SUPPORTING INFORMATION

Evaluation of Performance of Zero-Electrolyte-Discharge Air-Cathode Microbial Fuel Cell Based on Substrate Type

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1. Protocol for 16S Bacteria Species Barcoding

Genomic DNA extraction was done using commercial DNA extraction kit via beads bashing method. Purified PCR product was cloned into pJET1.2/blunt vector using commercial cloning kit according to the manufacturer's protocol. Positive clones were send for sequencing, and sequence results were then analyzed and BLAST in 16S ribosomal RNA sequences (bacteria only) database excluding uncultured bacteria bacterium (taxid:77133). Phylogeny tree was constructed using NCBI Blast Tree method.

2. Amount of Supplemented Substrate Stock Solution

At the end of each analysis period during the continuous operation, substrate stock solution needs to be supplemented into the electrolyte in order to maintain the substrate concentration and prevent working voltage from dropping. The amount of supplemented stock solution is calculated using the **Eq. S1**.

$$V = \frac{1000 \int_{t_1}^{t_2} \frac{V}{R} dt}{FCOD_{SS} \times CE_{Subs}} \quad \textbf{(S1)}$$

(V (mL): volume of added stock solution; t1, t2: the starting and ending point of last voltage monitoring period; F: Faraday's constant; COD_{SS} = 250 mg/mL, COD of stock solution; CE_{subs}:

average columbic efficiency of each substrate obtained in 3 consecutive operation cycles in batch mode)

Calculation of columbic efficiency is given by **Eq. S2**.

$$CE = \frac{8000 \times \int_0^t \frac{V}{R} dt}{FV \times \Delta COD} \times 100\% \quad \textbf{(S2)}$$

(V (V): Working voltage; R (Ω): external resistance; t (s): duration of data collection period, F: Faraday's constant; V (m^3): Volume of the MFC chamber; ΔCOD: the difference between the initial and final chemical oxygen demand of electrolyte)

3. Culturing of Anodic Bacteria and CFU vs. OD 600 Standard Curve

Anodic bacteria was cultured by cutting a small piece of acclimated anode and transferring the anode piece into 1L glass bottle filled with 50mM autoclaved PBS (pH = 7.1) medium of which the COD was adjusted to 5000 mg/L by adding glucose. The medium was kept inside a constant temperature shaker chamber with temperature and rotating speed controlled at 25 ^oC and 150 rpm. The medium was sampled using pipette at different growth phase of the bacteria, the samples were subjected to OD 600 measurements, manual colony forming unit (CFU) counting was performed by serial diluting the sample and plating the sample onto Luria-Bertani (LB) agar followed by culturing the agar plate for 24 hours under 30 ^oC. Standard curve between CFU and OD 600 was established by drawing CFU against OD 600 data on the same graph, and a correlation coefficient was obtained using linear model.

4. Impedance Analysis for Different Electrolyte pH and ionic strength

MFC reactors were one-to-one connected to external electrolyte reservoirs (600mL glass bottle) through clear silicone tube. Each glass bottle was filled with 500 mL of electrolyte, whose pH or ionic strength was modified by following methods,

pH: the pH of PBS electrolyte (50 mM) was adjusted by adding phosphoric acid (85%, Merck) to pHs of 7.0, 6.5, 6.0, 5.5 or by adding of sodium hydroxide (10 M) solution to pHs of 7.5, 8.0, 8.5, 9.0.

Osmolarity: the osmolarity of the electrolyte was adjusted by serial dilutions of the stock PBS puffer (400 mM) to concentration of 200 mM, 100 mM, 50 mM, 25 mM.

The COD of the electrolyte was adjusted by sodium acetate stock solution to 300 mg/L. The electrolyte was cycled between the MFCs and the reservoir using multi-channel peristaltic pump (Masterflex) at1.3 mL/min flow rate (**Fig. S2**). The MFC was operated with a 465Ω resistor for 24 hours and the resistor was then disconnected from the MFC. The electrochemical impedance was then analyzed in setting B.

5. Half-Cell Potential Measurement

The cell chambers of MFCs were thoroughly washed followed by soaking in PBS buffer for at least 24 hours after the continuous experiment. The MFCs were later subjected to batch reactions under similar experimental conditions (50mM PBS buffer, electrolyte COD 2000mg/L, 465Ω resistor) for 24 hours together with another MFC equipped with freshly made cathode. After the batch operation MFCs were subjected to HCP measurements of both anode and cathode using an Ag/AgCl (1M, 0.223V vs. SHE) reference electrode.

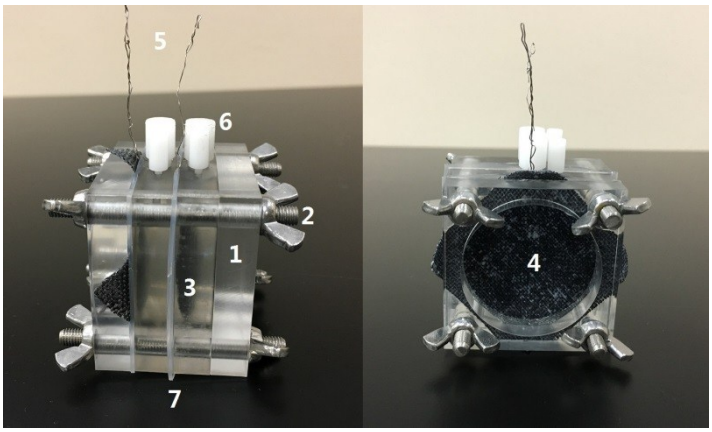

Fig S1. Image showing a typical air-cathode MFC assembly used in our experiment. Components: 1. acrylic parts; 2. screws and nuts; 3. anode; 4. air-cathode; 5. titanium wire; 6. delrin plugs 7. Silicon gasket.

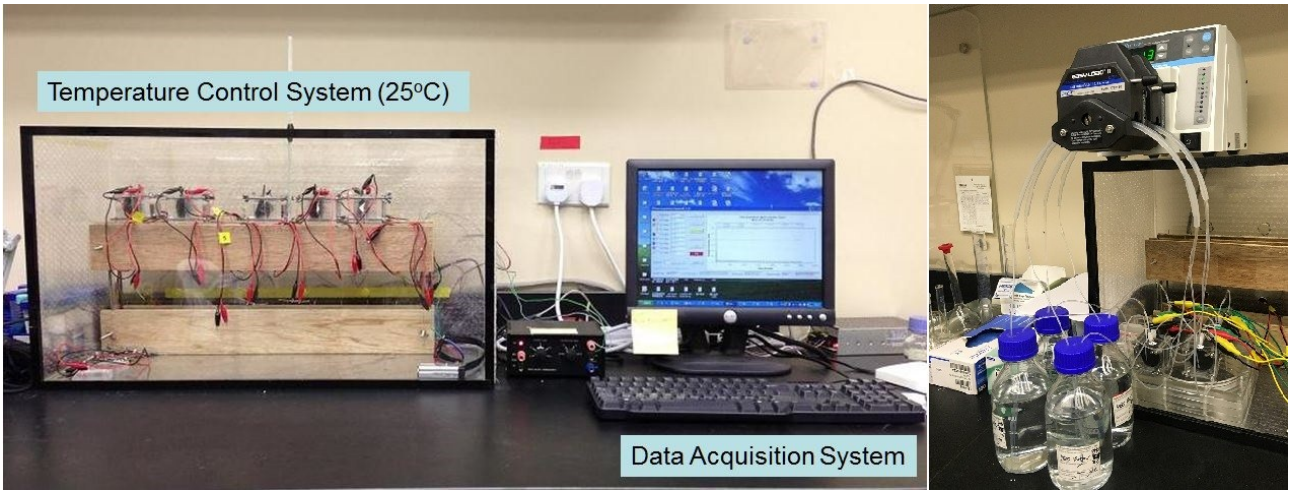

Fig. S2. Images of thermostatic chamber and data acquisition system set-up (left) and electrolyte cyclization system that connects MFCs and reservoirs using a multi-channel peristaltic pump (right).

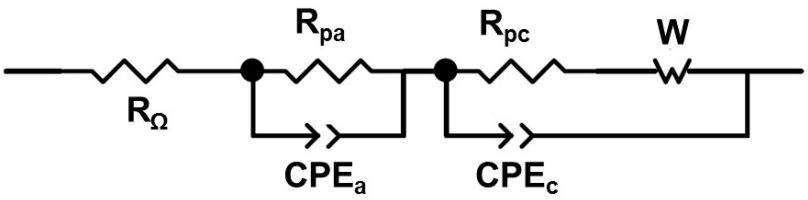

Fig. S3: Equivalent circuit used in our experiment for EIS data fitting. The full cell impedance takes contribution from both anode and cathode. R_{Ω}: ohmic resistance; R_{pa}, R_{pc}: polarization resistance of anode and cathode, CPE_a, CPE_c: constant phase element of anode and cathode; W: Warburg (diffusion) element.

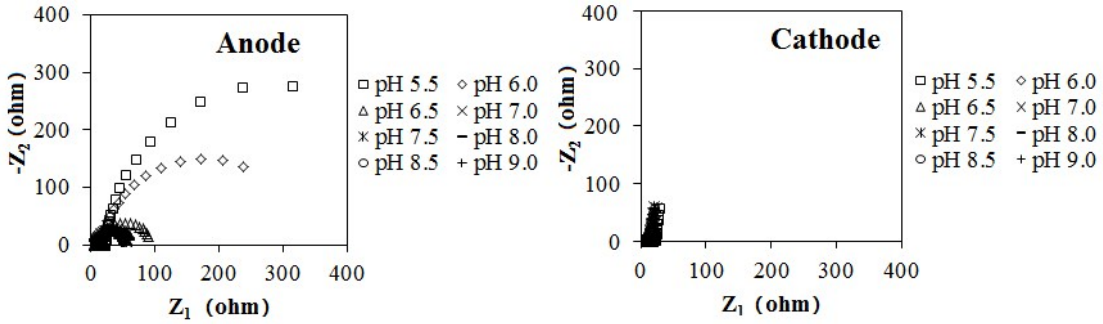

Fig. S4. Anode and cathode impedances measured under different electrolyte pH, measurements were performed in three-electrode mode with Ag/AgCl reference electrode in open circuit condition.

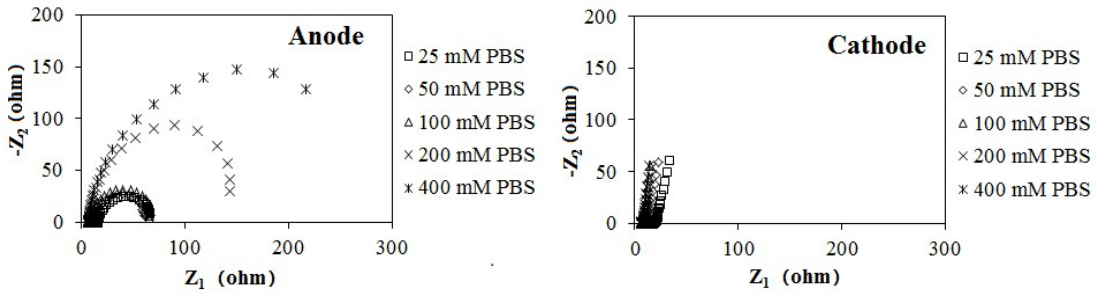

Fig. S5. Anode and cathode impedances measured under different electrolyte concentrations, measurements were performed in three-electrode mode with Ag/AgCl reference electrode in open circuit condition.

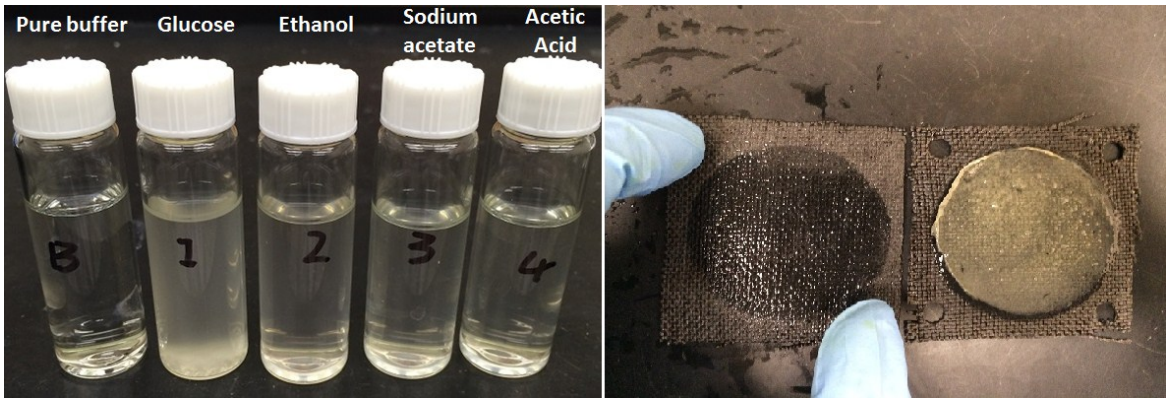

Fig. S6. Electrolyte collected at the end of experiment (left image) and comparison of a newly made air-cathode and cathode attached with biofilm (right image).

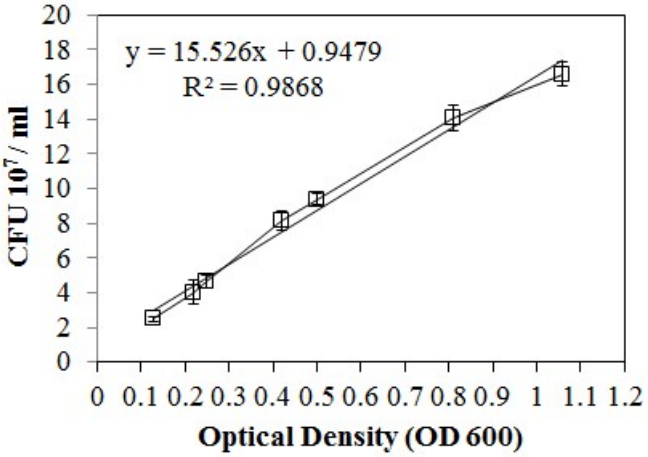

Fig. S7. Relationship between colony forming unit (CFU) and optical density of electroactive bacteria at 600 nm.

Table S1. Anodic polarization resistance calculated based on equivalent-circuit fitting of the obtained EIS data.

	R_p (Ω)		
	a zone	**d zone**	**g zone**
Glucose	8.7 ± 0.7	22.5 ± 1.0	228 ± 11.2
Ethanol	11.7 ± 0.6	13.2 ± 1.0	159.9 ± 12.0
AA	5.8 ± 0.2	6.8 ± 0.6	5.9 ± 0.7
SA	5.8 ± 0.2	6.8 ± 0.6	6.3 ± 0.9