Bioelectrochemistry 117 (2017) 48–56

Contents lists available at ScienceDirect

Bioelectrochemistry

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

Accelerating anodic biofilms formation and electron transfer in microbial fuel cells: Role of anionic biosurfactants and mechanism

Yunshu Zhang [a], Junqiu Jiang [a], Qingliang Zhao [a,*], YunZhi Gao [b], Kun Wang [a], Jing Ding [a], Hang Yu [a], Yue Yao [b]

[a] State Key Laboratory of Urban Water Resources and Environments (SKLUWRE), School of Environment, Harbin Institute of Technology, Harbin 150090, China
[b] School of Chemical Engineering and Technology, Harbin Institute of Technology, Harbin 150001, China

ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form 30 May 2017
Accepted 3 June 2017
Available online 08 June 2017

Keywords:
MFC
Anodic electron transfer
Rhamnolipid
Frumkin effect
Exoelectrogen

ABSTRACT

Anodic electron transfer is the predominant electricity generation process of MFCs. To accelerate anodic biofilms formation and electron transfer, 40 mg/L, 80 mg/L, and 120 mg/L of rhamnolipid biosurfactants were added to the anolyte, resulting in an increased abiotic capacitance from 15.12 F/m^2 (control) to 16.54 F/m^2, 18.00 F/m^2, and 19.39 F/m^2, respectively. Anodic biofilm formation was facilitated after dosing 40 mg/L of rhamnolipids on the 7th day after inoculation, resulting in an increased anodic biofilm coverage from 0.43% to 42.51%, and an increased maximum power density from 6.92 ± 1.18 W/m^3 to 9.93 ± 0.88 W/m^3. Furthermore, the adsorption of rhamnolipids on the anode caused the Frumkin effect, leading to a decrease of equilibrium potential from -0.43 V to -0.56 V, and an increase of exchange current density from 5.09×10^{-3} A/m^2 to 8.72×10^{-3} A/m^2. However, electron transfer was blocked when the rhamnolipid concentration was further increased to 80 mg/L, and 120 mg/L. Analysis of the anodic bacterial communities revealed that rhamnolipids facilitated the enrichment of exoelectrogen, increasing the total proportion from 65% to 81%. Additionally, biosurfactants were found to have significant impacts on the composition of exoelectrogens.

1. Introduction

Microbial fuel cells (MFCs) are constructed using microorganisms as catalysts to drive oxidation and reduction reactions at electrodes. Recently, more studies have been focused on wastewater treatment, sludge stabilization, bioremediation of contaminants and stabilization of recalcitrant compounds using MFCs to improve cost effectiveness and sustainability [1]. However, power generation remains a large challenge for practical MFC applications. During the power output process, electrons are produced by microorganism-mediated organic matter oxidation and are subsequently transferred to the anode. These electrons then flow through a circuit to the cathode, resulting in an overall energy loss [2]. In this electron transfer process, anodic extracellular electron transfer (EET) is the rate-limiting step during MFC start-up and is considered the most complex and important process [3]. Furthermore, anodic biofilms play important roles in EET. During the initial stage of MFC operation, anodic biofilms evolve to develop more uniform coverage as the current output increases [4,5]. Mature anodic biofilms of *Geobacter sulfurreducens* can also form nanowires to promote long-range electrical transfer and increase current output [6]. In particular, as the first step of anodic biofilms formation, the attachment of microorganisms onto anode surfaces significantly influences the start-up stage of MFCs. As microorganism increasingly attach onto the anode, the electron transfer rate is significantly enhanced with a corresponding decreasing start-up time [7]. Therefore, a number of methods have been used to enhance the attachment of microorganisms onto anode surfaces, such as anode surface modification, anode potential imposition, external resistance adjustment and use of gene-deficient microorganisms [8–10].

Surfactants have been confirmed to have the ability to change cell membrane ultrastructures to form trans-membrane channels, which are effective for enhancing microbial cell permeability, reducing membrane resistance, expediting mass transport through cell membranes, and increasing substrate degradation. Biosurfactants are a type of surfactant produced by bacteria, yeasts and fungi and have strong interfacial activity, moisturizing effect, emulsifying action and foaming properties [11]. The most studied biosurfactants are glycolipids, among which rhamnolipids are one of the best-known compounds [12]. Wen et al. [13] showed that open circuit voltage and maximum power density increased from 483 mV to 878 mV (1.8-fold) and from 22 W/m^3 to 275 W/m^3 (12.5-fold), respectively, in an air-cathode, single-chambered MFC as 0 to 80 mg/L rhamnolipids was added. Zheng et al. [14] constructed an overexpressed exoelectrogen *Pseudomonas aeruginosa* PAO1 for over-producing rhamnolipids, resulting in enhanced MFC power output and bacteria attachment on the anode.

Rhamnolipids have hydrophilic and hydrophobic groups, and the hydrophobic groups can be adsorbed onto the hydrophobic anode

---

[*] Corresponding author.
E-mail address: qlzhao@hit.edu.cn (Q. Zhao).

http://dx.doi.org/10.1016/j.bioelechem.2017.06.002

surface to change the characteristics of anode interfaces from hydrophobicity to hydrophilicity. Hydrophilic anodes can facilitate the attachment of microorganisms and the formation of anodic biofilms [8,15]. Meanwhile, the hydrophilic groups of rhamnolipids are easy to link the hydrophilic cell surface and make the cell surface more hydrophobic [16]. In other words, under the appropriate concentration, rhamnolipids can serve as a bridge to enhance the adsorption of microorganisms onto anode surface. Moreover, rhamnolipids can maintain open channels to enhance *P. aeruginosa* biofilms intercellular interactions and facilitate the attachment and migration of microorganisms to accelerate anodic biofilms formation and electron transfer [17]. For anodic reactions, the electron transfer between the anode and redox species can only occur at the Helmholtz plane, and the reaction rate is determined by the electric double layer (EDL) potential, which is the potential difference between the anode surface and outer Helmholtz plane (OHP). However, if the ion adsorption occurs on the anode surface, the ions arranged in the inner Helmholtz plane (IHP) results in the excess of ions in Helmholtz layer, which leads to a change of electrode potential and electrode reaction kinetics (Frumkin effect) [18]. As anionic biosurfactants, rhamnolipids are ionized in anolyte, resulting in the excess of anions on IHP and thus reducing the potential of the EDL and affecting the anodic electron transfer rate. Therefore, rhamnolipids have great potential to facilitate anodic microorganism adsorption and electron transfer. However, there have been few reports on enhancing anodic biofilms formation during MFC start-up and electron transfer efficiency through biosurfactant dosing [14].

This study was designed to elucidate the mechanism of anodic biofilms formation and electron transfer due to rhamnolipid addition during the start-up and stable stages of MFC operation. This study also attempted to determine the optimum concentration for rhamnolipid addition. The scopes of this work are to (1) analyze the electrochemical response of anodic rhamnolipid adsorption without inoculation, (2) investigate the influence of rhamnolipid dosing time on the start-up performance of MFCs, (3) verify the effect of rhamnolipid concentration on anodic electron transfer by applying the Bockris-Devanathan-Müller (BDM) model to simulate anodic electron transfer and to investigate the mechanism through electrochemical analyses, and (4) identify changes in anodic bacterial communities after rhamnolipid addition.

2. Methods and materials

2.1. Construction of air-cathode single chambered MFCs and operation

Air-cathode, single chambered MFCs were fabricated with Plexiglas with outside dimensions of 5-cm length, 5-cm width and 4-cm height and with effective working volumes of 28 cm^3 (built-in cylinder, 4-cm length and 3-cm diameter) [19]. Anodes were prepared from carbon fiber with a titanium wire as described by Logan et al. [20], having outer diameters and lengths of 3 cm. Cathodes were prepared from carbon cloth modified with 0.40 mg/m^2 of Pt catalyst. All MFCs cascading an external resistance of 1000 Ω were operated at 25 °C in a thermostatic room.

To verify the adsorption characteristics of rhamnolipids on anodes, 40 mg/L (Anode-R_{40}), 80 mg/L (Anode-R_{80}) and 120 mg/L (Anode-R_{120}) rhamnolipids (purchased from Victex Company, Daqing, China) were added to the anolytes of three non-inoculated MFCs, and one non-inoculated MFC without addition of rhamnolipids (Anode–NR) was served as the control. The rhamnolipids applied in this study were characterized as a mixture of mono-rhamnolipid congeners and di-rhamnolipid congeners, and the purity of rhamnolipids was 90 ± 5%. The physical and chemical properties of mono-rhamnolipid congeners and di-rhamnolipid congeners are similar [21], and the typical chemical structures of mono-rhamnolipid congeners and di-rhamnolipid congeners were shown in Fig. S1. The anolytes, containing 1 g/L glucose, 17.1 mg/L $Na_2HPO_4 \cdot 12H_2O$, 3.0 mg/L KH_2PO_4, 0.3 mg/L NaCl, 0.494 mg/L $MgSO_4 \cdot 7H_2O$, 0.01 mg/L $CaCl_2$ and 10 mL trace elements, were applied as described by Rabaey et al. [22]. The pH levels of the anodic electrolytes were maintained at 7.0–7.2.

To investigate the influence of dosing time on MFC start-up performance, four groups of MFCs were run in triplicate. Each MFC was identically constructed with 10% surplus sludge supernatant inoculation (centrifuged at 3500 rpm), which exhibited high resistance to process disturbances, high versatility to different types of substrates, and high power output yield. The sludge was anaerobic sludge collected from a secondary settling tank of the Wenchang Wastewater Treatment Plant in Harbin, China. One group of the MFCs served as controls (MFC_{NR}) to which no rhamnolipids were added. The other three MFC groups were dosed with 40 mg/L rhamnolipid prior to inoculation ($MFC_{R40\text{-}0d}$), on the 3rd day ($MFC_{R40\text{-}3d}$) and on the 7th day ($MFC_{R40\text{-}7d}$) after inoculation.

To study the effect of rhamnolipid concentration on anodic electron transfer during the stable stage of MFC operation, four groups of inoculated MFCs (3 MFCs in parallel for each group) with similar whole cell voltages and anode potentials on the 7th day of MFC operation were selected and given rhamnolipid doses of 0 (MFC_{R0}, control test), 40 mg/L (MFC_{R40}), 80 mg/L (MFC_{R80}) and 120 mg/L (MFC_{R120}), respectively, in the anodic electrolyte. When these MFCs reached steady stage, analyses were performed.

2.2. Electrochemical analyses and calculations

Real-time whole cell voltage was detected and recorded every 30 min by a data acquisition board (PISO-813, PCI-DAS Co., Ltd.) connected to a computer. The anode and cathode potentials were monitored by Ag/AgCl reference electrodes (+0.197 V vs. standard hydrogen electrode). Polarization, Tafel, and cyclic voltammetry (CV) curve measurements and electrochemical impedance spectroscopy (EIS) were conducted by an electrochemical workstation (CHI 660e, Chenhua, Shanghai). Polarization curves were calculated by linear sweep voltammetry (LSV) analysis. In LSV, the working electrode was the anode, and the counter electrode and reference electrode were the cathodes. The swept voltage range of LSV was −1.2 V to 0 V at a scan rate of 1 mV/s. Current density (j) and power density ($P = j \times V$) were calculated from the polarization curve data. *t*-tests were used to compare biofilm biomass and power density between two groups. Comparison P values were calculated with OriginPro 2015. Differences with P values <0.01 were considered very significant. Differences with P values between 0.01 and 0.05 were considered significant but not very obvious. Differences with P values >0.05 were considered to be not significant.

Tafel plots, CV curves, EIS, and open circuit potential curves were performed for three-electrode systems, with an anode as the working electrode, a Ag/AgCl electrode as the reference electrode and a cathode as the counter electrode. For the anodic Tafel plot, the initiation potential was φ_{OCP}-0.1 V (φ_{OCP} represents open circuit potential), and the final potential was $\varphi_{OCP} + 0.1$ V at a scan rate of 1 mV/s. The swept voltage range of CV was −0.6 V to 0.2 V at a scan rate of 5 mV/s. For EIS, the frequency range was 10 kHz to 10 mHz with an amplitude of 5 mV; the working potential was the φ_{OCP} of each MFC. For open circuit potential curves, the running time is 1200 s. and the sample interval is 1 s.

2.3. Microscopic examination of anodic biofilms

SEM (Quanta 200, FEI, USA) was used to image carbon fiber brush anodic biofilms. The anodic biofilms samples were pretreated as described previously [23]. All samples were fixed with 2.5% glutaraldehyde in 4 °C for 2 h, dehydrated with a gradient concentration of ethanol (20%, 50%, 70%, 85%, 95%, and 100%) and isoamyl acetate (100%) and dried in a vacuum oven at room temperature for 4 days. After the samples were dried, they were placed on aluminum foil and sprayed with alloy. The attached biomass was determined using modified bicinchoninic acid (BCA) protein assay kit (Sangon Biotech, Shanghai, China). The biomass samples were collected from the anode carbon fibers, which were cut off about 5 mm and rinsed with sterile distilled water to remove loosely attached cells