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Ohmic resistance affects microbial community and electrochemical kinetics in a multi-anode microbial electrochemical cell

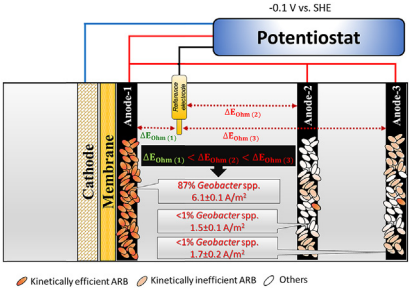
Bipro Ranjan Dhar [a], Hodon Ryu [b], Jorge W. Santo Domingo [b], Hyung-Sool Lee [a, *]

[a] Department of Civil & Environmental Engineering, University of Waterloo, 200 University Ave. West, ON N2L 3G1, Canada
[b] National Risk Management Research Laboratory, U.S. Environmental Protection Agency, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, USA

HIGHLIGHTS

- A multi-anode microbial electrochemical cell (MxC) was designed and operated.
- Microbial community and anode kinetics were evaluated for individual anodes.
- *Geobacter* species were dominant only on the anode closest to a reference electrode.
- Half-saturation anode potential was lower for the closest anode.
- High current density was produced only from the closest anode.

GRAPHICAL ABSTRACT



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ABSTRACT

Multi-anode microbial electrochemical cells (MxCs) are considered as one of the most promising configurations for scale-up of MxCs, but understanding of anode kinetics in multiple anodes is limited in the MxCs. In this study we assessed microbial community and electrochemical kinetic parameters for biofilms on individual anodes in a multi-anode MxC to better comprehend anode fundamentals. Microbial community analysis targeting 16S rRNA Illumina sequencing showed that *Geobacter* genus was abundant (87%) only on the biofilm anode closest to a reference electrode (low ohmic energy loss) in which current density was the highest among three anodes. In comparison, *Geobacter* populations were less than 1% for biofilms on other two anodes distant from the reference electrode (high ohmic energy loss), generating small current density. Half-saturation anode potential (E_{KA}) was the lowest at −0.251 to −0.242 V (vs. standard hydrogen electrode) for the closest biofilm anode to the reference electrode, while E_{KA} was as high as −0.134 V for the farthest anode. Our study proves that electric potential of individual anodes changed by ohmic energy loss shifts biofilm communities on individual anodes and consequently influences electron transfer kinetics on each anode in the multi-anode MxC.

1. Introduction

Our current society needs sustainable biotechnologies to build a green cycle at the intersection of economy, environment, and energy. Microbial electrochemical cells (MxCs) that produce value-added chemicals from organic waste and wastewater can be one of the biotechnologies to catalyze establishment of the green circle [1–3]. MxCs should produce high current density (the fast yield of value-added products) with an acceptable range of exogenous energy to deploy MxCs in field. The kinetics on anodes primarily limits

* Corresponding author.
E-mail address: hyungsool@uwaterloo.ca (H.-S. Lee).

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current density in external energy dependent-MxCs, since electrode catalysts and exogenous energy accelerate abiotic reaction rates associated with ohmic and cathodic limitations [1–5]. While anodic reaction rate significantly depends on biological kinetic parameters, nanomaterials and new designs have been applied for anodes to improve the reaction rate [1,4–7]. The physical and chemical approaches on anodes increased current density by 10–25 A/m^2 [6,7], but they would not be readily applicable for large-scale MxCs, due to cost and scale-up issues [4,6,7]. Multi-anode configurations employing inexpensive electrodes could be a technically robust and economically viable option for scale up of MxCs [8–14], since multiple electrodes can improve mass transfer and biofilm formation per volume of anode chambers (or MxCs), propelling anode kinetics without footprint increase of MxCs [8,12–14]. However, literature has shown non-linear or sometimes trivial increase of current density in multi-anode MxCs over single-anode ones [9–12].

The small contribution of multiple anodes to the overall current density implies sluggish kinetics on the anodes, especially supplemental anodes. No studies, however, have assessed anode kinetics for individual anodes in multi-anode MxCs. Each anode would have heterogeneous conditions for electron transfer in biofilm anodes, such as mixing conditions, local substrate concentration, local pH, and ionic resistance between anodes, which could cause inconsistent, slow kinetics on individual anodes. Among these heterogeneities in multiple anodes, it is evident that multi-anode configuration creates ionic resistance between anodes different from single anode. Ohmic resistance (mainly ionic resistance) can change electric potential of individual anodes in multi-anode MxCs when electrode distance is far from each other. In this circumstance, anode potential of individual anodes would not be equivalent in multi-anode MxCs, which can change microbial community in biofilm anodes, leading to different biological and electrochemical electron transfer kinetics on each anode. The contribution of additional anodes to overall current density would be small when kinetically inferior ARB are enriched on supplemental anodes. Anode potential is a key parameter for enrichment of kinetically efficient anode-respiring bacteria (ARB) in biofilm anodes from mixed-culture inocula [14–16], although other factors (e.g., electrode materials and structures, substrate type and concentration, pH, and temperature) can affect ARB community in biofilm anodes [1–5,17–19]. For example, *Geobacter* genus, one of the most kinetically efficient ARB, became dominant in biofilm anodes and generated high current density (~10 A/m^2) when mixed culture was inoculated at negative anode potential (−0.05 to −0.15 V vs. standard hydrogen electrode (SHE)) [14,15]. In comparison, more diverse ARB community was established at positive anode potential (+0.2 to +0.37 V vs. SHE), along with low current density (0.6–2 A/m^2) in MxCs [14,16].

Improvement of current density using multiple anodes is based on the assumption that individual anodes generate comparable current density, thereby increasing the overall current density in multi-anode MxCs. If different microbial communities are built on individual anodes in multi-anode MxCs probably caused by ohmic energy loss among anodes, biological and electrochemical kinetics may not be conserved throughout multiple anodes. Then, current density generated from individual anodes is inconsistent, and multi-anode MxCs would not improve current density proportionally to the number of anodes. However, no studies have explored this important aspect yet.

This study was conducted to characterize microbial community and electrochemical kinetics in individual anodes for a multi-anode MxC. First, we quantified the current density in each anode of the MxC. Second, we identified dominant ARB in biofilm anodes by targeting 16S rRNA. Third, we characterized half-saturation anode potential (E_{KA}) for individual anodes to evaluate electrochemical kinetics. Finally, the implication of ohmic energy loss and its impacts on performance of multi-anode MxCs was summarized.

2. Methods

2.1. Configuration of multi-anode microbial electrochemical cell (MxC)

A dual chamber microbial electrochemical cell (MxC) equipped with three anode modules was fabricated using plexiglass (Fig. 1a) and the working volumes of an anode and a cathode chamber were 300 mL and 100 mL, respectively. A stainless steel mesh was employed for the cathode (Type 304, McMaster Carr, OH, USA) (Fig. 1b). High-density carbon fibers (2293-A, 24A Carbon Fiber, Fibre Glast Development Corp., Ohio, USA) that were connected with stainless steel current collectors were used as the anode module (Fig. 1c). Prior to use, the carbon fibers were pretreated for 3 days with nitric acid (1 mol/L), acetone (0.17 mol/L) and ethanol (0.17 mol/L) for 1 day in series, and then washed with MilliQ water (18.2 MΩ-cm). Three anode modules (anode-1, anode-2, and anode-3) were installed in the anode chamber of the MxC (Fig. 1a) and they were connected via copper wires. An anion exchange membrane (AMI-7001, Membranes International Inc., USA) was used as a separator between the anode and the cathode chamber, and the geometric surface area of the membrane was 28.1 cm^2; current density was expressed per the membrane surface area in this study [20]. A reference electrode (Ag/AgCl reference electrode, MF-2052, Bioanalytical System Inc., USA) was placed between anode-1 and anode-2, and we reported electrode potential against standard hydrogen electrode (SHE) in this study. The distance between the reference electrode and anode-1 was 1.27 cm. The distances of the reference electrode from anode-2 and anode-3 were 3.81 cm and 6.35 cm, respectively (Fig. 1a).

2.2. Inoculation and operating conditions

We inoculated the MxC equipped with three anodes using 30 mL of anolyte from a mother MxC, which had been run with acetate medium [20] for over 1 year. The anode chamber was sparged with ultra-pure nitrogen (99.999%) for 20 min, and then the anode potential was set at −0.1 V vs. SHE using a potentiostat (BioLogic, VSP, Gamble Technologies, Canada). Current and applied voltage were recorded at every 120 s using EC-Lab for windows v 10.32 software in a personal computer connected with the potentiostat (BioLogic, VSP, Gamble Technologies, Canada). The MxC was operated in a temperature-controlled room at 25 °C. The anolyte was circulated using a peristaltic pump (Master Flex® L/S economy variable-speed drive, Cole-Parmer, Canada) at a flow rate of 25 mL/min for mixing. The cathode chamber was filled with tap water where hydrogen gas is produced, which allows us to focus on anodic reactions in the MxC [18]. The MxC was operated in batch mode for 3 days, and switched to continuous mode by feeding acetate medium (100 mM phosphate buffer) to the anode chamber at a flow rate of 15 mL/h with a peristaltic pump (Master Flex® L/S digital drive, Model 7523-80, Cole-Parmer, Canada); hydraulic residence time (HRT) in the anode chamber was kept at 20 h during the experiment. The average COD concentration of acetate medium was 2300 ± 40 mg COD/L.

At the constant anode potential and HRT, we started to run the MxC with three anode modules (anode-1, anode-2, and anode-3 for phase-1), then two modules (anode-1 and anode-2 for phase-2), and finally one module (anode-1 for phase-3). Anode modules were removed and reassembled in an anaerobic chamber (COY Type B Vinyl Anaerobic Chamber, COY Lab Products, USA) to avoid