Water Research 127 (2017) 230–238

Contents lists available at ScienceDirect

Water Research

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

Microbial activity influences electrical conductivity of biofilm anode

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ARTICLE INFO

Article history:
Received 29 May 2017
Received in revised form
11 October 2017
Accepted 12 October 2017
Available online 13 October 2017

Keywords:
Acidic biofilm
Biofilm anode
Biofilm conductivity
Extracellular electron transfer
Microbial electrochemical cell

ABSTRACT

This study assessed the conductivity of a *Geobacter*-enriched biofilm anode in a microbial electrochemical cell (MxC) equipped with two gold anodes (25 mM acetate medium), as different proton gradients were built throughout the biofilm. There was no pH gradient across the biofilm anode at 100 mM phosphate buffer (current density 2.38 A/m^2) and biofilm conductivity (K_{bio}) was as high as 0.87 mS/cm. In comparison, an inner biofilm became acidic at 2.5 mM phosphate buffer in which dead cells were accumulated at ~80 μm of the inner biofilm anode. At this low phosphate buffer, K_{bio} significantly decreased by 0.27 mS/cm, together with declined current density of 0.64 A/m^2. This work demonstrates that biofilm conductivity depends on the composition of live and dead cells in the conductive biofilm anode.

1. Introduction

Extracellular electron transfer (EET) is unique for anode-respiring bacteria (ARB) to balance intracellular reducing power using solid electron sinks (Torres et al., 2010; Lee et al., 2009, 2016; Rotaru et al., 2015). Microbial electrochemical cells (MxCs) employing ARB as an anode catalyst are very promising, since they are able to recover value-added products from organic waste and wastewater (Logan et al., 2015; Feng et al., 2016; Dhar et al., 2016a). High current density is essential for catalyzing MxC deployment in field, and thus understanding EET kinetics can be important for achieving high current density in MxCs. Among several EET mechanisms, conductive EET allows biofilm anodes to generate high current density in MxCs (Torres et al., 2010, 2008a; Lee et al., 2016; Marcus et al., 2007; Renslow et al., 2013). Electron conduction of EET can occur through electrically conductive pili (Reguera et al., 2005; Malvankar et al., 2011; Xiao et al., 2016), extracellular cofactors (Snider et al., 2012; Strycharz-Glaven and Tender, 2012; Yates et al., 2016; Phan et al., 2016), or its combination (Lee et al., 2016). The literature reported that the electrical conductivity of a *Geobacter sulfurreducens* biofilm anode was as high as ~5 mS/cm (Malvankar et al., 2012), and the conductivity of *Geobacter*'s pili is even higher (Adhikari et al., 2016). For *Geobacter*-enriched mixed culture biofilm anodes, high biofilm conductivity (K_{bio}) of 0.96–2.44 mS/cm was also reported (Lee et al., 2016; Dhar et al., 2016b). The literature has suggested EET would occur through electrically conductive pili in biofilm anodes having high K_{bio} (Malvankar et al., 2011, 2012; Xiao et al., 2016; Adhikari et al., 2016). In comparison, a low range of K_{bio} (0.0003–60 μS/cm) was observed for *Geobacter sulfurreducens* biofilm anodes when redox conduction via extracellular cofactors would be responsible for conductive EET (Yates et al., 2016; Phan et al., 2016). These K_{bio} values are several orders of magnitude lower than the highest K_{bio} in a *Geobacter* pure culture or enriched biofilm anode where electrically conductive pili would play an important role of EET. Unfortunately, there are no clear mechanisms accounting for substantial K_{bio} difference in electrically conductive biofilm anodes, in which two different EET pathways would occur. Conductive EET mechanisms seem more complicated than early research suggested (Torres et al., 2010; Reguera et al., 2005; Malvankar et al., 2011; Snider et al., 2012), and thus it is challenging to assess dominant EET mechanism for conductive biofilm anodes. However, modeling

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https://doi.org/10.1016/j.watres.2017.10.028
0043-1354/

approach based on biofilm conduction (i.e., the Nernst-Monod equation) suggests that K_{bio} would be higher than 0.5 mS/cm in biofilm anodes generating high current density without substantial energy losses (Marcus et al., 2007; Renslow et al., 2013), which emphasizes K_{bio} significance to success of MxCs as resource recovery wastewater treatment technologies.

Anode respiration allows ARB only to transfer electrons to the anode, accumulating substantial protons in biofilms. This unique respiration can acidify biofilm anodes (Torres et al., 2008b; Marcus et al., 2010, 2011; Franks et al., 2009). The challenge is that ARB's metabolism is seriously inhibited at acidic pH (Franks et al., 2009; Kim and Lee, 2010; Patil et al., 2011). ARB (e.g., *Geobacter*) grow well at neutral pH, although some alkaliphilic ARB (e.g., Geoalkalibacter spp.) were identified (Pierra et al., 2015; Yoho et al., 2015). For instance, the growth rate of *Geobacter sulfurreducens* at pH 6 was decreased to 80% over pH 7 (Franks et al., 2009). Literature also reported considerable decrease of current density in MxCs when bulk pH or local biofilm anodes were acidic (Franks et al., 2009; Kim and Lee, 2010; Patil et al., 2011). It is apparent that acidic pH seriously inhibits electron transfer rate from donor substrate to the anode in biofilm anodes.

Despite the significance of acidic pH for electron transfer rate in biofilm anodes, understanding of EET kinetics at acidic pH is very limited. Malvankar et al. (2012) reported the decrease of K_{bio} (from 5 to 0.25 mS/cm) and current density (from 10 to 2 A/m^2) for a thick biofilm (130 μm) in which mass transfer limitations of protons or substrate might decrease K_{bio} and current density. Interestingly, the conductive pili of *Geobacter sulfurreducens* showed higher electrical conductivity at pH 2 than neutral and alkaline pHs (Malvankar et al., 2011; Adhikari et al., 2016), which is opposite to K_{bio} reduction in acidic pH (Malvankar et al., 2012). Understanding of EET kinetics at acidic pH can be very important for MxC deployment in field where acidic biofilms can be built due to relatively low buffer conditions in wastewater. However, there are no clear, detailed information on K_{bio} and EET kinetics at acidic biofilm anodes.

In this study, we evaluated the change of biofilm conductivity in a steady-state biofilm anode in which different proton gradients were built using three phosphate buffer concentrations. First, current density, biofilm thickness, and proton gradients were quantified for individual phosphate conditions. Second, the metabolic activity of ARB within biofilm anodes was qualitatively compared between low and high phosphate buffer using confocal laser scanning microscopy (CLSM). Finally, we experimentally measured K_{bio} and half-saturation anode potential (E_{KA}) for the biofilm at the three phosphate buffer concentrations and discussed the implication of reduced K_{bio} at acidic pH.

2. Materials and methods

2.1. MxC configuration and operation

Three dual-chamber MxCs were constructed with plexiglass: two biotic and one abiotic MxCs (see Fig. 1(a)). The abiotic MxC identical to the two biotic MxCs was used for measuring ionic conductance to quantify intrinsic biofilm conductance. Two gold electrodes (width 9.5 mm × length 15 mm × thickness 10 μm) on a glass base with a non-conductive gap of 50 μm were designed as anodes to measure biofilm conductivity (Dhar et al., 2016b), and the total geometric surface area of the anodes was 2.85 cm^2 (see Fig. 1(b)). A porous graphite plate (Isomolded Graphite Plate 203101, Fuel Cell Earth, USA) was used as the cathode and anion exchange membrane (AMI-7001, Membranes International Inc., USA) was inserted between the anode and the cathode chambers as a separator. The working volumes of both chambers were 15 mL. A reference electrode (Ag/AgCl reference electrode, MF-2052, Bioanalytical System Inc., USA) was placed within less than 1 cm distance from the anodes to fix anode potential (E_{anode}) during experiments.

Two MxCs were inoculated with a biofilm anode collected from a mother MxC that had been operated with acetate medium (25 mM acetate) for over one year. The anode chambers were filled with 25 mM acetate medium (100 mM phosphate buffer, pH 7.25–7.4), and the cathode chambers were filled with tap water, producing H_2 gas; the literature provides the composition of acetate medium (Dhar et al., 2013). We fixed anode potentials (E_{anode}) at −0.2 V against standard hydrogen electrode (SHE) using a potentiostat (BioLogic, VSP, Gamble Technologies, Canada). The anode chambers were sparged with ultra-pure nitrogen (99.999%) for 5 min before operation of the MxCs. Current was recorded at every 2 min using EC-Lab for windows v 10.32 software in a personal computer connected to the potentiostat. After operating the MxCs for 5 days in batch mode (the maximum current density ~0.7 A/m^2), we continuously fed acetate medium to the MxCs at a flow rate of 5.8 mL/h using a peristaltic pump (Master Flex® L/S digital drive, Model 7523-80, Cole-Parmer, Canada). Hydraulic residence time (HRT) in the anode chambers was kept at 2.6 h, leading to substrate non-limiting conditions for anode-respiring bacteria (ARB). Phosphate buffer concentration in acetate medium was stepwise decreased from 100 mM to 2.5 mM (pH 7.2–7.4) to create different pH gradients throughout the biofilm anode of a MxC (MxC_{buffer}). At the steady-state biofilm anode for current density and effluent acetate concentration at each buffer concentration in the MxC_{buffer}, pH gradients throughout the biofilm anode, biofilm conductivity (K_{bio}), half-saturation anode potential (E_{KA}), and biofilm thickness (L_f) were measured. To mitigate the changes of biofilm community structure and biofilm thickness at 50 and 2.5 mM phosphate buffer, we completed the experiments for the low phosphate buffer concentrations in ~2 weeks.

2.2. Biofilm conductivity

The conductivity of the biofilm anode grown at different phosphate buffer concentrations in the MxC_{buffer} was measured using the two-probe measurement method (Malvankar et al., 2011; Lee et al., 2016; Dhar et al., 2016b). For the biofilm conductance measurement, the gold anodes and the cathode were disconnected temporarily (open circuit mode). Then, a linear sweeping voltage of 0–0.05 V in a step of 0.025 V was applied across two gold electrodes using a source meter (Keithley 2400, Keithley Instruments, Inc., USA), and the current was recorded in 1 min for each voltage. The voltage ramp was applied in 4–5 cycles until a steady-state current-voltage (I-V) response was obtained; we confirmed negligible effects of anode potential for the steady state I-V responses (Dhar et al., 2016b). Observed biofilm conductance ($G_{Biofilm\ (obs)}$, mS) was calculated from steady-state I-V curves (Dhar et al., 2016b; Lee et al., 2016). Ionic current can contribute to $G_{Biofilm\ (obs)}$ values, and therefore the ionic conductance ($G_{control}$, mS) was measured with the abiotic MxC (control) using the acetate medium and MxC effluent as electrolytes. Intrinsic biofilm conductance ($G_{Biofilm} = G_{Biofilm\ (obs)} - G_{control}$, mS) was quantified with Eq. (1) (Kankare and Kupila, 1992).

$$K_{bio} = G_{Biofilm}\frac{\pi}{L}\Big/ln\left(\frac{8L_f}{\pi a}\right) \quad (1)$$

where, $G_{biofilm}$ is intrinsic biofilm conductance (mS) ($G_{Biofilm} = G_{Biofilm\ (obs)} - G_{control}$), L_f is the biofilm thickness (μm), L is the length of the electrodes (1.5 cm), and a is half of the non-conductive gap between two electrodes (25 μm). For the steady-state biofilm