Electronic supplementary information for

"Kinetics and scale up of oxygen reducing cathodic biofilms"

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S1. Materials and Methods

S1.1. Mathematical modeling of the oxygen reduction kinetics in cathodic biofilms

The derivation of electrode kinetics follows the derivation of Butler-Volmer equation as described previously [1, 2]. Instead of using first order kinetics, we use Michaelis-Menten equation to describe biologically catalyzed oxygen reduction reaction.

Forward reaction: $\qquad$ ox + n e$^-$ → red

Reverse reaction: $\qquad$ red → ox + n e$^-$

The overall activation energy barrier for the cathodic reaction (ΔG_{Tc}) can be described as a summation reaction activation energy (ΔG_c) and part of the electrical energy activation barrier ($\alpha nF\Delta E$), where α is the transfer coefficient, F is Faraday's constant, n is the stoichiometric balance for the number of electrons transferred in the redox reaction, and ΔE is the electric potential barrier.

$$\Delta G_{Tc} = \Delta G_c + \alpha nF\Delta E$$

Because we set the electrode potential at 0.396 V below the standard reduction potential, the contribution of the anodic reaction to the current is ignored. The contribution of the anodic reaction to the current is less than 1% when the overpotential is less than -0.118 V [1].

Arrhenius equation is used to describe the reaction rate constant (k), where A' is the Arrhenius frequency factor, R is the universal gas constant, and T is temperature.

$$k = A'e^{\frac{-\Delta G_{activation}}{RT}}$$

Michaelis-Menten reaction is used to describe the rate of cathodic reactions (r_c).

$$r_c = \left\{-\frac{r_{max,c}\,[S]}{K_{M,c} + [S]}\right\} e^{\frac{-\Delta G_{activation}}{RT}} A$$

$$r_c = \left\{-\frac{r_{max,c}\,[S]}{K_{M,c} + [S]}\right\} e^{\frac{-(\Delta G_c + \alpha nF\Delta E)}{RT}} A$$

$$r_c = \left\{-\frac{r_{max,c}\,[S]}{K_{M,c} + [S]}\right\} e^{\frac{-\Delta G_c}{RT}} e^{\frac{-\alpha nF\Delta E}{RT}} A$$

$$r_c = -r_{max,c}\, e^{\frac{-\Delta G_c}{RT}} \frac{[S]}{K_{M,c} + [S]} e^{\frac{-\alpha nF\Delta E}{RT}} A$$

Where $r_{max,c}$ is the maximum cathodic reaction rate which includes the Arrhenius frequency factor (A'), $K_{M,c}$ is the Michaelis-Menten cathodic half-saturation constant, $[S]$ is the concentration the electron acceptor and A is the electrode surface area.

The cathodic current density (j_c) is then calculated using Faraday's constant

$$j_c = \frac{r_c nF}{A}$$

$$j_c = -nFr_{max,c}\, e^{\frac{-\Delta G_c}{RT}} \frac{[S]}{K_{M,c} + [S]}\, e^{\frac{-\alpha nF\Delta E}{RT}}$$

The potential between the electrode and the solution ΔE is composed of the equilibrium potential (ΔE_{eq}) and overpotential (η).

$$\Delta E = \Delta E_q + \eta$$

$$j_c = -nFr_{max,c}\, e^{\frac{-\Delta G_c}{RT}} \frac{[S]}{K_{M,c} + [S]}\, e^{\frac{-\alpha nF(\Delta E_q + \eta)}{RT}}$$

$$j_c = -nFr_{max,c}\, e^{\frac{-\Delta G_c}{RT}} e^{\frac{-\alpha nF\Delta E_q}{RT}} \frac{[S]}{K_{M,c} + [S]}\, e^{\frac{-\alpha nF\eta}{RT}}$$

$$j_c = -j_{o_{c,max}} \frac{[S]}{K_{M,c} + [S]}\, e^{\frac{-\alpha nF\eta}{RT}}$$

The overpotential is defined as the difference between working electrode potential (ε) subtracted from the standard reduction potential. Significant losses could happen in oxygen reduction reaction in bioelectrochemical systems. We use two terms to describe these losses as described previously: 1) a lumped constant potential term (E_L) and 2) an ohmic loss term characterized by a constant ohmic resistance (R_L) [3].

$$\eta = \varepsilon - E - E_L + j_c AR_L$$

$$j_c = -j_{o_{c,max}} \frac{[S]}{K_{M,c} + [S]}\, e^{\frac{-\alpha nF(\varepsilon - E - E_L + j_c AR_L)}{RT}}$$

S1.2. Schematic of the electrochemical cell used to grow and study cathodic biofilms
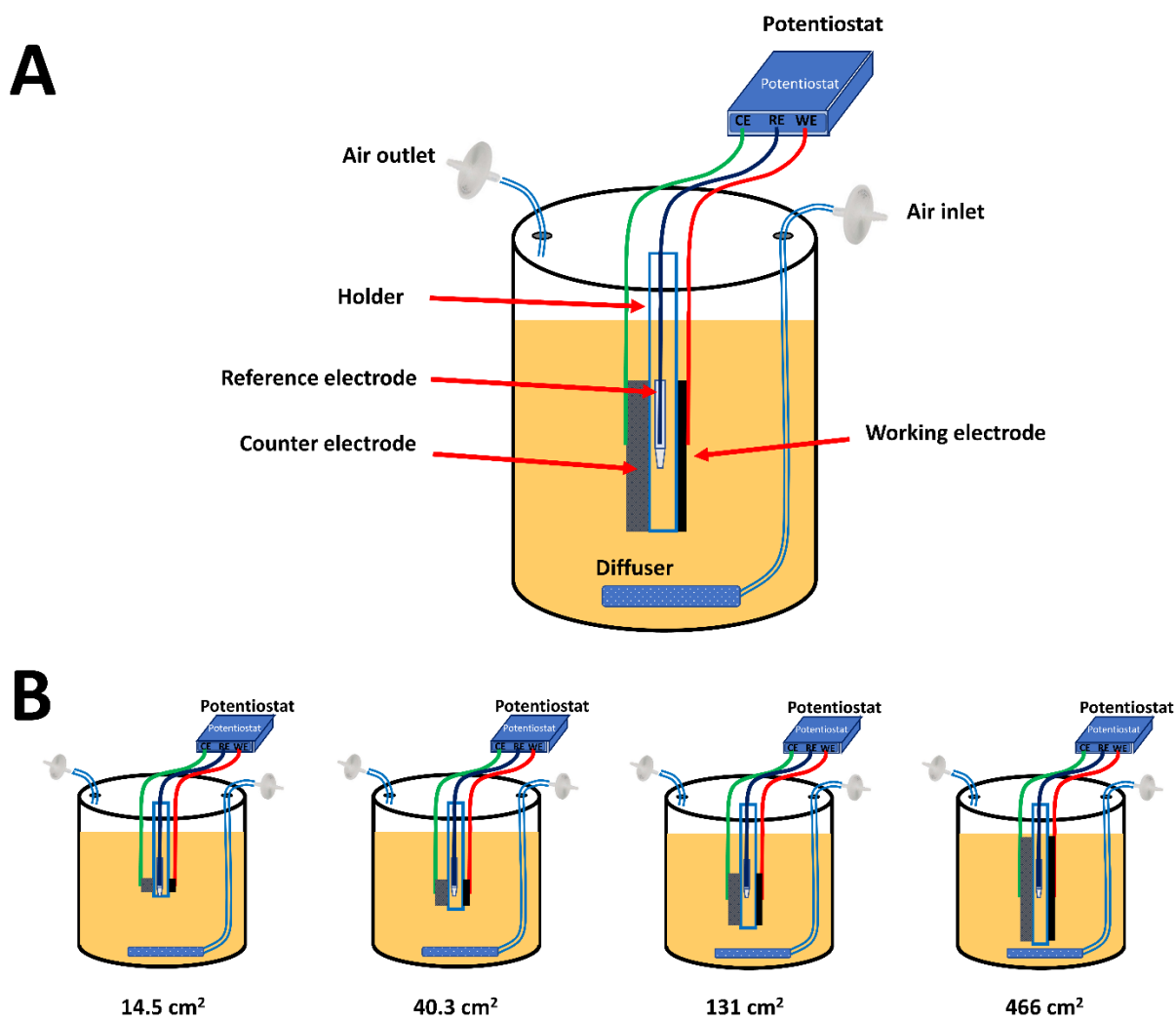

Figure S1. A) Schematic diagram of the electrochemical cell, including working electrode (WE), counter electrode (CE), reference electrode (RE), potentiostat, and air inlet and outlet. B) The experimental setup used to test the effect of electrode size on cathodic current density. Schematics are not drawn to scale.
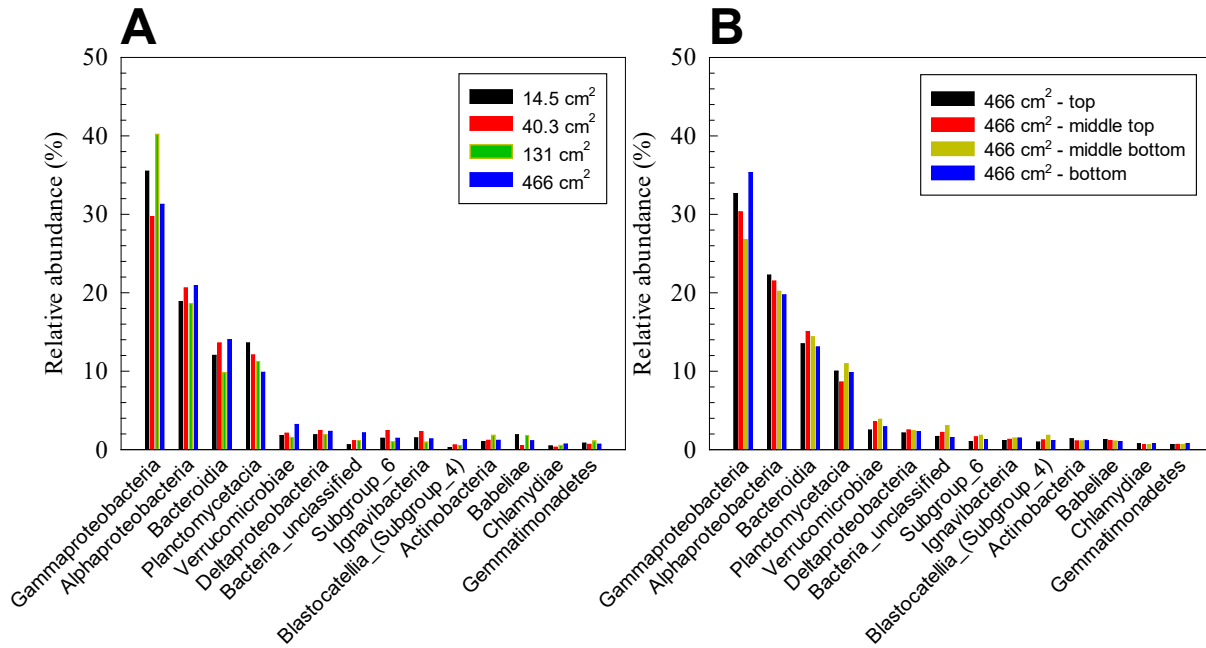
S2. Results.

Figure S2. Class-level 16S rRNA gene microbial community analysis of A) four oxygen-reducing cathodic biofilms (surface areas = 14.5 cm^2, 40.3 cm^2, 131 cm^2 and 466 cm^2), and B) a 466 cm^2 cathodic biofilm sampled at four different locations. The data show that the microbial community structure is independent of cathode size, and of the location within the cathode.

References:

1. Bard, A.J. and L.R. Faulkner, *Electrochemical Methods: Fundamentals and Applications, 2nd Edition*. 2008: Wiley. 864.
2. Lewandowski, Z. and H. Beyenal, *Fundamentals of biofilm research*. 2014, Boca Raton: CRC Press/Taylor & Francis Group.
3. Renslow, R., et al., *Oxygen reduction kinetics on graphite cathodes in sediment microbial fuel cells.* Phys Chem Chem Phys, 2011. **13**(48): p. 21573-84. DOI: 10.1039/c1cp23200b.