Electricity Generation by *Enterobacter sp.* of Single-Chamber Microbial Fuel Cells at Different Temperatures

Olga Tkach, Lihong Liu, and Aijie Wang

***Abstract*—Practical applications of microbial fuel cells (MFCs) for wastewater treatment will require operation of these systems over a wide range of temperatures. MFCs at room or higher temperatures (20–35°C) are relatively well studied compared those at lower temperatures. MFC performance was examined here over a temperature range of 5–25°C in terms of initial time needed for reproducible power cycles, and performance. We have investigated the *Enterobacter sp*. ALL-3 at the different temperatures and it was it was found more effective if the microorganism is able to transfer electrons directly (exo-electrogenic organism) via the cytochromes or the ubiquinone. These carriers of electrons form stable reversible redox couples, not biologically degraded and not toxic to cell. MFCs was originally launched at 10°C, followed by a decrease in temperature to 5°C and then the temperature was increased to 25°C, thus we have created the conditions to the natural fluctuations in temperature. This cell performance resembled that for MFC operated at 5°C (30 d). That for MFC operated at 10°C voltage of reactors increased to 525-530 mV, just after 8-10 days level decreased to <50 mV. A subsequent increase in temperature up to 25°C led to the fact that the voltage in the reactor dropped to 480 mV, but remained still high. The cycle has increased to 12-d. These results demonstrate that MFCs can effectively be operated over a wide range of temperatures, but our findings have important implications for the startup of larger scale reactors where low wastewater temperatures could delay or prevent adequate startup of the system.**

***Index Terms*—Cyclic voltammetry, *Enterobacter sp.*, microbial fuel cells, microbiological identification, polarization curve, power generation.**

I. Introduction

Energy has become an inevitable source for this modern world and fossil fuel is the main source for our energy needs. Fossil fuel is exhausted and therefore, we have to come up with new ideas for sustainable development. One of such methods will be receiving electricity from bacteria through the using of microbial fuel cell. Microbial fuel cells (MFC) can convert the chemical energy of organic matter into electric energy with the using of microorganisms, providing a method of simultaneous production of renewable energy during wastewater treatment [1]-[4].

Bacteria gain energy by the transferring electrons from an electron donor (glucose or acetate) to an electron acceptor (oxygen). The larger the difference in potential between donor and acceptor the bigger growth of the organism which can proportionally affect on the columbic efficiency and on the electricity generation. Hence microbial fuel cells are used of potential microbial energy to generate electricity.

There is the organism inoculated in pure or in mixed culture of the microbial fuel cell [5]. The yield of electricity is overwhelming in case of microbial fuel cell inoculated with the mixed culture. The product of one organism has been made useful by others [6]. For example Pseudomonas aeruginosa in a mixed consortium, produces pyocyanin and several more shuttling compound which are used by other electrochemically active organisms for electron transferring. But there are also some major setbacks with this approach which predominately includes the risk of contamination.

The microbial fuel cell is broadly classified as mediator less microbial fuel cell and mediator fuel cell. The mediator less microbial fuel cell is found to be more effective as the microorganism is able to transfer electrons directly (exoelectrogenic organism) via the cytochromes or the ubiquinone. These carriers of electrons are in form of stable reversible redox couples, not biologically degraded and not toxic to cell [7], [8]. Examples of such bacteria are Geobacter [9], [10] and Rhodoferax ferrireducens [11]. Further the sediments from eroded beds (marine as well as lake bed) have the consortia of exo-electrogenic organism [12]. These organisms have been reported to form biofilms on the electrode surface.

Power density produced by bacteria with pure compounds such as acetate has increased by nearly six orders of magnitude through improvements in reactor architecture [13], [14], optimization of solution chemistry [2], [15], and using new materials and modifying electrode surfaces [16], [17], [7], [18]. Characteristics of the substrates and system operation also can greatly affect power densities. These include solution pH [19], [20]; wastewater alkalinity, added buffers and their concentration, ionic strength, and solution conductivity [21], [15]; operation mode in terms of fed-batch or continuous flow [1], [22]; and specific organic matter species in the different types of wastewater and their degradation by products [2], [21] [23], [24].

Temperature is another important characteristic, but the most studies have examined performance at a single temperature, with typical temperatures chosen of room temperature or higher (20–35°C). When temperatures have

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been varied during a study, different results have been obtained relative to impact of temperature on performance, although in almost all cases lowering the temperature reduced performance. In two different studies with single-chamber MFCs operated in fed-batch mode, the power density decreased by 10% when the temperature was reduced from 32°C to 20°C [15], [25]. In another study with a single-chamber MFC operated with continuous mode, the power density decreased by 21% when the temperature decreased from 35°C to 24°C, but only by 5% when the temperature was decreased from 30°C to 24°C [26].

Bacteria, such as Enterobacter aerogenes (E. aerogenes) [27], Escherichia coli (E. coil) [28] и Clostridium butyricum [29], [30] may not act as catalysts for the production of electricity, thus, additional chemical catalyst is always required for oxidation of hydrogen, most of platinum (Pt). Interestingly, Zhang *et al*. (2006) reported that E. coli-catalyzed MFC with not-Pt anode was able to produce a maximum power density more than 600 mWm^2, assuming direct Biocatalysis for electricity production is possible with the help of E. coli.

Although there were lots of researches done on the basement of big variety bacteria and yeast for microbial fuel cells but almost no work was done in pure culture for fuel cell application, using Enterobacter cloacae. In this study we examined the performance of single-chamber MFC insulinaemia bacteria Enterobacter sp. in the range of working temperatures 5-25°C. We compared the performance of the system in these various initial temperatures, with the consequent increase of temperature by the following parameters of voltage, current and power density, and cyclic voltammetry.

II. Materials and Methods

A. Microbial Fuel Cell Construction and Operation

The single-chamber MFC includes an anode and cathode which are placed into bilateral water-tube column, presented by camera made of polypropylene in diameter of 6 cm and in length of 7 cm with an efficient working volume in 110 ml.

The anode was presented by carbon brush (40 mm in diameter and 40 mm in length; T700- 12 K, Toray Industries Co. Ltd., Japan). The cathode was presented by carbon cloth (W0S1002, CoTech Co., Ltd., 19.6 cm^2) with the water side coated with carbon black (Vulcan XC-72) and the catalyst (0.5 mg/cm^2 Pt) mixed with a Nafion binder, and the air side with four PTFE diffusion layers for preventing water leakage [17], [31]. Electrodes were connected from external side with concealed copper wire by dint of external load resistance (1000 Ω). Reference Ag/AgCl electrode (type 217, XianRen Industries Co., Shanghai, China) was installed into the anodic chamber for conducting of electrochemical measurements. Prior to construction for MFC, 70% C_2H_5OH was completely filled in chambers for 1 day to guarantee complete sterilization [32]. After sterilization, chamber was cleaned up by deionized-and-distilled water and all reactors were autoclaved before inoculation into chamber. All tests were performed in duplication by means of two sets with use of same equipment, performance and filling.

B. Inoculation and Growth Media

The activated sludge was taken from the Harbin Wenchang Wastewater Treatment Plant. This sludge was adapted into an acetate medium for electricity generation by seed bacterial cultures. They were taken from activated sludge and were pre-cultured in PBS medium containing 2 g/L acetate for 2 months. Half of microbial culture broth was replaced with fresh medium every 5 days to maintain maximal metabolic activity of bacterial cells. PBS medium contained (per liter) NaAc 2.0 g, NH_4Cl 0.62 g, KCl, 0.26 g, NaH_2PO_4 4.9 g, Na_2HPO_4 9.15 g, mineral solution 12.5 ml, and Wolfe's vitamin solution 5ml, with pH adjusted to 7.0.

Then, cultured microbial cells were added into MFCs for cell propagation after 2-months their pre-culturing. At the same time, in MFC, half of the medium in the reactor was replaced by fresh medium when cell voltage was dropped to 50mV. The MFC system was considered as ready for under steady-state operation when its maximum voltage output was stable and reproducible after three cycles of medium replacement.

C. Electrochemical Analysis

The cells voltage was measured automatically (setting at one data point per minute) through an acquisition data system (DAS 5020; Jiehan Technology Corporation) for external resistance Rout = 125 mV. The power of density (P) for MFC of fed-batch mode could be calculated as per Eq. (1):

$$P = \frac{UI}{Area} \tag{1}$$

where, *U* is the voltage between anode and cathode (V), *I* is the current (A), *Area* is denoted as an anode area (19.625 cm^2);

The evaluation of voltammetry by linear sweeping (LSV) was performed with a potentiometer/galvanometer (CHI 440, CH Instrument Inc., Austin, TX, USA). The voltage and current were recorded by LSV at a scan rate of 1 mV/s. The value of output power was calculated as Vcell × Icell. The density of current and density of power were calculated on cathodic area base (19.6 cm^2). For analysis of electrochemical response the system of three electrodes was prepared in (for) the anode compartment. The curves of polarization, namely, the density of current against the potential (vs. Ag/AgCl/sat'd KCl) were obtained by LSV at a scan rate of 1 mV s^{-1}.

The electrochemical impedance spectroscopy (EIS) experiments were performed at the end of tests. ''ZahnerTM IM6ex'' potentiostat-AC frequency analyzer equipment was used for the EIS experiments, and the results were analyzed with using of ''Thales1'' software. The frequency of the AC signal was varied from 100 kHz to 10 mHz with an amplitude of 5 mV.

An impedance experiment was performed under galvanostatic closed circuit conditions at 400 mA for the mature biofilms. The initial electrical potential for anode tests was at þ0.5 V, while that for cathode tests it was at þ0.25 V. To ensure steady state during galvanostatic operation, the MFCs were allowed to equilibrate for 10 min between each of current setting before applying the AC signal.

Cyclic voltammetry (CV) was carried out using a potentiometer (CHI 627C; CH instrument, USA) connected to a personal computer (CHI627C Electrochemical Analyzer) with a scan rate of 0.1 V s^{-1}, ranging from −0.45 to 0.6 V. A conventional three-electrode set-up was employed, with the anode as the working electrode, an Ag/AgCl reference electrode, and platinum wire as the counter electrode. CV is a standard tool in electrochemistry, and could provide valuable insight into the electron transfer interactions between microorganisms or microbial biofilms and microbial fuel cells. Hence, voltammograms were obtained during a period of maximum current generation to detect the electrochemical properties of the MFCs.

D. Microbiological Isolation and 16S rRNA Phylogenetic Analysis

The rationale to isolate dominant electrochemically active strain(s) is straightforward as follows: dispersing of the sample; isolating the developed colonies, and finally, conducting isolate identification via SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and 16S rRNA gene analysis [32]. Microbial samples obtained from the well-propagated biofilm on the anode of MFCs were first used to isolate the predominant bacteria for bioelectricity generation. Isolated samples were then spread onto the solidified agar medium containing iron-containing culture medium contained (per liter) $NaHCO_3$ 2.5g, NH_4Cl 1.5 g, KH_2PO_4 0.6 g, KCl 0.1 g, yeast extract 0.01g, ferric citrate 12.28 g, acetate 0.82 g with pH adjusted to 6.8 and plates were cultivated at 10°C for the propagation of colonies. This procedure of strain isolation through agar plates was carried out at least three times to ensure the purity of isolates. After screening upon the most dominant isolates (e.g., large-sized colonies) via SDS-PAGE, protein spectra (i.e., translation profiles) of these isolates were found all similar to be possibly genetically identical.

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 below conditions: 5 min of denaturation at 94°C, followed by 35 cycles at 94°C for 45 s, 55°C for 45 s and 72°C for 90 s, 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 ligated to vector pMD19 and cloned into *Enterobacter sp.* DH5a competent cells following the manufacturer's protocol. Hundred plasmids containing positive insert from this sample were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster, CA) with 27F primer. 16S rRNA gene Sequences were analyzed using the ''BLASTN'' search tools (http://www.ncbi.nlm.nih.gov/blast) and ''EzTaxon'' server [33]. 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 4.0 software, and distances were calculated using the ''Kimura 2'' parameter distance model, also. A phylogenetic tree was built using by the neighbor-joining method. Each of dataset was bootstrapped over 1000 times [34].

The microstructure of the biofilm on the anode surface was examined using scanning electron microscopy (SEM, JSM-6330F, JEOL, Tokyo, Japan). The SEM samples were pretreated following the procedure described by Zhang et al. (2008).

III. Results and Discussion

A. MFCs Performance

Voltage-t curves cell performances at various temperatures reactor is shown in Fig. 1. As seen in Fig. 1, voltage reactors has increased, to a maximum of 500mV during feeding, after 8-10 days its level was reduced to <50 mV. The maximum stress level was stable up to 500 mV and maintained around that value after all acetate was consumed. This cell performance resembled that for MFC operated at 5°C (30 d). That for MFC operated at 10°C voltage of reactors increased to 525-530 mV, just after 8-10 days level decreased to <50 mV. A subsequent increase in temperature up to 25°C led to the fact that the voltage in the reactor dropped to 480 mV, but remained still high. The cycle has increased to 12-d.

As the result of research we found that the change of temperature does not greatly effect on changes in indicators of power. But the best results of stable power for the production of the original operating temperatures we have managed to achieve at 10°C. It is important that we have found that if the MFCs started up at the lowest temperatures of 5°C and 10°C it did not produce an appreciable power even after very long operation times. This shows that the initial temperature has great impact on the initial formation of an exoelectrogenic biofilm. This shows that, once formed, the bacteria were able to operate at different temperatures.

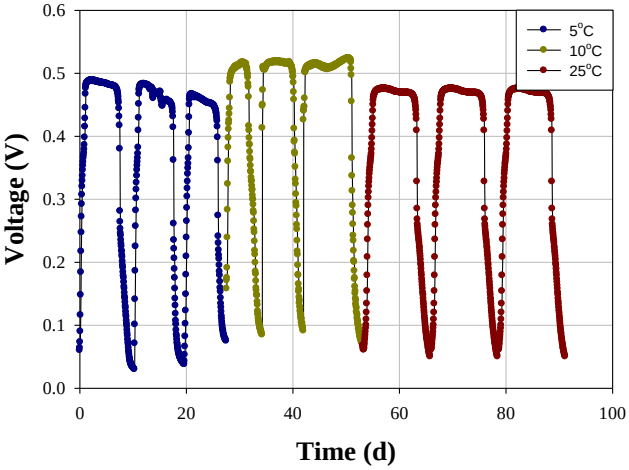

Fig. 1. Cell performances at different temperatures.

B. Electrode Characteristics

The main characteristics of MFCs are presented at Table I. All these parameters were calculated by voltage and power density charts which were produced by different reactors and are shown at Fig. 2.

TABLE I: COMBINED ELECTRODES CHARACTERISTICS OF EXAMINED MFCS (PAGE 4, ELECTRODE CHARACTERISTICS)

$T^o C$	Activation losses (mV)	Open-circle voltage (mV)	Maximum power density (mW/m^2)	Current at maximum power density (A/m^2)	Voltage at maximum power density (mV)	Short circuit current density (A/m^2)
5	90	100	293	0.29	79	0.424
10	184	200	213	1.45	147	2.26
25	157	168	84	0.66	126	0.95

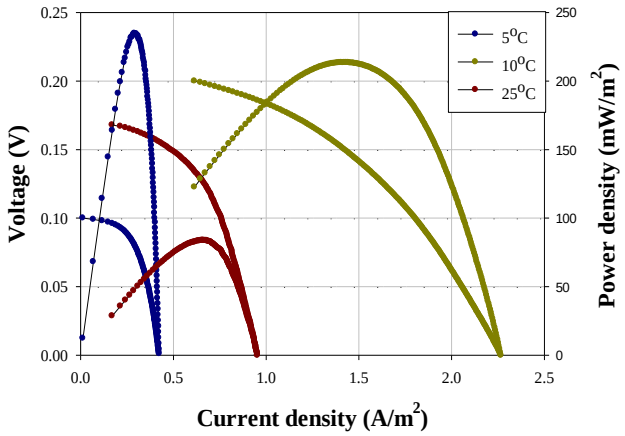

Fig. 2. Polarization curve and power density curve at different temperatures.

For MFC 5°C the open-circle voltage (OCV) was 100mV. Reducing of external load yielded an increase in electrical current with reduction of cell voltage. The mild initial drop in voltage indicates the presence of activation losses for the present MFC at approximately 90 mV. When the reaching was V = 79 mV and I = 0.29 Am^{-2}, the power of density peaked at 243 mWm^{-2}. It was found, that further decreasing in external loading reduces the power of density. The density of short circuit current (I_{scc}) was 0,424 Am^{-2}. No cell power shoot how it was proposed by Nien *et al*. [35], [36] and it was noted for the present MFC.

For MFC 10°C the open-circle voltage (OCV) was 200 mV. The mild initial drop in voltage indicates the presence of activation losses for the present MFC at approximately 184 mV. When reaching was V = 147 mV and I = 1.45 Am^{-2} the power of density peaked at 213 mWm^{-2}. Also, it was found that further decreasing in external loading reduces the power density as in the previous group. The density of short circuit current (I_{scc}) was 2.26 Am^{-2}.

For MFC 25°C the open-circle voltage (OCV) was 168 mV. The mild initial drop in voltage indicates the presence of activation losses for the present MFC at approximately 157 mV. When the reaching was V = 126 mV and I = 0.66 Am^{-2}, the power of density peaked at 84 mWm^{-2}. It was investigated as in others previous cases that further decreasing in external loading reduced the power of density. The density of short circuit current (I_{scc}) was 0.95 Am^{-2}.

The main characteristics of MFCs are presented at Table I.

C. Cyclic Voltammetry (CV)

CV was performed to characterize the electrochemical activity of the anodic biofilm. Fig. 3 gives the CV results for the anode in the E. sp.ALL-3–MFC. The measurements of cyclic voltammetry (CV) both of redox activities of components in anodic medium and mediators bound to the bacterial in anodic chamber were evaluated. CV was performed in the anode medium with pure bacteria culture.

After 15 days MFC 5°C operation, the voltammogram recorded a strong oxidation peak at 0.4 V (vs. SCE) in the forward scan and a strong reduction two peaks at 0.45 and 0.12 V (vs. SCE) in the reverse scan, indicating electrochemical activity of the biofilm on the anode surface. It appears that under long-term electrochemical tension, E.sp. ALL-3 grew on or became attached on the anode to form a biofilm and developed a capability for electrochemical activity. After the temperature in the reactor was raised to 10°C another dimension CV was recorded an oxidation peak at 0.39 V in the forward scan and a reduction two peaks at 0.38 and 0.16 V. When the temperature in the reactor has raised up to 25°C there was an increase of peak current in an oxidation peak at 0.4 V in the forward scan with the reduction of two peaks, on minor at 0.42 and 0.18 V. These results are indicating the electrochemical activities bacteria and the presence of redox active compounds in the anode biofilm, which may be involved in extracellular process of electrons transfer.

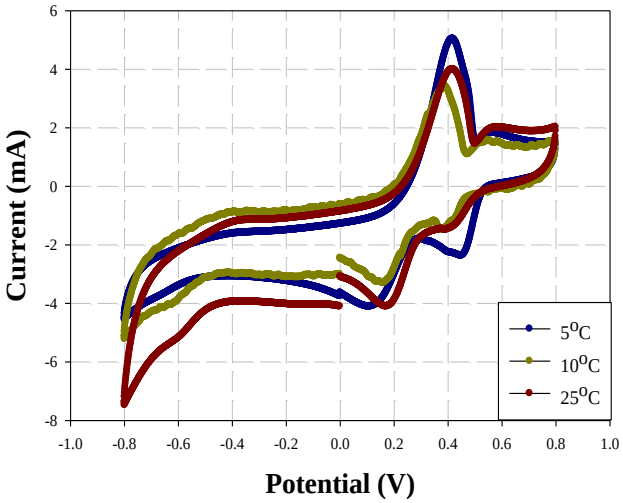

Fig. 3. Cyclic voltammograms for different temperatures.

D. Culture Isolation Studies

After all the tests on completion of work, at which point a piece of carbon brush in the anode was sampled and analyzed under a scanning electron microscope. The cells in the anodic biofilm have tiny wires (20-50 nm) connecting it, to provide the conducting paths for enhancing electron transfer to anode surface (Fig. 4). The anode MFC was covered with bacterial cells long rods. Apparently, the SEM observation further demonstrated the formation of a biofilm structure on the electrode surface over a short period of time.

Fig. 6 shows the morphological characteristics of the strain, long rod, weeks flagella, no capsule, long 2-4 μm.

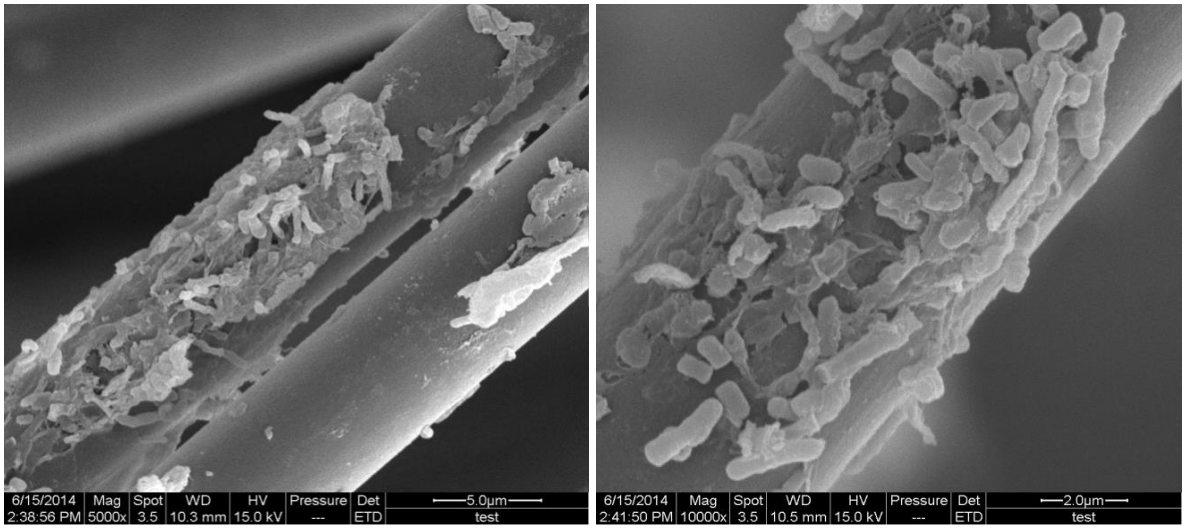

Fig. 4. SEM photographs for anodic biofilms at the end of tests.

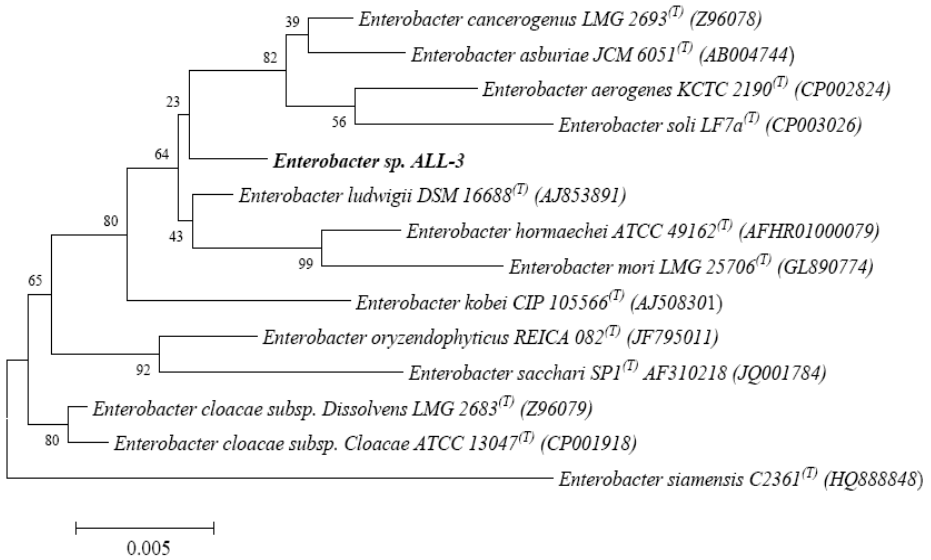

Fig. 5. *Enterobacter sp* ALL-3, based on the 16S rDNA sequences of Enterobacteriacae showing the phylogenetic position of the isolated strain (page 5, Culture isolation studies).

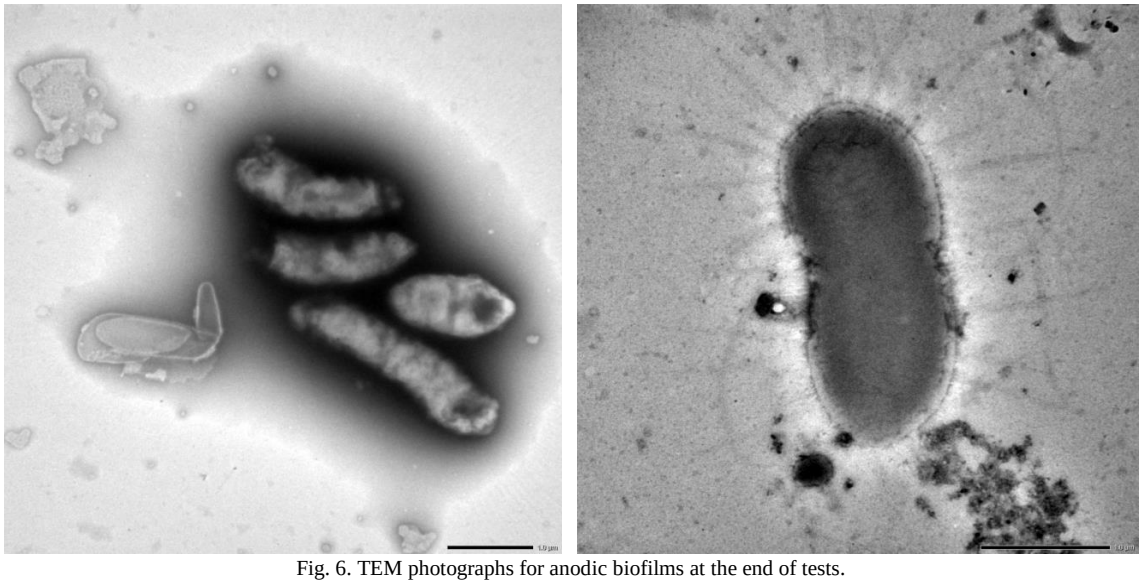

Fig. 6. TEM photographs for anodic biofilms at the end of tests.

The 16S rRNA gene clone libraries for anodic biofilm of MFC yielded 30 operational taxonomic units (OTUs), and that for anodic biofilm of MFC produced 32OTUs based on100 random selected clone sequences (Table II). The ribotypes were identified phylogenetically and were grouped by phylum. The total frequency for a given phylogenic group was calculated.

Enterobacter sp. ALL-3 was isolated with activated sludge

taken from the Harbin Wenchang Wastewater Treatment Plant and identified by 16s rRNA sequence. Enterobacter sp. ALL-3 was maintained in minimal agar medium. 16S rRNA gene sequencing was carried out to identify the isolated strain and the 1037 bp sequence was determined. Based on the similarity of the 16S rRNA gene, the isolate was found to be analogous to *Enterobacter ludwigii DSM 16688* (99.53%), *Enterobacter cloacae subsp. Dissolvens LMG 2683* (99.25%), *Enterobacter cloacae subsp. Cloacae ATCC 13047* (99.13%) and *Enterobacter cancerogenus LMG 2693* (99.05%). A phylogenetic tree was constructed and it is shown in Fig. 5. The isolate was determined to belong to E. sp. based on the results of 16S rRNA gene sequencing, and was designated, Enterobacter sp. ALL-3 (GenBank accession number: KC211019). The numbers at the nodes indicate the levels of bootstrap support based on a neighbor-joining analysis of 1000 resampled datasets: only the values ≥ 99% are given.

TABLE II: SIMILARITY OF THE 16S RRNA GENE SEQUENCES OBTAINED FROM CLONE LIBRARY OF ANODE BRUSH AND THEIR CLOSELY MATCHED SPECIES. (PAGE 5, CULTURE ISOLATION STUDIES)

Name	Accession	Similarity(%)	Diff/Total nt	Ref
Enterobacter ludwigii DSM 16688$^{(T)}$	AJ853891	99,53	7/1478	[1]
Enterobacter cloacae subsp. Dissolvens LMG 2683$^{(T)}$	Z96079	99,25	11/1476	[2]
Enterobacter cloacae subsp. Cloacae ATCC 13047$^{(T)}$	CP001918	99,13	13/1498	[3]
Enterobacter cancerogenus LMG 2693$^{(T)}$	Z96078	99,05	14/1471	[4]
Enterobacter asburiae JCM 6051$^{(T)}$	AB004744	98,95	15/1422	[5]
Enterobacter hormaechei ATCC 49162$^{(T)}$	AFHR01000079	98,6	21/1498	[6]
Enterobacter aerogenes KCTC 2190($^{T)}$	CP002824	98,4	24/1498	[7]
Enterobacter oryzendophyticus REICA_082$^{(T)}$	JF795011	98,36	23/1404	[8]
Enterobacter kobei CIP 105566$^{(T)}$	AJ508301	98,28	25/1450	[9]
Enterobacter soli LF7a$^{(T)}$	CP003026	98,2	27/1498	[10]
Enterobacter mori LMG 25706$^{(T)}$	GL890774	98,2	27/1498	[11]
Enterobacter sacchari SP1$^{(T)}$	JQ001784	97,53	37/1497	[12]
Enterobacter siamensis C2361$^{(T)}$	HQ888848	97,06	42/1429	[13]

IV. CONCLUSION

It was shown that the production of electricity using MFC with pure culture *Enterobacter sp. ALL-3* was under the strong influence of environment temperature. MFC achieved reproducible production of electricity at temperature of 10^{o}C, as it was temperature the minimum operation cycle. For MIFC, which produces power at each temperature, the amount of power was not proportional to the temperature. Maximum peaks power of density and cyclic voltammetry were observed at a temperature of 5^{o}C.

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REFERENCES

[1] Y. Ahn and B. E. Logan, "Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures," *Bioresour. Technol.* vol. 101, no. 2, pp. 469–475, 2009.

[2] Y. Feng, X. Wang, B. E. Logan, and H. Lee, "Brewery wastewater treatment using air-cathode microbial fuel cells," *Appl. Microbiol. Biotechnol.* vol. 78, no. 5, pp. 873–880, 2008.

[3] H. Liu and B. E. Logan, "Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane," *Environ. Sci. Technol.* vol. 38, no. 14, 4040–4046, 2004.

[4] B. Min and B. E. Logan, "Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell," *Environ. Sci. Technol*., vol. 38, no. 21, pp. 5809–5814, 2004.

[5] V. N. Sharma and M. Abhilasha, "Bioelectricity generation from paper industry waste using a microbial fuel cell by Clostridium species," *J Biochem Tech*, vol. 1, no. 2, pp. 4-52, 2009.

[6] K. Rabaey, N. Boon, S. D. Siciliano, M. Verhaege, and W. Verstraete "Biofuel cells select for microbial consortia that self-mediate electron transfer," *Appl Environ Microbiol*, vol. 70, no. 9, pp. 5373-5382, 2004.

[7] D. H. Park and J. G. Zeikus, "Improved fuel cell and electrode designs for producing electricity from microbial degradation," *Biotechnol Bioeng*, vol. 81, no. 3, pp. 348-355, 2003.

[8] I. Willner, E. Katz, F. Patolsky, and A. F. Buckmann, "Biofuel cell based on glucose oxidase and microperoxidase-11 monolayerfundionalized electrodes," *J. Chem. Soc. Perkin Trans*, vol. 2, no, 8, pp. 1817-22, 1998.

[9] D. R. Bond and D. R. Lovley, "Electricity production by Geobacter sulfurreducens attached to electrodes," *Appl. Environ Microbiol*., vol. 69, no. 3, pp. 1548-1555, 2003.

[10] H. Yi, K. P. Nevin, B. C. Kim, A. E. Franks, A. Klimes, L. M. Tender *et al*., "Selection of a variant of Geobacter sulfurreducens with enhanced capacity for current production in microbial fuel cells," *Biosens Bioelectron*, vol. 24, no. 12, pp. 3498-3503, 2009.

[11] S. K. Chaudhuri and D. R. Lovley, "Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells," *Nat Biotechnol*, no. 21, pp. 1229-1232, 2003.

[12] L. M. Tender, C. E. Reimers, H. A. Stecher, D. E. Holmes, D. R. Bond, D. A. Lowy *et al*., "Harnessing microbially generated power on the seafloor," *Nat Biotechnol*, vol. 20, no. 8, pp. 821-825, 2002.

[13] B. E. Logan and J. M. Regan, "Electricity-producing bacterial communities in microbial fuel cells," *Trends Microbiol*., vol. 14, no. 12, pp. 512–518, 2006.

[14] B. E. Logan and J. M. Regan, "Microbial fuel cells — Challenges and applications," *Environ. Sci. Technol*. 2006b, vol. 40, no. 17, pp. 5172–5180.

[15] H. Liu, S. Cheng, and B. E. Logan, "Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration," *Environ. Sci. Technol*., vol. 39, no. 14, pp. 5488–5493, 2005.
[16] S. Cheng and B. E. Logan, "Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells," *Electrochem. Commun*., vol. 9, no. 3, pp. 492–496, 2007.
[17] B. E. Logan, S. Cheng, V. Watson, and G. Estadt, "Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells," *Environ Sci Technol*., vol. 41, no. 9, pp. 3341–3346, 2007.
[18] F. Zhang, S. Cheng, D. Pant, G. V. Bogaert, and B. E. Logan, "Power generation using an activated carbon and metal mesh cathode in a microbial fuel cell," *Electrochem. Commun.*, vol. 11, no. 11, pp. 2177–2179, 2009.
[19] A. P. Borole, H. O'Neill, C. Tsouris, and S. Cesar, "A microbial fuel cell operating at low pH using the acidophile Acidiphilium cryptum," *Biotechnol. Lett.*, vol. 30, pp. 1367–1372, 2008.
[20] Y. Fan, E. Sharbrough, and H. Liu, "Quantification of the internal resistance distribution of microbial fuel cells," *Environ. Sci. Technol*., vol. 42, no. 21, pp. 8101–8107, 2008.
[21] L. Huang and B. E. Logan," Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell," *Appl. Microbiol. Biotechnol*., vol. 80, no. 2, pp. 349–355, 2008a.
[22] L. Huang and B. E. Logan," Electricity production from xylose in fed-batch and continuous-flow microbial fuel cells," *Appl. Microbiol. Biotechnol*., vol. 80, no. 4, pp. 655–664, 2008b.
[23] H. Liu, R. Ramanarayanan, and B. E. Logan, "Production of electricity during wastewater treatment using a single chamber microbial fuel cell," *Environ. Sci. Technol*., vol. 38, no. 7, pp. 2281–2285, 2004.
[24] B. Min, J. R. Kim, S. Oh, J. M. Regan, and B. E. Logan, "Electricity generation from swine wastewater using microbial fuel cells," *Water Res*., vol. 39, no. 20, pp. 4961–4968, 2005.
[25] X. Wang, Y. Feng, and H. Lee," Electricity production from beer brewery wastewater using single chamber microbial fuel cell," *Water Sci. Technol*., vol. 57, no. 7, pp. 1117–1121, 2008.
[26] H. Moon, I. S. Chang, and B. H. Kim, "Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell," *Bioresour. Technol*., vol. 97, no. 4, pp. 621–627, 2008.
[27] S. Tanisho, N. Kamiya, and N. Wakao, "Microbial fuel cell using Enterobacter aerogenes," *Bioelectrochem. Bioenerg*., vol. 21, pp. 25–32, 1989.
[28] U. Schröder, J. Nieben, and F. Scholz, "A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude," *Angew. Chem. Int. Ed*., no. 42, pp. 2880–2883, 2003.
[29] J. Niessen, U. Schröder, F. Harnisch, and F. Scholz, "Gaining electricity from in situ oxidation of hydrogen produced by fermentative cellulose degradation," *Lett. Appl. Microbiol*., no. 41, pp. 286–290, 2005.
[30] J. Niessen, U. Schröder, and F. Scholz, "Exploiting complex carbohydrates for microbial electricity generation — A bacterial fuel cell operating on starch," *Electrochem. Commun*., vol. 6, pp. 955–958, 2004.
[31] S. A. Cheng, H. Liu, and B. E. Logan, "Increased performance of single-chamber microbial fuel cells using an improved cathode structure," *Electrochem. Commun*., vol. 8, pp. 489–494, 2006.
[32] B. Y. Chen, W. M. Chen, F. L. Wu, P. K. Chen, and C. Y. Yen, "Revealing Azo-dye decolorization of indigenous aeromonas hydrophila from fountain spring in Northeast Taiwan," *J. Chin. Inst. Chem. Eng.*, vol. 39, p. 495, 2008.
[33] J. Chun, J. H. Lee, Y. Jung, M. Kim, S. Kim, B. K. Kim, et al. "EzTaxon: a web-based tool for the identification of prokaryotes based on 16Sribosomal RNA gene sequences," *Int J Syst Evol Microbiol*, vol. 57, pp. 2259-2261, 2007.
[34] K. Tamura, J. Dudley, and M. Nei, "MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0.," *Mol Biol Evol*, vol. 24, pp. 1596-1599, 2007.
[35] P. C. Nien, C. Y. Lee, K. C. Ho, L. H. Liu, A. J. Wang, N. Q. Ren *et al*., "Power overshoot in two-chambered microbial fuel cells (MFC)," *Bioresour Technol*, vol. 102, pp. 4742-4746, 2011.
[36] L. H. Liu, C. Y. Lee, K. C. Ho, P. C. Nien, A. Su, A. J. Wang *et al.*, "Occurrence of power overshoot for two-chambered MFC at nearly steady-state operation," *Int. J. Hydrogen Energy*, vol. 36, pp. 13896-13899, 2011.

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