Bacillus Biocathode improves Electricity Generation with Microbial Fuel Cells

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ABSTRACT

In a microbial fuel cell (MFC), biocathodes alleviate the need to use noble catalysts to reduce oxygen, which substantially increases the viability and sustainability of a MFC. Three electrochemically active strains of bacteria are isolated from the electroactive biofilm formed in the sediment of a MFC cathode and identified as Bacillus, according to 16S rDNA sequence analysis and biochemical, physiological, and morphological characteristics. Electrochemically active of every strain was detected by cyclic voltammograms methode. The performance of a biocathode in the terminal electron-accepting process that is biocatalyzed by these Bacillus bacteria is investigated. The maximum power density of biocathode MFC is 2.4 times more than that of an abiotic cathode MFC. This study examines a widespread property among bacteria such as Bacillus that can utilize carbon substrates for cathode O2 reduction, thus developing new and interesting routes in the field of electroactive bacteria research.

Keywords: *Bacillus* Biocathode, Microbial fuel cell, 16S rDNA, PCR

1 INTRODUCTION

The microbial fuel cell (MFC) has been one of the most promising technologies for power generation in recent years[1-4]. This novel technology has shown great promise for the practical application of simultaneous electricity production and waste treatment. The MFC presents an exciting and sustainable hope to bioenergy. However, there still remain certain limitations to overcome for this technology to become a viable alternative to traditional energy production processes. Potential loss at both cathode and anode, as well as power generation for longer periods of time, are some of the major factors that lead to lower electron transfer efficiency. Potential losses occur at the cathode surface due to the poor efficiency of cathodic oxygen reduction. Many different measures have been taken to reduce the cathodic activation overpotential. For example, an electron transfer mediator can reduce the loss in cathodic activation energy. $K_3[Fe(CN)_6]$ has a wide range of applications as both a mediator and acceptor, and can reduce transfer resistance and increase open circuit potential[5]. The addition of a catalyst in the cathode compartment for electron transfer from the cathode to oxygen also helps decrease the activation overpotential. Metallic catalysts (such as Pt, At, PbO2), metal-based catalysts, and biological catalyst proteins can also decrease activation barriers in the cathode. The use of platinum as a catalyst for oxygen reduction is popular but expensive, and thus unfeasible for large scale application[6]. Such disadvantages can be overcome by cathodes based on biocatalysts. Recent studies with biological cathodes have sparked interest for an inexpensive and sustainable alternative to chemical cathodes. MFC performance can be increased by inoculating the cathode with microorganisms[7-10]. These biocatalysts retrieve electrons directly from the cathode, which are then transferred to a final electron acceptor such as oxygen, nitrogen, or sulfur. A few reports on operation with a biocathode MFC show higher electrogenesis over abiotic cathode operations. Carbajosa et al. reported that cathodic Acidithiobacillus ferrooxidans biofilm achieved an increase in the current output compared with a non-catalyzed graphite cathode[11]. It has recently been shown that white-rot fungus can be combined with a biocathode to increase MFC power output[12]. Aerobic biocathode[13] operating with passive oxygen transfer in microbial fuel cells have been designed and analyzed by Xia et al. These studies suggest that oxygen reduction on the cathode is directly catalyzed by the biofilm. Thus, biocathodes were developed those use as catalysts to assist electron-transfer highlight a promising route to improving MFC performance.

Biocathodes are a stable and feasible way to enhance MFC power generation. Strong electron acceptor conditions prevail at the cathode with aerobic metabolism and facilitate the gradual reduction of protons, leading to stable electron transfer for longer periods of time[14]. However, research on biocathodes is in its infancy. The performance of biocatalysts as a terminal acceptor has not yet been fully exploited, and there are several constraints to be overcome. A diverse

range of electrochemically active microorganisms has been utilized in MFC systems, including: (i) oxygen reduction on the cathode directly catalyzed by the *Enterobacter sp. E1* biofilm[15]; (ii) electrochemical reduction of oxygen catalyzed by a wide range of bacteria, including Gram-positive bacteria[16] such as Kingella denitrificans, Staphylococcus carnosus, and Bacillus subtilis;(iii) electrochemical reduction of oxygen catalyzed by Pseudomonas aeruginosa. In addition, Nimje et al. reported that Bacillus subtilis used in MFC showed better electrochemical performances[17] and elucidated its biocatalyst mechanism. Bacillus can be obtained from aerobic sludge, substantially reducing costs and improving the practical applicability and sustainability of the MFC.

In this study, three bacterial strains were isolated from the electroactive biofilm formed in the sediment microbial fuel cell (sMFC) cathode. They were identified as Bacillus using 16S rDNA sequencing and physiological and biochemical tests. A biocathode MFC based on the oxygen reduction biocatalysis of Bacillus was developed, and its performance was investigated. We evaluated the influence of Bacillus in the terminal electron-accepting process on the electrogenic activity of the MFC. The MFC's performance during operation with varying Bacillus strains was evaluated and compared with abiotic performance through electrochemical analysis for both anode and cathode chambers.

2 EXPERIMENTAL

2.1 Strain isolation and characterization

Electrochemical strains were isolated from the electroactive biofilm formed in the sediment microbial fuel cell (SMFC) cathode. A light microscope and Gram stain set were used to determine the Gram reaction. Catalase and oxidase activities and other characteristics were determined using standard methods[18].

16S rDNA was amplified using universal bacterial primers 27F: 5'-AGAGTTTGATCCTGGCTCA-3' and 1492R: 5'- GGTTACCTTGTTACGACTT-3' primer pairs. 16S rDNA sequencing analysis was queried against the GenBank[18]. A Neighbor-Joining tree was constructed with the MEGA5 program[19].

2.2 Biocathode-MFC setup

A double-chambered membrane MFC was constructed from two glass bottles of 250 mL capacity joined together with a glass bridge containing a proton exchange membrane (PEM, Nafion 117, Dupont Co., USA; inner diameter=3 cm)[20]. A schematic of the "H"-shaped biocathode MFC configuration is illustrated in Fig. 1.

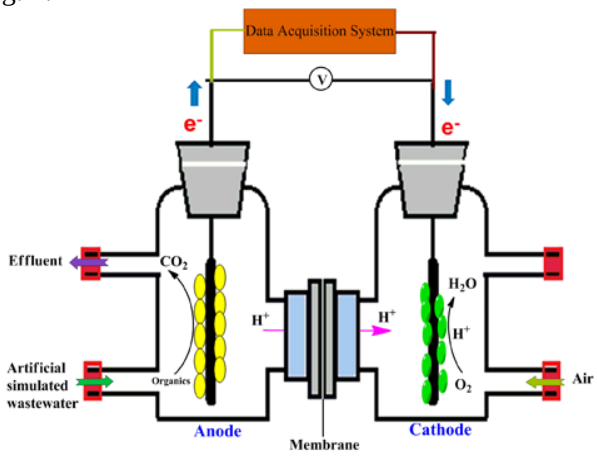

Fig.1 Schematic of the biocathode MFC configuration

2.3 Electrochemical analysis of biocathode

The measurement and determination of the internal resistance were performed according to the power density peak method. The Ag/AgCl electrode was used as a reference to measure the anode and cathode potentials. COD was determined using the closed reflux method mentioned in the Standard Methods[21]. Coulomb efficiency (CE) was calculated as reported previously[22-24]. Electrochemical impedance spectroscopy (EIS) experiments (0.1 to 10^5Hz, 5 mV) were carried out to verify the characteristics of the biocathode.

2.4 Scanning electron microscope (SEM)

The samples were fixed with 2% glutaraldehyde and 1% osmium tetroxide, and then dehydrated in increasing concentrations of ethanol[25]. The morphology of the electrode surface was investigated with a scanning electron microscope (SEM) (Hitachi S-4800).

2.5 Protein content analysis

In the end, the cathode carbon felt was dipped into deionized water for ultrasonic treatment (100 W, 30 min). The biomass loaded on the cathode carbon felt surface was suspended in water. After centrifugation of the supernatant for 2 min at 2000 rpm, 0.5 mL of 0.1mol/L NaOH was added to the tube. The mixtures were boiled for 20 min. Finally, the biomass protein was determined with the modified Lowry method[26].

3 RESULTS AND DISCUSSION

3.1 Strain isolation and characterization

Three strains were isolated from the cathode biofilm of sMFC, and named B1, B2, and B3. Identification with a light microscope showed that all strains had Gram-negative short rod morphology with spores. The colony morphology of all strains is shown in Table S1. Tests for catalase, glycolysis, Voges–Proskauer reaction, amylolytic enzyme, and lysozyme of the three strains were positive, while indole production and tyrosine hydrolysis were negative (Table S2). A 16S rDNA target fragment was PCR-amplified using every strain genome DNA as a template and 16S rDNA universal primers 27F/1492R. The sequence similarity of the 16S rDNA gene was compared with organisms from GenBank.

A phylogenetic tree showed the relationships between strains and related species based on this sequence (Fig. 2). Near neighbors of the isolated strains were all Bacillus, and results of physiological and biochemical properties and 16S rDNA analysis indicated that the three electrochemical strains all belonged to Bacillus

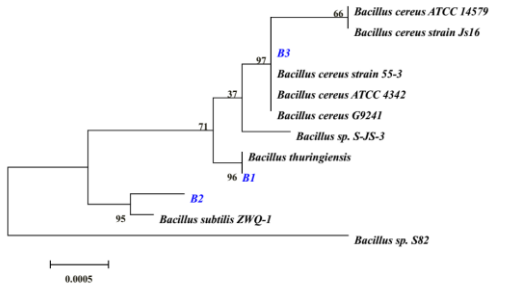

Fig.2 Phylogenetic tree showing the relationships between strains and related species based on 16S rRNA gene sequence.

3.2 Performance of biocathode MFC

All MFCs were operated for about 48 days. As shown in Fig. 3, stable voltages were achieved. The longer startup period of the biocathode MFC was possibly due to the fact that the microorganisms in it were enriched on both the anode and cathode surfaces.

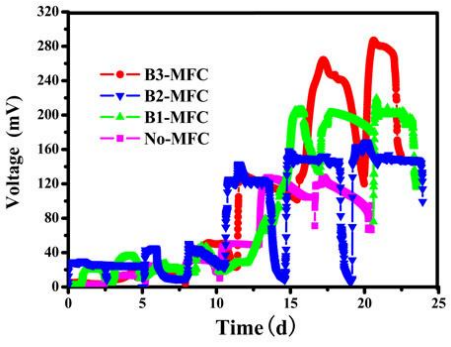

Fig. 3 Voltage generation of the MFCs with different biocathode. The MFCs were connected through a resistance of 1000 Ω.

The lag period was about 20 days. All the MFCs voltages output were lower. It was possibly that the anode electro-biofilms from the anaerobic sludge played a decisive role in the start of the system and maximum output voltage (0-20 days). Biocathode had significant effect on the startup time in this system. From 20-48 days all MFCs voltages quickly reached their respective maximum voltages, suggesting that the biocathode began to work. When all MFCs reached the maximum voltages, the anode and cathode potentials were detected.

With time, the cathodic inoculum adapted to the system's microenvironment, resulting in competent metabolic activity in the chambers for the terminal electron acceptor. This could be explained by the fact that cathode potential regulates the thermodynamic energy available for cathode bacteria to grow[27]. Since the potential of each anode was similar, the divergent performance of the MFCs was primarily caused by different adsorptions of microorganism biofilms on the cathode surface[28], attributed to bacterial adhesion and prolonged contact time[29]. Thus, the differential catalytic activity of bacterial strain biofilms changed the activation energy for electron transfer in the biocathodes, with the B3 strain exhibiting the best performance in electricity generation.

The power density curves of the biocathode and abiotic MFC are drawn at their stable stage (Fig. 5). Significant differences in power generation were found in maximum power densities 59.45, 43.27, 69.39 and 28.98 mW/m^2 for B1-MFC, B2-MFC, B3-MFC and No-MFC, respectively.

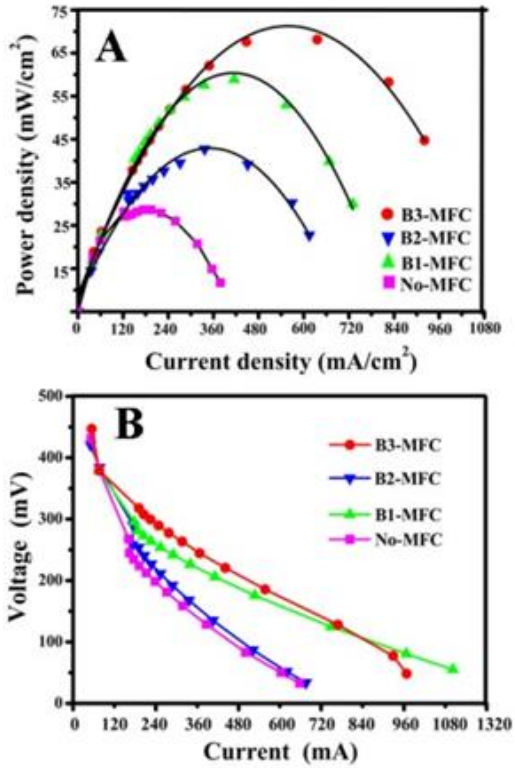

Fig.5 Power density (A) and polarization (B) curves of the different biocathode MFC and abiotic cathode MFC. Cell potential difference and power density generated in the MFC.

The different adhesive abilities on the electrode of B1, B2 and B3 could be responsible for the electrochemical activity. Biofilms on the cathode surface can be clearly seen with scanning electron micrograph (SEM) images (Fig. 6). The abiotic cathode had a smooth and clean surface (Fig. 6D), and the biocathodes were covered with biofilm (Fig. 6A, 6B, 6C). Some solid crystalline particles were scattered on the surface of each graphite fiber. The B3 biofilm attached to the electrode was more intensive and uniform (Fig. 6C), and the slimmest biofilm was the B2 (Fig. 6B). From protein content analysis, we can found that biomass concentration on Fig. 6C was the highest, and the slimmest biomass concentration was on Fig.6B.

4 CONCLUSIONS

Aerobic bacteria *Bacillus* B1, B2, B3 showed good oxygen catalytic reduction ability, and acted as favorable biofilm, which indicated they are suitable for biocathodes in MFC, with greatly enhanced power densities. The maximum power density of biocathode MFCs were 105%, 49% and 139% higher than the abiotic cathode MFC, demonstrating that the biocathode MFC exhibited better performance in electricity generation. EIS demonstrated that the microorganisms' catalytic activity in the biocathode was comparable with that of the abiotic cathode. In such a novel system, the electron transfer was supported by the metabolism of aerobic bacteria, and thus improved the reduction efficiency of oxygen in order to increase electricity production. The present work demonstrated that the aerobic bacteria *Bacillus*, isolated from electroactive biofilm formed in a SMFC cathode, was capable of catalyzing the electrochemical reduction of oxygen to enhance electricity generation in the MFC.

REFERENCES

[1] H. Liu, B. E. Logan, Environ. Sci. Technol. 2004, 38, 4040-4046.
[2] C. Abourached, et al., Water Res. 2014, 51, 228-233.
[3] R. Katrin, et al., Electrochim. Acta. 2014, 55, 115-125.
[4] Doherty, L., et al., Water Res. 2015, 85, 38-45.
[5] S.E. Oh, et al., Environ. Sci. Technol. 2004, 38, 4900-4904.
[6] B. E. Logan, et al., Water Res. 2005, 39, 942-952.
[7] G.W. Chen, et al., Appl. Microbiol. Biotechnol. 2008, 79, 379-388.
[8] L.X. Huang, et al., Environ. Sci. Technol. 2011, 45(11), 5025-5031.
[9] J.M. Pisciotta, et al., Appl. Environ. Microbiol. 2012, 78(15), 5212-5219.
[10] Z. Zaybak, et al., J. Biotechnol. 2013, 168, 478-485.
[11] S. Carbajosa, et al., Biosens. Bioelectron. 2010, 26, 877-880.
[12] C. Wu, et al., Appl. Energ. 2012, 98, 594-596.
[13] X. Xia, et al., Environ. Sci. Technol. 2013, 47(4), 2085-2091.
[14] S. Srikanth, V.S. Mohan, Bioresource. Technol. 2012, 119, 241-251.
[15] S. Parot, et al., J. Appl. Microbiol. 2009, 106, 1350-1359.
[16] C. Amandine, et al., Electrochem. Commun. 2010, 12, 505-508.
[17] V.R. Nimje, et al., J. Power. Sources. 2009, 190, 258-263.
[18] J.M. Luo,et al., Bioresource. Technol. 2013, 139, 141-148.
[19] T.S. Song, et al., Bioresource. Technol. 2012, 108, 68-75.
[20] D.R. Lovley, K.P. Nevin, Curr. Opin. Biotechnol. 2011, 22, 441-448.
[21] C. Peter, et al., Environ. Sci. Technol. 2007, 41, 7564-7569.
[22] H.S. Lee, et al., Water. Res. 2008, 42, 1501-1510.
[23] J. Sun, et al., J. Power. Sources. 2009, 187, 471-479.
[24] C. Fang, et al., Appl. Biochem. Biotechnol. 2011, 164, 464-474.
[25] T.S. Song, et al., J. Chem. Technol. Biotechnol. 2012, 87, 1436-1440.
[26] B. Frolund, et al., Appl. Microbiol. Biotechnol. 1995, 43, 755-761.
[27] X. Xia, et al., Bioresource. Technol. 2012, 120, 26-33.
[28] C. Li, L.L. Ding, et al., Bioresource Technol. 2012, 116, 459-65.
[29] C. Amandine, et al., Electrochimica. Acta. 2010, 55, 4902-4908.