Performance of low temperature Microbial Fuel Cells (MFCs) catalyzed by mixed bacterial consortia

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Abstract: Microbial Fuel Cells (MFCs) are a promising technology for treating wastewater in a sustainable manner. In potential applications, low temperatures substantially reduce MFC performance. To better understand the effect of temperature and particularly how bioanodes respond to changes in temperature, we investigated the current generation of mixed-culture and pure-culture MFCs at two low temperatures, 10°C and 5°C. The results implied that the mixed-culture MFC sustainably performed better than the pure-culture (*Shewanella*) MFC at 10°C, but the electrogenic activity of anodic bacteria was substantially reduced at the lower temperature of 5°C. At 10°C, the maximum output voltage generated with the mixed culture was 540-560 mV, which was 10%-15% higher than that of *Shewanella* MFCs. The maximum power density reached $465.3 \pm 5.8 \text{ mW/m}^2$ for the mixed culture at 10°C, while only $68.7 \pm 3.7 \text{ mW/m}^2$ was achieved with the pure culture. It was shown that the anodic biofilm of the mixed culture MFC had a lower overpotential and resistance than the pure culture MFC. Phylogenetic analysis disclosed the prevalence of *Geobacter* and *Pseudomonas* rather than *Shewanella* in the mixed culture anodic biofilm, which mitigated the increase of resistance or overpotential at low temperatures.

Keywords: microbial fuel cell; psychrophilic bacteria; *Shewanella*; low temperature; bioelectrochemical

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Introduction

Microbial Fuel Cells (MFCs) have garnered tremendous research attention over the last decade due to their low energy cost during wastewater treatment. In MFCs, the microorganisms at the anode convert chemical energy into electrical energy by oxidizing organic compounds present in wastewater and use the anode as the final electron acceptor (Torres et al., 2009). Although a great deal of technical development of MFC reactors at laboratory scale has been achieved, the construction and operation of larger-scale systems still face inherent challenges before they can become an efficient and economical alternative to the current commercialized wastewater treatment technologies (Logan, 2008). Temperature fluctuation is one of several problems that must be solved in order to achieve the successful operation of large-scale MFCs. Optimal temperatures for MFCs enable shorter start-up times for reaching reproducible voltage generation and better anode performance, since anodic biofilm formation is faster and enzymes are more active. However, practical application of MFCs in wastewater treatment requires operation at ambient temperatures (Ahn and Logan, 2010; Cheng et al., 2011; Michie et al., 2011; Patil et al., 2010). Low temperature is considered a vital factor affecting the electrochemical properties of anode-respiring microbes, and is an important factor in achieving stable current generation in practical MFC applications, and changes in many related key properties such as microbial anode potential and internal resistance can bring about a drastic change in their performance (Wang et al., 2010a, 2010b).

Recent research has implied that at lower temperatures the reactor must generate less electric current (Liu et al., 2012). Usually it is suggested that starting an MFC at moderate temperatures (around 22°C) is possible for practical operation in wastewater treatment, but a long lag phase would occur during start up under these conditions (Min et al., 2008). MFC operation at low temperature (15°C) has not been successful, with low power output being obtained even after several loadings. Adding certain auxiliary chemicals, such as trehalose, could maintain anode biofilm activity at 0°C (Linji et al., 2014). However, Jadhav and Ghangrekar (2009) have reported enhanced MFC performance at low (10°C) rather than at high temperatures (20-35°C). With decrease in temperature, the growth of methanogens was

suppressed and a larger fraction of the substrate was available to the electrogenic population, resulting in an increase in current and CE. Therefore, there are still gaps remaining in the understanding of bioelectrochemical property changes concerning anodic microbial communities under low temperature conditions.

To date, discrete entities of bacterial species of genus *Geobacter* (Richter et al., 2008), *Enterobacter* (Rezaei et al., 2009), *Shewanella* (Firer-Sherwood et al., 2008; Watson et al., 2010) and *Bacillus* (Nimje et al., 2009) have been tested with respect to generation of electrical current or maximization of the power output of MFCs. Recently, MFCs inoculated by mixed microbial communities have garnered much attention owing to their stability, robustness due to nutrient adaptability, stress resistance, and general tendency to produce higher current densities than those with pure cultures (Lanthier et al., 2008). A study conducted by Larrosa-Guerrero et al. (2010), in single and double-chambered MFCs using mixed bacterial consortia at 4°C, revealed that MFCs can be successfully operated at low temperatures and that psychrophilic bacteria were present in the mixed consortia. These results support the proposition that employing mixed cultures in reactors will be more beneficial and advantageous than pure cultures.

In order to understand the differences in electric current production at low temperature by a mixed-culture system compared to pure cultures, it is important to study the behavior of the system individually and synergistically using a pure culture as control. Such comparisons are needed to provide insights into the effects of mixed cultures on power production at low temperature. The aim of this study was to evaluate MFC performance in terms of overpotential and internal resistance at the low environmental temperatures of 10 and 5°C. The anode performance of the MFCs was evaluated by polarization measurements, electrochemical impedance spectroscopy and cyclic Voltammetry tests.

1. Materials and methods

1.1. Microbial fuel cell construction and operation

Single-chambered MFCs were constructed of polypropylene with an effective working volume of 110 mL (Wang et al., 2010c; Liu et al., 2012). A photograph of the reactor is shown in **Fig. 1**. The anode was a carbon fiber brush (40 mm in diameter and 40 mm in length; T700-12K, Toray Industries Co. Ltd., Japan). The cathode was made of a piece of

carbon cloth (W0S1002, CoTech Co., Ltd.) with an effective area of 19.6 cm². The water-facing side of the cathode was coated with carbon black (Vulcan XC-72) and catalyst (0.5 mg/cm² Pt), and the air-facing side consisted of four PTFE diffusion layers (Cheng et al., 2006; Logan et al., 2007). Electrodes were connected by insulated copper wire with external load resistance of 1000 Ω . A Ag/AgCl reference electrode (type 217, XianRen Industries Co., Shanghai, China) was installed into the anodic chamber to aid in the electrochemical measurements. For pure culture experiments, the polypropylene tubes were sterilized before use.

1.2 Inoculation and startup of MFCs

The activated sludge for this research as seed bacterial culture was procured from Harbin Wenchang Wastewater Treatment Plant (Wang et al., 2014) and was mixed with an acetate medium for electricity generation. The culture medium for the mixed culture was prepared in phosphate buffer solution (PBS) containing 2 g/L of acetate. The MFC startup was conducted at 10°C initially. Half of the microbial culture broth was replaced with fresh medium every five days to maintain maximum metabolic activity. PBS medium consisted of NH₄Cl 0.62 g/L, KCl, 0.26 g/L, NaH₂PO₄ 4.9 g/L, Na₂HPO₄ 9.15 g/L, mineral solution 12.5 mL/L and Wolfe's vitamin solution 5 mL/L (pH 7.0). When the voltage production dropped to 50 mV, half of the medium was replaced by fresh medium. The MFCs were considered ready for steady-state operation when their voltage output was stable after three cycles of medium replacement. After achieving stable bioelectrochemical performance at 10°C, continuous operation was carried out at 5°C. All temperature conditions of 10 and 5°C in this experiment were controlled by a constant temperature incubator (BI-250A, STIK, USA) as described in a previous study (Kong et al., 2014).

Shewanella sp. ALL-2 was extracted from the mixed bacterial culture in the activated sludge (Tkach et al., 2014), and was used to assemble pure-culture MFCs as control reactors in this study. The PBS medium for pure culture MFCs was prepared and stored in sterile containers before inoculation, and all instruments and solutions were exposed to UV to avoid contamination. The cultured *Shewanella* sp. were inoculated into MFCs, and the same operation for mixed-culture MFCs was carried out at the same time at low temperatures.

1.3. Electrochemical analysis

The cell voltage was measured automatically (one data point per minute) through a data acquisition system DAS 5020; Jiehan Technology Corporation. The electrical power density P for MFCs in the batch-fed mode was calculated as Eq. (1)

$$P = \frac{UI}{\text{Area}} \quad (1)$$

where U (V) is the voltage between anode and cathode, I (A) is the current in, and Area is anode area, equal to 19.625 cm².

Linear sweep voltammetry (LSV) was performed using a potentiostat-galvanostat model CHI 440, CH Instrument Inc., Austin, TX, USA. The voltage and current were recorded by LSV at a scan rate of 1 mV/sec, and power was calculated by multiplying voltage and current. The current density and power density were calculated from the area of the cathode (19.625 cm²). A three-electrode system in the anode compartment was used for analysis of electrochemical response (Cai et al., 2016). Polarization curves were obtained once every 20 min by varying the resistance of the MFC circuit in a descending order. The electrochemical impedance spectroscopy (EIS) experiments were performed at the end of the experiment with “Zahner TM IM6ex” potentiostat-AC frequency analyzer equipment, and the results were analyzed using the “Thales1” software. Impedance measurements were conducted with a frequency range from 10 MHz to 100 kHz using a sinusoidal perturbation with amplitude of 5 mV. In addition, two-electrode experiments were conducted for evaluation of the ohmic internal resistance of the MFCs. The anode resistance and the total ohmic resistance are represented as the Z_{re} (Real) axis of the Nyquist plot. The resistance to anode charge transfer was determined using circle fit software (Hutchinson et al., 2011), using the diameter of the semicircle of impedance data in the Nyquist plot.

Cyclic Voltammetry (CV) was performed using a potentiostat (PGSTAT 128N, Metrohm Autolab, Netherlands) (Hong et al., 2011). The anode was used as the working electrode, the cathode as the counter electrode and the Ag/AgCl electrode as the reference electrode. CVs were conducted from -0.6 V as initial anode potential to +0.6 V as final potential for 4 cycles at a scan rate of 1 mV/sec. The first derivative of the CV was derived by plotting the slope of each CV data point (DI/DE) vs the potential, and was determined at high frequencies where the impedance data crosses the axis.

1.4. Phylogenetic analysis

The process of identification of the dominant electrochemically active microbes consisted of: dispersion of the samples; isolation of the developed colonies and isolate identification by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and 16S rRNA gene analysis (Liu et al., 2013). Microbial samples obtained from the well-developed biofilm on the anode of MFCs were used for the identification of predominant bacteria capable of bioelectricity generation. The samples were then spread on solidified agar medium plates with iron-containing medium with NaHCO₃ 2.5 g/L, NH₄Cl 1.5 g/L, KH₂PO₄ 0.6 g/L, KCl 0.1 g/L, yeast extract 0.01 g/L, ferric citrate 12.28 g/L, and acetate 0.82 g/L, and pH was adjusted to 6.8. The screening for the most dominant and genetically identical isolates was done by SDS-PAGE and protein spectra (i.e., translation profiles).

The bacterial 16S rRNA gene clone libraries were constructed by using universal primer sets 27F (50-AGAGTTTGATCC TGGCTCAG-30) and 1492R (50-GGTTACCTTGTTACGACTT-30). PCR-amplification was performed following the conditions: 5 min of denaturation at 94°C, followed by 35 cycles at 94°C for 45 sec, 55°C for 45 sec and 72°C for 90 sec, with a final extension at 72°C for 10 min. The PCR products were purified on a 1% agarose gel, extracted with a UNIQ-10 gel-extraction kit (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China), then they were ligated to vector pMD19 and cloned into *E. coli* DH5a competent cells in compliance with the manufacturer's protocol. One hundred plasmids containing positive inserts from these samples were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster, CA) using 27F primer. Then 16S rRNA gene sequences were analyzed using the "BLASTN" (<http://www.ncbi.nlm.nih.gov/blast>) search tools and "EzTaxon" server (Chun et al., 2007). Alignments with different 16S rRNA gene sequences from GenBank were performed using "Clustal X" 1.8.3 with default settings. The phylogenetic characteristics were analyzed with "MEGA" version 6.0 software, and distances were calculated with help of the "Kimura 2" parameter distance model. A phylogenetic tree was built by the neighbor-joining method. Each dataset was bootstrapped over 1000 times (Tamura et al., 2007).

2. Results and discussion

2.1. MFC performances at low temperatures

The voltages produced by different reactors at two temperatures are shown in **Fig. 2**. At 10°C, the voltage of reactors increased to the maximum level during the feeding and after 8-10 days it decreased to less than 100 mV for both pure and mixed bacterial cultures. The voltage of the mixed-culture MFC gradually increased from 500 to 565 mV after each feed replacement; whereas a stable output voltage generation of 500 mV was achieved by the control pure culture MFC. These results indicated that there was an ongoing process of formation of a mature biofilm on the anode. On the 30th day the temperature was reduced to 5°C in order to investigate the impacts of low temperature on electricity generation and culture vitality. Electrogenic activities of all MFCs were substantially reduced at 5°C. There was a 12% reduction of the peak output voltage compared to that of 10°C. For the pure-culture control MFC, the duration of peak output voltage was noticeably reduced from 10 to 5 days, indicating a reduction of output duration time at low temperatures. Before every feed replacement, the COD removals were up to 90%-95%, which led to a drop of output power at the end of each batch cycle in **Fig. 2**.

For the mixed-culture MFC at 10°C, the open circuit voltage (OCV) was 327 ± 11 mV (**Table 1**). When the temperature was reduced from 10 to 5°C, the OCV decreased to 99 ± 8 mV, while the OCV measured was 168 ± 11 mV in the control MFC of the pure culture at 10°C, and the OCV was also reduced to 110 ± 5 mV at 5°C. High OCV was obtained possibly due to the fact that anode-respiring bacteria can accumulate a charge on the electrode without electron transport (Esteve-Núñez et al., 2008), but electron loss was less effective in the mixed culture than in the pure culture. Lower temperature decreased the OCV. The decrease in working voltage in the low current region indicated larger activation losses; hence the MFC with *Shewanella* had greater energy losses than the mixed-culture MFC. This can also serve as substantiation for the dramatic change in OCV and maximum power density of the mixed culture MFC after temperature reduction from 10 to 5°C. In a study on the ability of bacteria to accumulate charge, it was found that *G. sulfurreducens* had enough electron-storage capacity to eliminate the need to respire over a period of 8 min when there was no electron acceptor available (Esteve-Núñez et al., 2008).

For the pure culture at 10°C, after the output voltage reached 128 ± 10 mV, the power density reached a maximum of 83.7 ± 4.0 mW/m² (**Fig. 3**). The short-circuit current density (I_{sc}) attained a value of 1.72 ± 0.12 A/m² (**Table 1**). When temperature was reduced from 10

to 5°C, the maximum power density was reduced to $68.7 \pm 3.7 \text{ mW/m}^2$ at the voltage of $69 \pm 5 \text{ mV}$, and I_{sc} was $0.95 \pm 0.05 \text{ A/m}^2$. However, the mixed culture showed a much higher output power density at 10°C. The voltage reached values as high as $232 \pm 7 \text{ mV}$, and the power density reached $465.3 \pm 5.8 \text{ mW/m}^2$. I_{sc} was $3.06 \pm 0.56 \text{ A/m}^2$. When the temperature was reduced from 10 to 5°C, the maximum power density was only $61.6 \pm 3.5 \text{ mW/m}^2$ when the voltage was substantially decreased to $65 \pm 4 \text{ mV}$, and I_{sc} was $1.49 \pm 0.72 \text{ A/m}^2$. The results implied that the MFC with the mixed culture performed better than the pure culture MFC at 10°C, but a lowering of the temperature to 5°C substantially reduced the electrogenic activity of both mixed culture and pure culture.

2.2. Electrode characteristics

As shown in the **Table 2**, values of R_{in} were respectively calculated for individual electrodes with the same equation ($R_{\text{in}} = R_{\text{s}} + R_{\text{p}}$). Values of R_{s} and R_{p} were obtained through the “ZSime” program based on the analysis described in previous studies (He et al., 2009). Setting the circuit module let the program return a table with values of R_{s} and R_{p} . Nyquist plots for the pure-culture MFC at 10°C showed that the internal resistance R_{in} reached 46 Ω for the anode, and it was approximately 25 Ω for the cathode (**Fig. 4**). At 5°C, there was a significant rise in the anode internal resistance up to 152 Ω ; but a relatively stable resistance was presented by the mixed culture MFC. The control MFC with pure culture had an anode internal resistance of 31 Ω at 10°C, but this increased to 37 Ω at 5°C, and the cathode internal resistances were all approximately 44 Ω at both 10°C and 5°C. This indicated that the anode internal resistance was quite sensitive to temperature. Moreover, a huge increase in the anode internal resistance occurred in the pure culture MFC when the temperature was reduced from 10°C to 5°C. On the other hand, at the cathode, low temperature led to a relatively small increase in cathode internal resistance for all MFCs, but the pure-culture cathode internal resistance still varied much more than for the mixed culture.

The anodes of mixed-culture MFCs also achieved lower charge transfer resistances than pure-culture MFCs at 10°C (**Fig. 4**). The mixed-culture charge transfer resistances of $1.5 \pm 1 \text{ }\Omega$ and $3.5 \pm 1 \text{ }\Omega$ were achieved at 10°C and 5°C respectively. However, the anode for the pure culture had a slightly larger charge transfer resistance of $20 \pm 1 \text{ }\Omega$ at 10°C, but a greatly increased charge transfer resistance of $77 \pm 3 \text{ }\Omega$ at 5°C. The anode charge transfer resistance is

a useful tool for evaluating the stability of the anode biofilm. Liu et al. (2005) operated batch-fed MFCs to evaluate the effects of reactor configuration, temperature and ionic strength on power generation. They reported that temperature can be a vital factor affecting the biological activities of bacteria in the biofilm, which in turn may cause an impact on the charge transfer ability and internal resistance of the electrodes. So, these factors were found to be interrelated.

2.3. Cyclic voltammetry analysis of anode

Cyclic voltammetry (CV) data are shown in **Fig. 5** for the voltage overpotential output of the anodic biofilm of both the reactors at 10°C and 5°C. **Fig. 5a** shows that the voltage overpotential for the mixed culture at 10°C was 300 mV, whereas it was 400 mV for the pure culture. **Fig. 5b** shows no significant peak voltage outputs. Thus, it was evident that the mixed-culture anode was better than the pure-culture one, with a low overpotential. This aspect was supported by Selembo et al. (2010), where they have suggested that electrodes that produce the lowest overpotentials can be selected for bioelectrochemical reactors. Similar findings were reported by Sangeetha et al. (2015), where a Ni electrode that produced the lowest overpotential was chosen for upflow bioelectrochemical reactors.

2.4 Microbiological identification of dominant strain in mixed-culture MFC

The 16S rRNA gene libraries for clones of the MFC anode biofilm with active sludge gave 59 operational taxonomic units (OTUs), based on a random sample of 100 clone sequences. Ribotypes were identified phylogenetically and were grouped according to the type or class (for *Proteobacteria*) using a global sequence alignment for taxonomic approach. The total ratio for this phylogenetic group was calculated (**Fig. 6**), and in the result, the dominant phyla were *Geobacter psychrophilus*, *Pseudomonas caeni*, *Simplicispira psychrophila*, *Comamonas badia* and *Geobacter chapellei*. To determine which bacterial strains played the role of key species in the mixed culture MFC, we conducted purification for the predominant species in the mixed culture.

Mechanisms proposed for electron transfer by *Shewanella* sp. have included direct transfer by cell-surface contact (Marsili et al., 2008), self-produced mediators/electron shuttles (Lanthier et al., 2008; Biffinger et al., 2007), and bacterial nanowires (Goby et al., 2006). Some of these can operate and enrich in psychrophilic conditions. Liu et al. (2012, 2013) constructed and operated two single-chambered MFCs with mixed culture at 15°C and

25°C. They reported that the MFC with psychrophilic bacteria was better in performance, with low anodic resistance and better electricity generation, and this condition was attributable to a better enrichment of psychrophilic bacteria like *Shewanella psychrophila* and *Geobacter psychrophilus* in the anodic biofilm. So, we also confirmed that the reason for the better performance of the mixed-culture MFC compared to the pure-culture MFC in this study may be due to the enrichment of psychrophilic bacteria.

In this study, phylogenetic analysis disclosed the prevalence of *Geobacter* and *Pseudomonas*, rather than *Shewanella*, in the mixed-culture anodic biofilm at low temperatures. Exo-electrogenic bacteria like *Geobacter*, *Shewanella* and *Pseudomonas* have been identified by Geochip-based functional gene analysis in the bioelectrochemical systems by Liu et al. (2010, 2012). *Geobacter* sp. have been widely studied due to their electro-active ability for current generation in MFCs (Bond and Lovley, 2003; Liu et al., 2004). It was reported by Nevin et al. (2005) that *Geobacter psychrophilus* is an iron-reducing psychrophilic bacteria which can be grown at low temperatures from 4°C-10°C. *Pseudomonas* were also found to be one of the dominant bacteria in the anodic biofilm of the mixed-culture MFC in this study. Though *Pseudomonas* sp. were abundantly found in the bioelectrochemical systems, they were low-power-density producing microbes. However, they were considered to generate self-produced mediators like pycocyanin, which can be used by other microbes for anodic respiration. So, the above-mentioned studies give evidence that the psychrophilic bacteria in the anodic biofilm of the mixed-culture MFCs were electro-active, which mitigated the increase of resistance or overpotential at low temperatures.

Actually, low temperatures (4°C or 10°C) will lead to a long start-up process for MFCs due to slow growth of anode-respiring bacteria. Research has pointed out that low temperature MFCs did not produce appreciable power unless they were first operated at 30°C before being switched to the lower temperatures (Cheng et al., 2011). In this study, *Geobacter* and *Pseudomonas* were the most dominant species under low temperature conditions, but other anaerobic bacteria were retarded at low temperature compared with their microbial abundance at room temperature. Similar results were also obtained in microbial electrolysis cells, which enriched the dominant populations belonging to δ -*Proteobacteria* and γ -*Proteobacteria* at both room temperature and low temperature, but *Bacteroidetes* were reduced by 50% when temperature decreased to 4°C (Lu et al., 2011).

Since only acetate was used as a carbon source, however, a relatively high functional and phylogenetic diversity of microorganisms was successfully formed in the mixed-culture biofilm (Liu et al., 2010). In recent studies of mixed cultures, microbial community analysis showed that planktonic bacteria with exoelectrogenic ability contributed to a lower system resistance, which was positive for electron transfer for anode biofilms (Wang et al., 2010a). Lately, the contribution of bacteria without the ability for anode respiration in bioelectrochemical systems has been discussed. It was found that the part of the microbial communities that did not participate in extracellular electron transfer was maintained in the anaerobic niche of electrode biofilms, where they could provide labile products for electrode-respiring bacteria (Liu et al., 2016a, 2016b). The diversity of the community may be maintained through utilization of compounds excreted by electrogenic bacteria, or by growth on the biomass of other cells by nonacetate-utilizing bacteria (Liu et al., 2010). This is consistent with the better performance of mixed-culture than pure-culture MFCs at low temperatures. Therefore, characterization of the mixed microbial communities in different MFC environments and analysis of the roles of the different microbial groups in substrate oxidation and power production are also needed in the future.

3. Conclusions

In this research work, the performance of single-chamber MFCs has been evaluated at the low temperatures of 5°C and 10°C. Although lower temperature decreased electrogenic bacterial activity, the mixed-culture exhibited a higher electricity output capability than the pure-culture MFC, which led to a lower internal resistance and overpotential for mixed-culture MFC anodic performance compared to the pure-culture MFC at 10°C. It was found that the enrichment of *Pseudomonas*, *Geobacter* etc. in the mixed culture could maintain microbial functions or interspecies cooperation, which supported sustainable electron generation and anodic activities. However, a substantial performance decline occurred with low output voltage for both mixed-culture and pure-culture MFCs when the temperature was further reduced to 5°C.

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Table 1 Cell performance under different operational temperatures

Reactor	Temp. (°C)	OCV (V)	Maximum power density (mW/m ²)	I_{sc} (A/m ²)	Voltage at maximum power density (mV)
Mixed culture	10	327±11	465.3±5.8	3.06±0.56	232±7
	5	99±8	61.6±3.5	1.49±0.72	65±4
Pure culture	10	168±11	83.7±4.0	1.72±0.12	128±10
	5	110±5	68.7±3.7	0.95±0.05	69±5

Error bar: The data were measured in at least three replicate batch operations.

OCV: open circuit voltage; I_{sc} : short-circuit current density

Table 2 Electrode resistances of the examined MFCs (Ω)

Reactor	Temp. (°C)	R_s		R_p		R_{in} (an./cath.)
		Anode	Cathode	Anode	Cathode	
Mixed culture	10	29	35	2	9	31/44
	5	35.5	36	1.5	8	37/44
Pure culture	10	44	23	2	2	46/25
	5	150	35	2	2	152/37

R_s : solution resistance (ohmic resistance); R_p : Polarization resistance (charge transfer resistance); R_{in} : internal resistance

Fig. 1 Photograph of the Microbial fuel cell used in this work.

Fig. 2 Output voltage generation of MFCs operated under different temperatures (1000 Ω). The switch of the operational temperature was carried out on day 30 and is indicated by the vertical red line.

Fig. 3 Polarization curve and power density curve for different reactors under two operating temperatures: (a, b) 10°C, (c, d) 5°C.

Fig. 4 The Nyquist plots of anode impedance spectra for reactors at 10°C (a) and 5°C (b)

Fig. 5 The cyclic voltammograms of anodes operated at (a) 10°C and (b) 5°C

Fig. 6 Phylum distributions in the anode biofilm