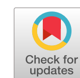
Bioelectrochemistry 120 (2018) 145–149

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Enhanced bioelectricity generation of air-cathode buffer-free microbial fuel cells through short-term anolyte pH adjustment



Yueping Ren *, Jinli Chen, Xiufen Li *, Na Yang, Xinhua Wang

Jiangsu Key Laboratory of Anaerobic Biotechnology, Jiangsu Cooperative Innovation Center of Technology and Material of Water Treatment, School of Environmental and Civil Engineering, Jiangnan University, Wuxi, Jiangsu 214122, China

A R T I C L E I N F O

Article history:
Received 25 July 2017
Received in revised form 13 December 2017
Accepted 15 December 2017
Available online 16 December 2017

Keywords:
Microbial fuel cell
Buffer-free
Anolyte acidification
Initial pH adjustment

A B S T R A C T

Short-term initial anolyte pH adjustment can relieve the performance deterioration of the single-chamber air-cathode buffer-free microbial fuel cell (BFMFC) caused by anolyte acidification. Adjusting the initial anolyte pH to 9 in 5 running cycles is the optimum strategy. The relative abundance of the electrochemically active *Geobacter* in the KCl-pH 9-MFC anode biofilm increased from 59.01% to 75.13% after the short-term adjustment. The maximum power density (P_{max}) of the KCl-pH 9-MFC was elevated from 316.4 $mW \cdot m^{-2}$ to 511.6 $mW \cdot m^{-2}$, which was comparable with that of the PBS-MFC. And, after the short-term adjusting, new equilibrium between the anolyte pH and the anode biofilm electrochemical activity has been established in the BFMFC, which ensured the sustainability of the improved bioelectricity generation performance.

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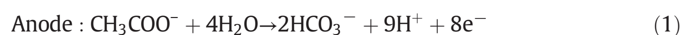
1. Introduction

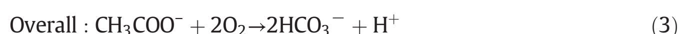
Microbial fuel cell (MFC) is widely regarded as a promising wastewater treatment technology as it integrates sewage treatment with electric energy generation [1,2]. However, high operating cost and low electric power are still the main restrictive problems to its extensive practical application [3–5]. Ionic strength and anolyte pH are two significant factors influencing the performance of the MFCs [6,7]. For practical wastewater processing MFCs, ionic strength can be provided by the naturally contained or additive inorganic salts but the anolyte acidification is inevitable because of the absence of buffers, which restricts the growth and activity of the electrochemically active bacteria (EAB) and results in performance deterioration [8–10].

In air-cathode MFC, the anode reaction produces protons (H^+) and HCO_3^-/H_2CO_3 (dissolved CO_2), and the oxygen reduction reaction (ORR) at cathode directly consumes H^+ through the acidic pathway or generates OH^- through the alkaline pathway [5,11]. Theoretically, anolyte acidification cannot be very serious because the overall cell reaction accumulates HCO_3^- (Eqs. (1)–(3)) [11,12] which was considered as natural buffer substances [13,14].



$$\text{Anode}: CH_3COO^- + 4H_2O \rightarrow 2HCO_3{}^- + 9H^+ + 8e^- \quad (1)$$

$$\text{Cathode}: O_2 + 4H^+ + 4e^- \rightarrow 2H_2O / O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (2)$$



$$\text{Overall}: CH_3COO^- + 2O_2 \rightarrow 2HCO_3{}^- + H^+ \quad (3)$$

However, in reality, the sluggish transfer of H^+ [15] and the lagged behind ORR usually result in sharp pH decline (from 7 to 5.4) at the initial electrogenesis stage [8,9,16], which seriously restricted the proliferation and activity of EAB on the anode surface and caused the deteriorated electric power [17]. Thus, deliberately breaking the restriction to promote the formation of a robust and electrochemically active anode biofilm at the initial electrogenesis stage is the crux to further improve the electric power of BFMFCs.

Neutral and weak alkaline environment are considered as can benefit the growth of EAB biofilm [18,19], however, adjusting the anolyte pH all along the running of BFMFCs is unpractical, especially for large-scale MFCs. In previous works, we found that short-term alkaline intervene can promote the proliferation of EAB in the anode biofilm and improve the electric power of BFMFCs [16]. However, the optimum adjusting strategy, including the optimal initial pH and the shortest adjusting duration that BFMFC needed to reach the optimal electric power, is still unknown. Therefore, in this paper, the anolyte initial pH values of three air-cathode single chamber KCl anolyte BFMFCs were adjusted to 8, 9 and 10 in short-term (1 to 5 running cycles) to relieve the anolyte acidification at the preliminary electrogenesis stage so as to promote the formation of robust EAB biofilms. The optimal anolyte pH adjusting strategy has been determined. The variations of the electrochemical activity and microbial community characters of the anode biofilms after the short-term anolyte pH adjustment have been systematically investigated. This method can significantly improve the bioelectricity

* Corresponding authors.
E-mail addresses: ypren@jiangnan.edu.cn (Y. Ren), xfli@jiangnan.edu.cn (X. Li).

https://doi.org/10.1016/j.bioelechem.2017.12.007
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generation of BFMFC and contribute to the large-scale application of BFMFCs in waste water treatment.

2. Materials and methods

2.1. The configuration of MFCs

The devices applied are the typical cubic single-chamber membrane-less MFCs with a cylindrical chamber of 28 mL, the electrode distance of 4 cm and two round electrodes of 3 cm in diameter. The graphite felt was used as the anodes (Hesen Electric Appliance Co., Ltd., Shanghai, China). Activated carbon (AC) air-cathode composed of AC catalyst layer and gas diffusion layer were prepared through the rolling-press method [20,21]. The external resistances connected to the MFCs are 500 Ω.

2.2. The inoculation and operation of MFCs

MFCs were inoculated and operated according to the previously reported method [16]. Anaerobic sludge (Tailake Newtown Sewage Treatment Plant, Wuxi, China) was used as inoculum. All the MFCs ran at constant temperature (30 °C) in sequencing batch mode. The anolytes of BLMFCs are composed of KCl (50 mM), sodium acetate ($1\ g\cdot L^{-1}$), vitamin ($5\ mL\cdot L^{-1}$) and trace mineral ($12.5\ mL\cdot L^{-1}$) solutions. The operation of BFMFCs included two stages. In Stage I, at the beginning of each running cycle, the initial pH of the KCl anolytes in three BLMFCs were adjusted to 8, 9 and 10 with NaOH solution ($1\ mol\cdot L^{-1}$) respectively in five running cycles, and the MFCs were named as KCl-pH 8-MFC, KCl-pH 9-MFC and KCl-pH 10-MFC. The BFMFC ran with pristine KCl anolyte was named as KCl-MFC, and its anolyte initial pH was 7. The MFCs with phosphate buffer (50 mM) was named as PBS-MFC. In Stage II, all the KCl-pH-MFCs ran in regular sequencing batch mode with pristine KCl anolytes. To determine the shortest pH adjusting duration that BFMFC needed to reach the optimal electric power, the anolyte initial pH of five BFMFCs were adjusted to 9 for 1 to 5 running cycles and named as KCl-pH-1C, KCl-pH-2C, KCl-pH-3C, KCl-pH-4C and KCl-pH-5C.

2.3. Measurements and analysis

The output voltages (U) were recorded by a 34972A data collection instrument (Agilent, USA) at 30 min interval. The electric power and the polarization properties were evaluated when the electrogenesis of the MFCs became stable. The power density and the polarization curves were plotted with the voltages and electrode potentials recorded at different external resistors (10,000 Ω to 68 Ω). The pH detector (405-DPAS-SC-K8S/225, Mettler Toledo) was used to measure the anolyte pH at 30 min intervals.

Electrochemical measurements were conducted with the CHI660D (Chenhua Instruments Co. Ltd., China) electrochemical workstation. The anode of each MFC, Pt wire electrode and saturated calomel electrode (SCE) were the working electrode, the counter electrode and the reference electrode in the three-electrode system, respectively. Cyclic voltammograms (CVs) of the anodes were performed in the potential range of −0.8 V and 0.5 V at the scan rate of $5\ mV\cdot s^{-1}$. Electrochemical impedance spectra (EIS) were tested in the frequency range of 0.01 to 10^5 Hz with an amplitude of 5 mV. The inorganic carbon (IC) concentrations of the anolytes at the running time of 0 h, 11 h and 22 h of a single running cycle were tested with the TOC-V_{CPH} total organic carbon (TOC) analyzer (Shimadzu, Japan). The concentrations of HCO_3^- and H_2CO_3 were calculated according to the carbonic acid dissociation equilibrium and the detailed calculation procedure can be found in the Supplementary material.

High-throughput sequencing was conducted to analyze the microbial community characteristics. The microorganism DNA on each anode was extracted with the EZNA®Soil DNA Kit (Omega Biotek, Norcross, USA) [22]. PCR amplification of the 16Sr RNA gene (V4-V5 region) was performed (95 °C for 3 min, 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and final extension at 72 °C for 10 min) with the ABI GeneAmp® 9700 PCR system (Applied Biosystems, USA) with primers 338F and 806R. After purified with AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA), PCR products were quantified with the QuantiFluor™-ST fluorometer (Promega, USA). High-throughput sequencing was carried out on Illumina Miseq PE300 platform. Operational taxonomic units (OTUs) were clustered with 97% similarity by the QIIME software. The taxonomy of each 16Sr RNA gene sequence was analyzed against the silva SSU115 database with a confidence threshold of 70% [23].

3. Results and discussion

3.1. Anolyte pH variation and performance of BFMFCs

In Stage I, the overall anolyte pH of the BFMFCs in each running cycle is significantly influenced by the adjusted initial value (Fig. 1a). The anolyte pH of the KCl-MFC, KCl-pH 8-MFC and KCl-pH 9-MFC first rapidly decline due to the accumulation of H^+ and then gradually rise up as the consuming of H^+ directly through the ORR or neutralized by the OH^- that generated by the ORR, as the ORR at cathode is lagged behind the substrates degradation at anode in BFMFCs [9,16]. The KCl-pH 10-MFC anolyte pH rapidly decreased and then stabilized around 6.9 in each running cycle, indicating that the OH^- added at the beginning of each running cycle is enough to neutralize the H^+ generated

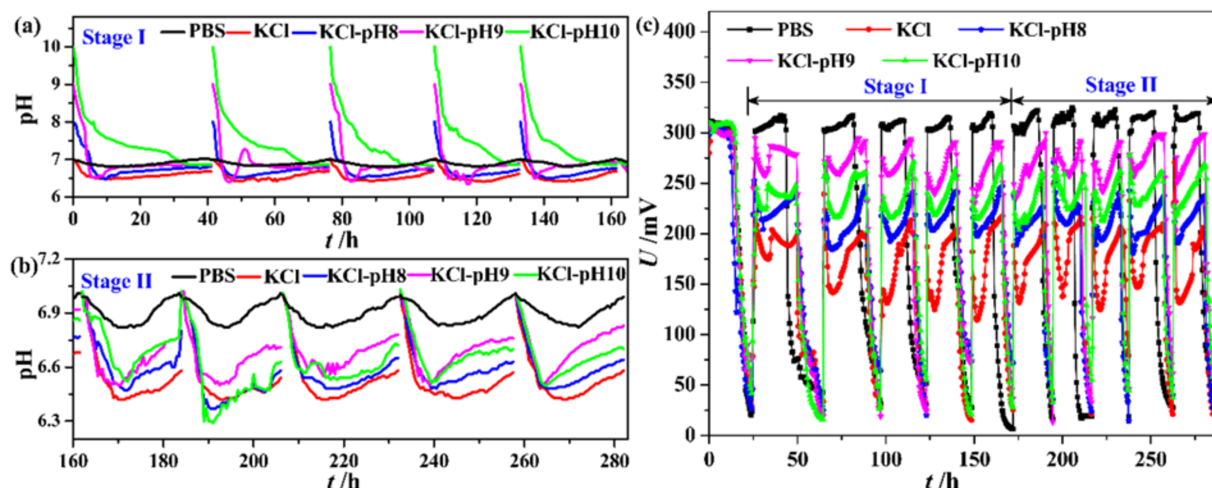

Fig. 1. The anolyte pH variations in (a) Stage I and (b) Stage II, and (c) the U of the MFCs.

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