE I

Distance between anode and cathode and the total resistance (R), which is the sum of ohmic resistance (R_Ω) and polarization resistance of anode (R_P^a) and cathode (R_P^c), in reactors treated with different depths of soil and water

Treatment[a)]	Distance	R_Ω	R_P^a	R_P^c	R
	cm			Ω	
3S+3W	6	36.0	37.3	416.5	489.8
5S+3W	8	43.6	67.6	362.1	473.3
5S+6W	11	42.0	194.1	319.0	555.1
5S+9W	14	45.0	526.5	402.2	973.7
7S+3W	10	53.6	52.9	611.5	718.0

[a)] 3S+3W = 3 cm soil with 3 cm overlaying water; 5S+3W = 5 cm soil with 3 cm overlaying water; 5S+6W = 5 cm soil with 6 cm overlaying water; 5S+9W = 5 cm soil with 9 cm overlaying water; 7S+3W = 7 cm soil with 3 cm overlaying water.

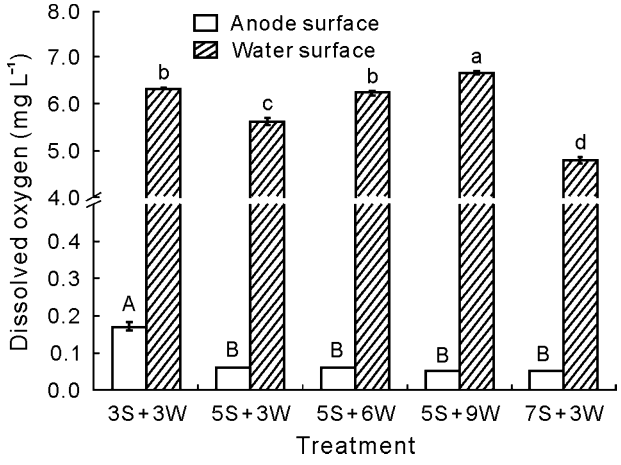

Fig. 4 Dissolved oxygen concentrations at the bottom of microbial fuel cell reactors and at water surface as affected by different depths of soil and water. 3S+3W = 3 cm soil with 3 cm overlaying water; 5S+3W = 5 cm soil with 3 cm overlaying water; 5S+6W = 5 cm deep soil with 6 cm overlaying water; 5S+9W = 5 cm soil with 9 cm overlaying water; 7S+3W = 7 cm soil with 3 cm overlaying water. Vertical bars represent standard errors of means. Bars with the same letter are not significantly different ($P < 0.05$, $n = 3$) within anode surface (uppercase letter) or within water surface (lowercase letter) by Duncan's test.

soil depth. A possible reason was that the dissolved soil organic matter and oxygen demand increased in overlaying water with soil addition and thus the cathodic reaction rate declined. Since there was no replicate for impedance measurements, the significance of the impedance data was unknown.

Open circuit voltage (OCV) and power output

The OCV showed a trend of 5S+3W (517 mV) > 7S+3W (451 mV) > 3S+3W (350 mV), and 5S+3W > 5S+6W (506 mV) > 5S+9W (470 mV) (Fig. 5). The OCV in fumigated control was around 15 mV, indicating that the microorganisms played a crucial role in power generation. The power density of MFCs with different soil and water depths showed the same trend as OCV. The maximum power density and power of 5S+3W was 0.72 mW m^{-2} and 0.11 W, respectively. The concentration of dissolved oxygen on the anode surface of 3S+3W was 0.17 mg L^{-1}. It decreased to 0.06 mg L^{-1} in 5S+3W and 0.05 mg L^{-1} in 7S+3W (Fig. 4). A thicker layer of soil could more effectively limit oxygen diffusion to the anode surface, however, the internal resistance increased with more soil and water. The 5S+3W treatment effectively reduced oxygen content on the anode surface and meanwhile, had a relatively low resistance compared to other treatments (Table I), thus generating the highest voltage and

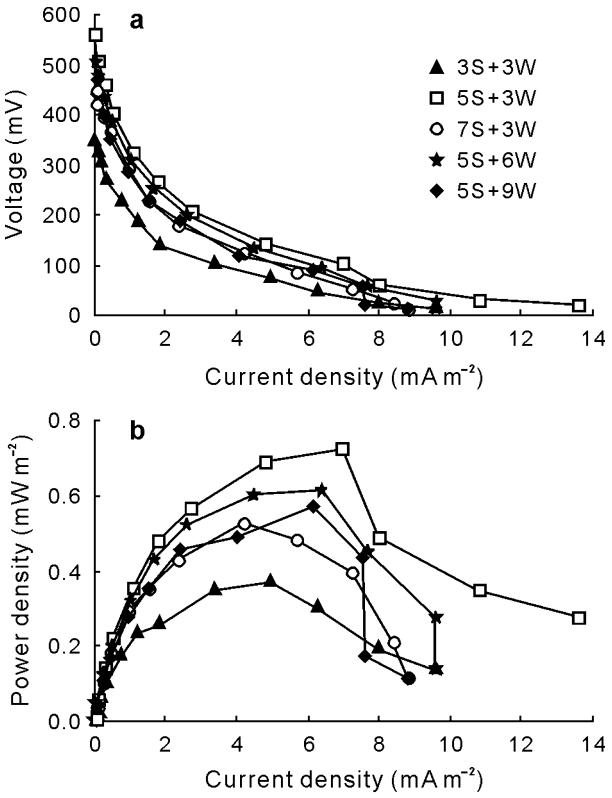

Fig. 5 Polarization curve (a) and power density (b) of microbial fuel cells in reactors as affected by different depths of soil and water. 3S+3W = 3 cm soil with 3 cm overlaying water; 5S+3W = 5 cm soil with 3 cm overlaying water; 7S+3W = 7 cm soil with 3 cm overlaying water; 5S+6W = 5 cm soil with 6 cm overlaying water; 5S+9W = 5 cm soil with 9 cm overlaying water.

power. The power output could be further improved by adopting proper methods. An increase of the anode area could probably improve the columbic efficiency (Deng *et al.*, 2012). The decomposition rate of organic matter can be accelerated at a temperature of over 30 °C (Kirschbaum, 1995) and the reduction rate of oxygen on cathode can be improved by more effective electrode materials (Zhao *et al.*, 2005). Tender *et al.* (2008) used a sediment MFC that produced 30 mW to power a meteorological oceanographic buoy. Theoretically, the power can be possibly achieved with soil, which will function in a land ecosystem. The power can be harvested and stored for further needs. Moreover, the MFCs in our study were membrane free so that a large proportion of cost would be exempted in scaling up single-chamber MFCs (Deng *et al.*, 2012).

Dynamic performance of an MFC

The voltage of the MFC with an external load of 500 Ω and the air temperature was recorded during the 30 d operation (Fig. 6). The voltage increased from 45 mV to a maximum of 162 mV during the first 9 d and then fluctuated around 100 mV. The voltage exhibited circadian oscillation that reached a maximum a little after noon (around 1:00–3:00 p.m.) and declined to the minimum at early morning (around 3:00–5:00 a.m.), except in the first 2 d when the cell voltage continuously increased. Correlation analysis showed that the voltage significantly correlated ($r = 0.51$, $P < 0.01$) with the air temperature. This is possibly because the metabolic activities of electrogenic bacteria are temperature dependent.

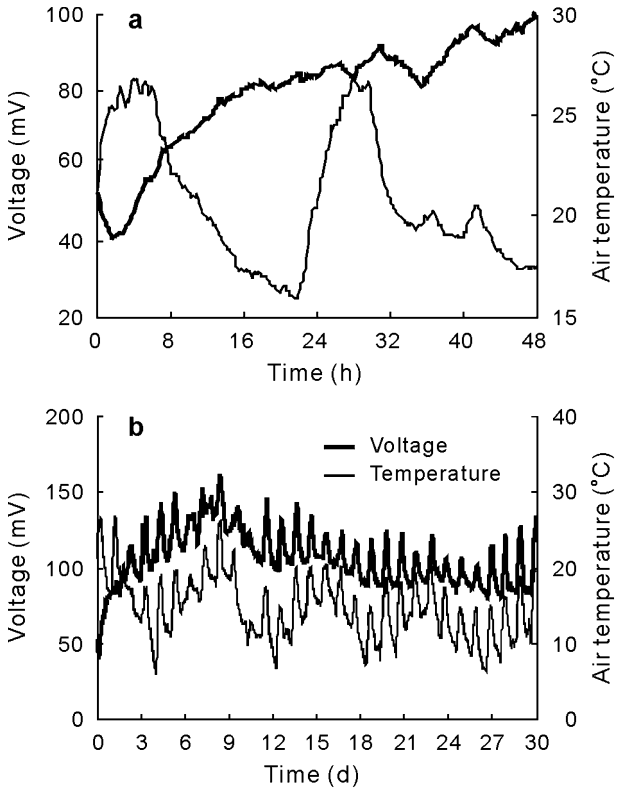

Fig. 6 Variation of the cell voltage under an external load of 500 Ω in the reactor treated with 5 cm soil and 3 cm overlaying water and air temperature during the first 48 h (a) and 30 d (b) of operation. All data were recorded every 10 min.

The concentration of DOC averaged from three sampling points decreased from 63.4 to 55.2 mg kg^{-1} during the 30 d. However, the difference did not reach the significant level ($P > 0.05$). The pH before operation (pH 6.41) was significantly higher ($P < 0.05$) than that after 30 d (pH 6.24). A similar result was found by Parot *et al.* (2008), who reported that the pH value of compost decreased from 7.7 to 7.4 after current generation. It may be due to the H^+ accumulation during the fermentation of soil organic compounds.

According to Eq. 3, the soil totally generated a total of 541 C or 5.62×10^{-3} mol electrons in the 30 d operation. Theoretically, 1 mol of organic carbon generated 4 mol electrons when oxidized into CO_2, and thus 1.41×10^{-3} mol or 16.92 mg organic carbon in soil was exhausted, taking up at most 2.7% of the 634 mg DOC in the MFC with 10 kg soil.

Bacterial community

The 16S rRNA gene sequences from the soil covered a wider range of bacterial taxonomy than those from the anode (Tables II and III). Studies have shown that the microbial richness on the anode was lower than present in the surrounding media due to the enrichment of electrogenic bacteria (Holmes *et al.*, 2004). In both clone libraries, sequences resembling *E. coli* were the most unexpectedly encountered, comprising 85% and 67% of sequences recovered from the anode and the soil, respectively. The *E. coli* being a major group was likely because the soil was from a farmland which was fertilized with manure. The enrichment of *E. coli* on the anode was due to extracellular electron transfer (Zhang *et al.*, 2008; Qiao *et al.*, 2009). The number of clones with > 97% similarity to *Deltaproteobacteria* was 5 from the anode and 2 from soil. *Geobacter* and other *Deltaproteobacteria* were known exoelectrogens (Phung *et al.*, 2004). In addition, *Bacteroidales* (Rismani-Yazdi *et al.*, 2007), *Flavobacteria* (Zhang *et al.*, 2011) and *Gemmatimonas* cells were found on the anode or generate power in pure culture (da Rosa, 2010). A clone from the anode was affiliated to iron-reducing bacterium, which was due to direct electron transfer to the anode. The presence of these bacterial groups on the anode indicates that soil microbial community may contain diverse electrogenic bacteria, tho-

TABLE II

Sequence affinity of 99 clones representing major bacterial groups on the anode

No. of clones	Reference	Identity	Division
1	Uncultured *Alphaproteobacterium* (JN409250)	100%	*Alphaproteobacteria*
1	*Sphingomonas* sp. (JQ660148)	99%	
1	*Mesorhizobium* sp. (JQ885930)	98%	
5	*Escherichia coli* (JQ912540)	100%	*Gammaproteobacteria*
37	*Escherichia coli* (JX096398)	99%	
42	*Escherichia coli* (JQ936087)	99%	
1	Uncultured *Deltaproteobacterium* (GU236077)	95%	*Deltaproteobacteria*
1	Uncultured *Deltaproteobacterium* (EF521024)	94%	
3	Uncultured *Geobacter* sp. (HQ875546)	98%	
2	Uncultured *Geobacter* sp. (JN091629)	97%	
1	Uncultured *Chloroflexi bacterium* (HQ162740)	97%	*Chloroflexi*
1	Uncultured *Bacteroidales bacterium* (GU472709)	100%	*Bacteroidetes*
1	Uncultured *Flavobacteria bacterium* (EU298065)	98%	
1	Uncultured *Gemmatimonas* sp. (JF703464)	97%	*Gemmatimonadetes*
1	Iron-reducing bacterium (FJ802353)	99%	Unknown

TABLE III

Sequence affinity of 95 clones representing major bacterial groups in soil

No. of clones	Reference	Identity	Division
2	Uncultured *Proteobacterium* (HQ658851)	98%	*Proteobacteria*
1	Uncultured *Alphaproteobacterium* (JQ861384)	99%	*Alphaproteobacteria*
1	*Chelatococcus* sp. (AM412118)	100%	
2	*Sphingomonas* sp. (JQ660148)	99%	
1	Uncultured *Betaproteobacterium* (GU257706)	98%	*Betaproteobacteria*
1	*Ralstonia* sp. (GU966534)	99%	
1	Uncultured *Gammaproteobacterium* (GQ242901)	94%	*Gammaproteobacteria*
25	*Escherichia coli* (JX096398)	100%	
6	*Escherichia coli* (FJ997270)	99%	
31	*Escherichia coli* (JQ936087)	99%	
2	*Escherichia coli* (JQ912540)	99%	
1	*Rhodanobacter* sp. (FJ772029)	100%	
1	Uncultured *Deltaproteobacterium* (EF663513)	96%	*Deltaproteobacteria*
1	Uncultured *Desulfuromonadales bacterium* (JN692205)	99%	
1	Uncultured *Geobacter* sp. (FR774780)	98%	
2	Uncultured *Actinobacterium* (GU936357)	100%	*Actinobacteria*
1	Uncultured *Actinobacterium* (JQ400598)	100%	
1	Uncultured *Actinobacterium* (GU936366)	97%	
1	*Streptomyces* sp. (JF439427)	99%	
1	*Nocardioides mesophilus* (JQ899251)	99%	
1	Uncultured *Nitrospirae bacterium* (JN408986)	100%	*Nitrospirae*
1	Uncultured *Acidobacteria bacterium* (EF075172)	99%	*Acidobacteria*
1	*Acidobacteriaceae bacterium* (HQ995661)	99%	
1	Uncultured *Acidobacteria bacterium* (EF457486)	100%	
1	*Acidobacteriaceae bacterium* (AB245338)	100%	
1	*Roseomonas* sp. (HQ436503)	100%	*Chloroflexi*
1	Uncultured *Chloroflexi bacterium* (AB433048)	96%	
1	Uncultured *Chloroflexi bacterium* (JQ071694)	99%	
1	*Virgibacillus* sp. (GQ889491)	96%	*Firmicutes*
1	*Bacillus* sp. (JN202607)	100%	
1	Uncultured *Bacillaceae bacterium* (EU862155)	100%	
1	Uncultured *Ktedobacteria bacterium* (HQ674967)	95%	Unknown

ugh the anaerobic condition, substrates and temperature in soil may not be the most favorable for electrogenic bacteria (Kim *et al.*, 2012). Optimization of the growth condition for dominant electrogenic bacteria would improve the power output. In addition, fungal strains were found to generate current, such as *Saccharomyces cerevisiae* (Walker and Walker Jr., 2006) and *Hansenula anomala* (Prasad *et al.*, 2007), which

were frequently found in soil. In our study, soil pH at the anode decreased during the 30 d and there may be a shift from bacteria to fungi. Future study may investigate into the electrogenic fungi in soil and their contribution in power generation.

CONCLUSIONS

The power generation was largely influenced by factors of soil and water depths and temperature. There was a trade-off between internal impedance and soil depth or anaerobic condition of anode. In our study, the proper soil depth was 5 cm, which may vary with soil types. *E. coli* and other electrogenic bacteria dominated the anode biofilm. It was suggested that the performance of MFCs, including power generation and the efficiency of soil remediation, could be improved under optimized living conditions for dominant electrogenic bacteria. Although the power output was low, soil microbial activities may be monitored by electrical signals, as illustrated by the results of positive relationship between temperature and cell voltage. Our results also suggest that the limitation for power generation mainly lies in the slow oxygen reduction rate at cathode. The application of high active electrode material together with sufficient anode area and optimal temperature could significantly improve the performance of soil MFCs.

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