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Supporting Information

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Limitations for Current Production in *Geobacter sulfurreducens* Biofilms

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Determination of buffer concentration for maximal current production

High buffer concentration was used in the experiments in order to avoid interference due to pH gradients inside the biofilm. A concentration of 100mM buffer was chosen, as it was found that under that condition current did not increase either with agitation or addition of buffer:

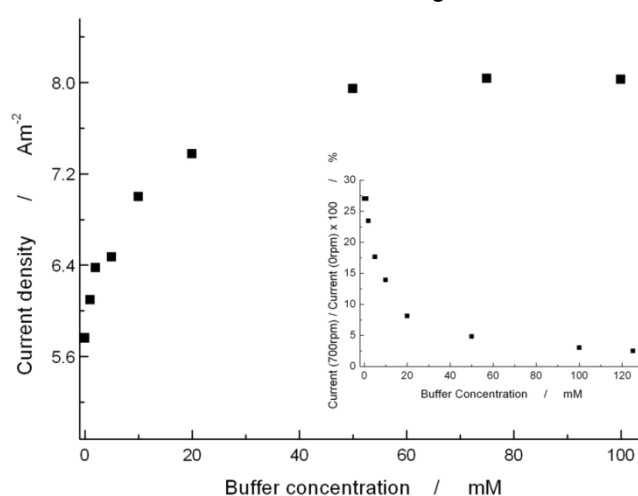


Figure S1 – Current density as a function of buffer concentration at 700rpm. Inset: increase in current when comparing no agitation with 700rpm as a function of buffer concentration.

Biofilm thickness determination

Biofilm thickness was determined by measuring the resistance between the non-polarized working electrode and a microelectrode connected to a micromanipulator using a multimeter (Fluke 289). The microelectrode was initially positioned perpendicular to the biofilm, at a distance of about 0.5mm. The micromanipulator allowed approaching to the working electrode with 5 μ m steps. With every step the resistance between the electrodes became lower and, once the working electrode was touched, the measured resistance was in the order of the 5-10 ohms. The thickness of the biofilm was calculated from the number of steps between the first sudden change in the resistance (touching the biofilm) and the last change (touching the electrode). The measurement was repeated at different positions on the biofilm surface in order to get a representative value. The reported value is the average of more than 5 independent measurements and agrees with typical thickness values reported for biofilms grown under similar experimental conditions and measured by confocal microscopy.^[1]

Calculation of electrons diffusivity (D_E) and total amount of mediators in the biofilm (Med_{tot})

The diffusivity of electrons inside the biofilm was calculated by linear regression of peak currents obtained in cyclic voltammeteries performed at different scan rates under electron donor

depletion^[2] (Figure S1). The total amount of mediators in the biofilm was estimated from the area of peaks on those voltammtries.

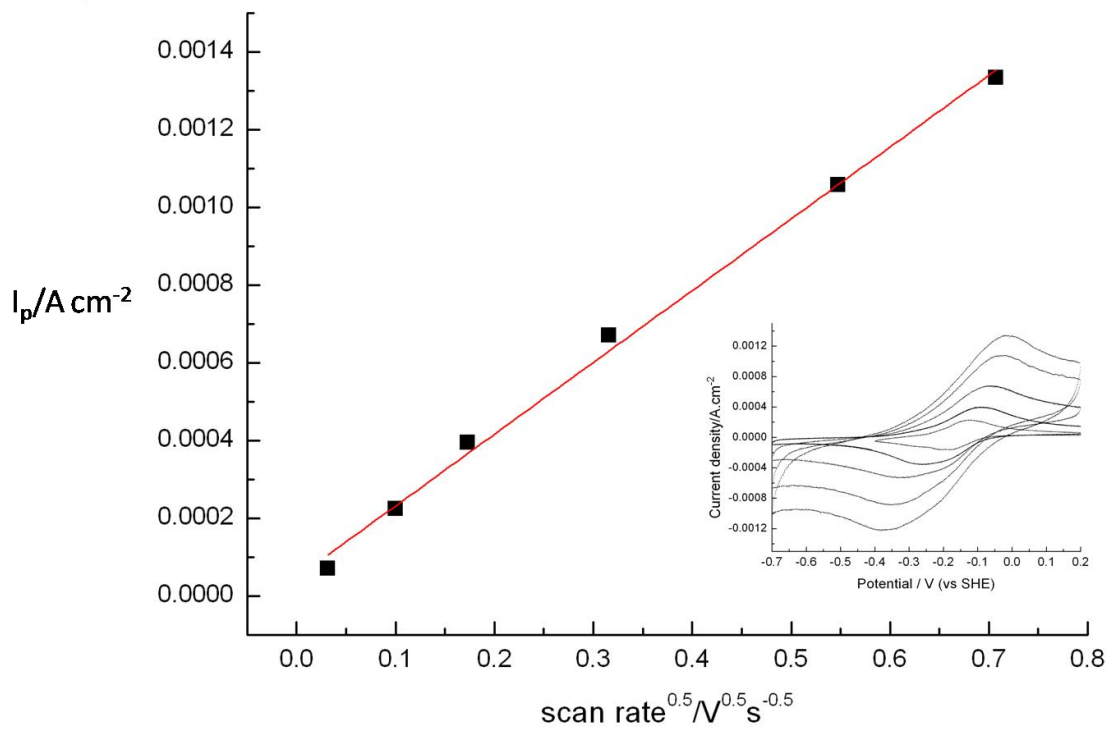


Figure S2 – Representative peak current densities (I_p) against square root of scan rate for biofilm under non turnover conditions (filled squares) and linear regression (slope= 0.00185 ± 0.00005). Inset: cyclic voltammtries under no turnover conditions at the different scan rates.

From the slope of the linear regression an electron diffusivity of $4.5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ can be estimated.

From Nernst-Einstein relation:^[3]

$$\sigma = \frac{n e^2 D}{k_B T}$$

where n is the number density of electron carriers ($1.8 \times 10^{18} \text{ cm}^{-3}$ in our case), e is the charge of the electron (C), D is the diffusivity of electrons, k_B is Boltzmann constant and T is the absolute temperature, an equivalent conductivity of 0.055 mS cm^{-1} can be estimated.

When performing cyclic voltammtries, the displacement x of a molecule transported by diffusion, depends on the diffusion coefficient D and on the time taken to scan the voltammogramm t (which in turn depends on the scan rate):^[4]

$$\sqrt{\langle x^2 \rangle} = \sqrt{2Dt}$$

As seen in inset of Figure S2, in the performed experiments both the cathodic and anodic scan span for about 900mV. The time taken to scan the voltammogramm was then, 1.8s for the fastest scan rate used (500 mV s^{-1}) and 900s for the slowest one (1 mV s^{-1}). Considering the measured electron diffusivity of $4.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ the diffusion layer can be approximated to 40um for the former case and 900um for the latter. Then, it can be concluded that electron transport (see Figure 1) follows a diffusive behavior in all the biofilm

Derivation of the rate equation

From steps 3 and 4 the rate equation that relates current production in each position of the biofilm with the amount of oxidized cytochromes and the concentration of electron donor can be obtained.

Considering that, at steady state, the amount of reduced and oxidized cells is constant in each part of the biofilm, the rate of oxidation and reduction of cells should be equal and that the cells and a mass balance of total cells (Mic_{tot}):

$$k_{ac}[Ac][Mic_{ox}] = k_{omc}[[Mic_{tot}] - [Mic_{ox}]] [Med_{ox}]$$

From where

$$[Mic_{ox}] = \frac{\frac{k_{omc}}{k_{ac}} [Med_{ox}][Mic_{tot}]}{[Ac] + \frac{k_{omc}}{k_{ac}} [Med_{ox}]}$$

Replacing this expression in the mass balance of acetate in the layer z:

$$\frac{d[Ac]}{dt} = -k_{ac}[Ac][Mic_{ox}] = \frac{-k_{omc}[Mic_{tot}][Ac][Med_{ox}]}{[Ac] + \frac{k_{omc}}{k_{ac}} [Med_{ox}]}$$

Which has the functional form of the Monod equation,^[2] but considers also the dependence with the concentration of oxidized mediator outside the cell.

Validity of hydrodynamic equations

Oxidation of ferrocyanide was used as control to test the validity of the hydrodynamic equations used in the model to account for mass transport to the biofilm via convection. The modeled values of current density for different rotating speeds were compared with experimental values. The agreement between modeled values and experimental results (Figure S3) indicates that convective equations used in the model, can accurately describe the dependence of the mass transport to the electrode with the rotating speed of the electrode.

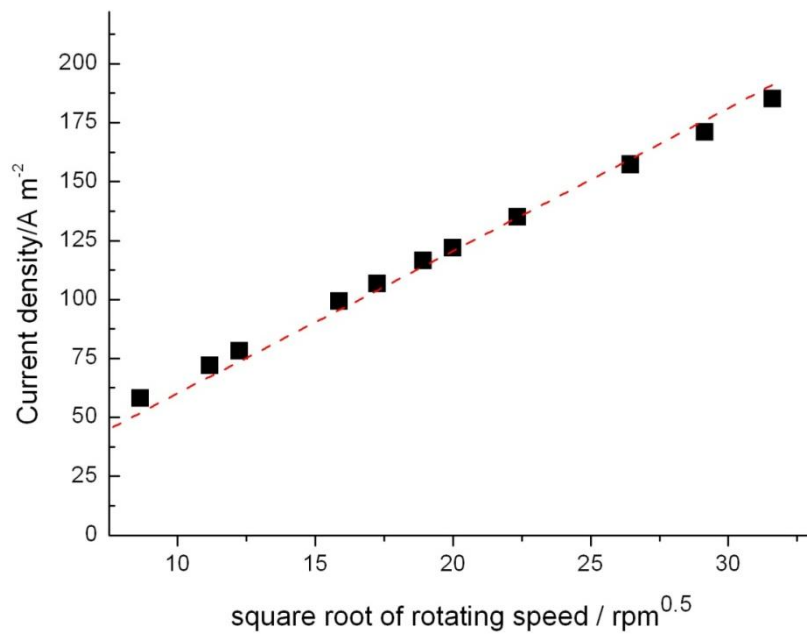


Figure S3 – Modeled (dashed line) and experimental (black squares) current densities as a function of rotating speed for oxidation of 1mM Ferrocyanide in 500mM KCl. Same experimental setup and hydrodynamic equations as for *Geobacter* biofilm were used.

Analysis of the rate of convective flux under different experimental conditions

In Figure S2 the convective flux, normalized by the maximum convective flux, is shown as a function of the rotating speed, for different acetate concentrations. As can be seen in the figure, under each of the experimental conditions the convective flux is far from its maximum theoretical value, which means that, as mentioned in the text, this process does not limit current production in any of the analyzed experimental conditions.

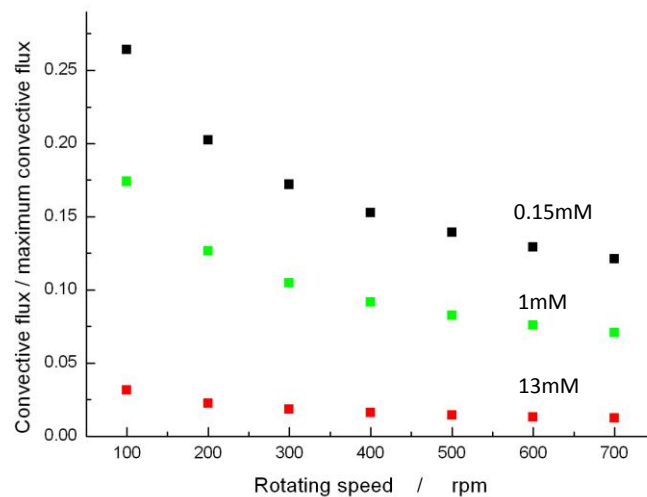


Figure S4 – Convective flux normalized by the maximum convective flux as a function of the rotating speed, for different acetate concentrations.

Experimental and modeled currents at different acetate concentrations and rotating speeds

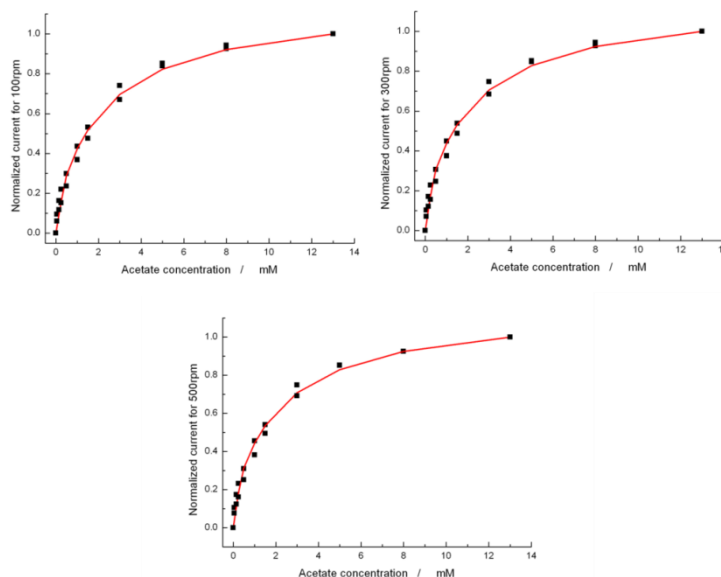


Figure S5 – Experimental (black squares) and modeled (red lines) current densities, normalized by the value at 13mM, as a function of acetate concentration at 100, 300 and 500rpm

Response to changes in electrode potential of biofilms with different conductivities

Modeling work by Torres and colleagues showed that in order to explain the voltammetric response of the biofilm, the conductivity should be 0.5mS cm^{-1} or higher.^[5] Interestingly, as, such results can also be accounted by the electron hopping mechanism, with a conductivity one order of magnitude lower (inset of Figure 3).

According to the electron hopping mechanism, if the conductivity of the biofilm (diffusivity of electrons) is decreased, the thickness of respiring biofilm (and the current) becomes smaller. This situation cannot be alleviated by an increase in the applied potential, as once the interfacial cytochromes are completely oxidized, no further increase in current is possible. Interestingly, in opposition to results obtained by Torres and colleagues, the saturation of current occurs at potentials about 100mV higher than E_0 , regardless the conductivity. Besides, the model based on electron hopping mechanism predicts that the conductivity modifies the maximum current that the biofilm can produce (see figure S6).

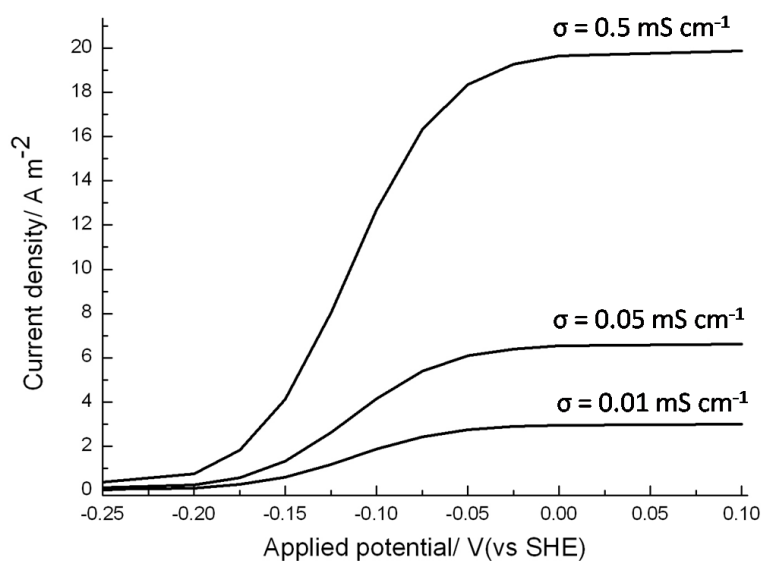


Figure S6 – Modeled current densities for biofilms with different conductivities, considering electron hopping mechanism.

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