Electronic Supplementary Material (ESI) for Energy & Environmental Science

Supplementary Information

Charge accumulation and electron transfer kinetics in *Geobacter sufurreducens* biofilms

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Derivation of the expression for the current transient

The electron transfer process from Acetate to the electrode is explained with a 4 step mechanism. All four steps are considered to follow elementary kinetics. Mass transfer of the substrate to the biofilm is considered fast enough and, therefore, is not rate limiting. The reactions taking place on each step are as follows:

$$\frac{1}{8}Ac^- + Mic_{ox} \xrightarrow{k_{Ac}} Mic_{red} + \frac{1}{4}CO_2 + H^+ \qquad Step\,1$$

$$Mic_{red} + Omc_{ox} \xrightarrow{k_{mic}} Mic_{ox} + Omc_{red} \qquad Step\,2$$

$$Omc_{red} + Omc^*_{ox} \xrightarrow{k_{omc}} Omc_{ox} + Omc^*_{red} \qquad Step\,3$$

$$Omc^*_{red} \xrightarrow{k_{int}} Omc^*_{ox} + e^- \qquad Step\,4$$

Where Mic_x stands for internal cell cytochromes haems, Omc_x for matrix cytochromes haems and Omc_x^* for interfacial cytochromes haems. The subscripts red and ox stand for reduced and oxidized states.

Discharging the biofilm

During the discharge process the current can be separated into two main contributions: the re-oxidation of the reduced cytochromes haems (including interfacial, matrix and internal cell cytochromes) and the current produced by substrate metabolization. As charge is stored in the form of reduced cytochromes haems, the current will depend on the oxidation rates of these species. The balances of the reduced cytochromes haems have to be solved in order to obtain the time dependence of the total current.

Interfacial cytochromes current

Considering that each step of the mechanism follows elementary kinetics, the balance for the interfacial reduced cytochromes haems yields:

$$\frac{dOmc^*_{red}}{dt} = k_{omc}.Omc_{red}.\left(Omc^*_{tot} - Omc^*_{red}\right) - k_{int}.Omc^*_{red} \qquad \text{(SI1)}$$

Since this species is in direct contact with the electrode, when working with sufficiently oxidative potential for the discharge, the re-oxidation of the interfacial cytochromes (step 4) will be faster than any other electron transfer process taking place in the biofilm. Thus, it can be considered that $k_{int} >> k_{omc}$, and Eq. SI 1 simplifies to:

$$\frac{dOmc^*_{red}}{dt} = -k_{int}.Omc^*_{red} \qquad k_{int} >> k_{omc} \qquad \text{(SI2)}$$

Solving this equation for Omc^*_{red}, the current produced by the oxidation of the interfacial cytochromes haems can be calculated:

$$I_{Omc*} = F.k_{int}.Omc*_{red0}.\exp(-k_{int}.t) \quad \text{(SI3)}$$

Being Omc^*_{red0} the number (in mol) of electrons stored in interfacial cytochromes haems at the time of electrode re-connection (discharge start time).

Matrix cytochromes current

The balance for the matrix reduced cytochromes yields:

$$\frac{dOmc_{red}}{dt} = k_{mic}.Mic_{red}.(Omc_{tot} - Omc_{red}) - k_{omc}.Omc_{red}.(Omc^*_{tot} - Omc^*_{red}) \quad \text{(SI4)}$$

Electron transport from the cell interior to the outer mediators (Step 2) implicates a series of membrane transport processes which are considered slower than the transfer in between cytochromes (Step 3). Thus, taking $k_{omc} >> k_{mic}$ the above equation simplifies to:

$$\frac{dOmc_{red}}{dt} = -k_{omc}.Omc_{red}.(Omc^*_{tot} - Omc^*_{red}) \quad \text{(SI5)}$$

As it was argued before, interfacial cytochromes haems (Omc^*) are almost instantaneously oxidized due to the highly oxidative applied potential. Then, it can be considered that during the discharge process of the matrix cytochromes haems (Omc), all the interfacial cytochromes haems are already oxidized ($Omc^*_{red}=0$). This simplifies the above equation to:

$$\frac{dOmc_{red}}{dt} = -k_{omc}.Omc^*_{tot}.Omc_{red} \quad \text{(SI6)}$$

It is important to note that according to Eq SI6 and the above mentioned assumptions and considering that the total number (in mol) of interfacial cytochromes haems (Omc^*_{tot}) is constant in time, the second order rate constant k_{omc} can be redefined as a pseudo first order rate constant ($k_{omc}.Omc^*_{tot}$)

From Eq SI6 the current produced by re-oxidation of matrix cytochromes haems can be easily found:

$$I_{Omc} = F.k_{omc}.Omc^*_{tot}.Omc_{red0}.\exp(-k_{omc}.Omc^*_{tot}.t) \quad \text{(SI7)}$$

Being Omc_{red0} the number (in mol) of electrons stored in matrix cytochromes at the time of electrode re-connection (t=0).

Cell metabolism and internal cell cytochromes current

Acetate concentration is thought to be constant during the discharge process. Thus, the dependence of the oxidation of cell internal cytochromes with acetate is implicitly incorporated on the rate constant k_{ac}, and the balance of reduced internal cytochromes (Mic_{red}) yields:

$$\frac{dMic_{red}}{dt} = k_{ac}.(Mic_{tot} - Mic_{red}) - k_{mic}.Mic_{red}.\left(Omc_{tot} - Omc_{red}\right) \quad \text{(SI8)}$$

In order to solve Eq. SI8 it is considered that matrix cytochromes re-oxidation (Step 3) is faster than both acetate uptake and internal cytochromes oxidation (Steps 1 and 2, respectively). Thus, it can be considered that the matrix cytochromes haems are already oxidized (no stored charge) during the internal cell cytochromes haems re-oxidation (Omc_{red}=0). With this simplification Eq. SI8 yields:

$$\frac{dMic_{red}}{dt} = k_{ac}.(Mic_{tot} - Mic_{red}) - k_{mic}.Omc_{tot}Mic_{red} \quad \text{(SI9)}$$

Considering that the total number (in mol) of matrix cytochromes haems (Omc_{tot}) is constant over time, the second order rate constant k_{mic} is redefined as a pseudo first order rate constant ($k_{mic}.Omc_{tot}$).
This is the expression of a reversible reaction,[1-2] with forward and backward rates constants $k_{mic}.Omc_{tot}$ and k_{ac}, respectively:

$$Micred \underset{k_{Ac^-}}{\overset{k_{mic}.Omc_{tot}}{\rightleftarrows}} Mic_{ox} + e^-$$

Taking Mic_{red0} as the initial number (in mol) of reduced internal cell cytochromes haems, the above equation can be easily solved for Mic_{red} to give:

$$Mic_{red} = Mic_{red0}.\exp\left[-(k_{mic}.Omc_{tot} + k_{ac})t\right] + \frac{k_{ac}Mic_{tot}}{k_{mic}.Omc_{tot} + k_{ac}}.\left[1 - \exp\left[-(k_{mic}.Omc_{tot} + k_{ac})t\right]\right] \quad \text{(SI10)}$$

The current produced by cells internal cytochromes haems re-oxidation and acetate metabolization will be:

$$I_{cells} = F.k_{omc}.Omc_{tot}.Mic_{red} \quad \text{(SI11)}$$

Combining Eq. SI11 with Eq. SI10 yields:

$$\frac{I_{cells}}{F} = k_{mic}.Omc_{tot}.Mic_{red0}.\exp\left[-(k_{mic}.Omc_{tot} + k_{ac})t\right] + \frac{Mic_{tot}}{\frac{1}{k_{ac}} + \frac{1}{k_{mic}.Omc_{tot}}}.\left[1 - \exp\left[-(k_{mic}.Omc_{tot} + k_{ac})t\right]\right] \quad \text{(SI11)}$$

Total current

The time dependence of the total current is obtained as the addition of the contributions from: the re-oxidation of interfacial cytochromes (Eq. SI3), the re-oxidation of matrix cytochromes (Eq. SI7), and the re-oxidation of internal cytochromes and acetate uptake and metabolization (Eq. SI11):

$$\frac{I_{total}}{F} = \frac{I_{Omc^*} + I_{Omc} + I_{Cells}}{F} =$$
$$= k_{int}.Omc^*_{red0}.\exp(-k_{int}.t) + k_{omc}.Omc^*_{tot}Omc_{red0}.\exp(-k_{omc}.Omc^*_{tot}.t) +$$
$$+ k_{mic}.Omc_{tot}.Mic_{red0}.\exp\left[-(k_{mic}.Omc_{tot} + k_{ac})t\right] + \frac{Mic_{tot}}{\frac{1}{k_{ac}} + \frac{1}{k_{mic}.Omc_{tot}}}.\left[1 - \exp\left[-(k_{mic}.Omc_{tot} + k_{ac})t\right]\right] \quad \text{(SI12)}$$

Kinetic parameters and charge distribution determination

In order to obtain the values of the rate constants for every step in the mechanism and the distribution of charge within the biofilm, experimental currents were fitted using Origin 8.0 software with an equation of the form:

$$I_{total} = A.\exp(-k_1.t) + B.\exp(-k_2.t) + C.\exp\left(-k_3 t\right) + D.\left[1-\exp\left(-k_3 t\right)\right] \quad \text{(SI13)}$$

which reproduces the functional form of the total current (Eq. SI12) , being:

$$A = F.k_{\text{int}}.Omc^{*}_{red0} \qquad B = F.k_{omc}Omc^{*}_{tot}.Omc_{red0} \qquad C = F.k_{mic}.Omc_{tot}.Mic_{red0}$$

$$D = F.\frac{Mic_{tot}}{\frac{1}{k_{ac}} + \frac{1}{k_{mic}.Omc_{tot}}} \qquad k_1 = k_{\text{int}} \qquad k_2 = k_{omc}Omc^{*}_{tot} \qquad k_3 = k_{mic}.Omc_{tot} + k_{ac}$$

$$F = Faraday\ Constant$$

Once the parameters of equation SI13 are known, the rate constants of steps 3 and 4 are directly obtained from the fitting parameters t_1 and t_2. In order to find the values of the rates constants of step 1 and 2 (k_{ac} and $k_{mic}.Omc_{tot}$), their relative values have first to be determined, because the fitting parameters functional form does not allow a direct calculation. This can be done using previous fluorescence analysis of *G. Sulfurreducens* cells performed by Esteve-Nuñez et al. (2008).[3] In that work the authors measured the fluorescence of *Geobacter* cells in three conditions: a) in the presence of $K_3Fe(CN)_6$ where internal cytochromes are totally oxidized, b) in lack of electron acceptor (totally reduced cytochromes) and c) in standard growth medium (steady state conditions). As fluorescence emission is proportional to the amount of reduced species, the total amount of reducible haems in the cells Mic_{tot} is proportional to the difference in the fluorescence of fully oxidized and fully reduced cells. In the same way, the total number of haems in the cells (Mic_{tot}) is proportional to the difference in the fluorescence of fully oxidized and fully reduced cells. In the same way, by comparison of fluorescence data from fully reduced cells and those in the standard growth medium the fraction of oxidized haems in steady state conditions $Mic_{ox(eq)}$ can be obtained. Using fluorescence data obtained by Esteve-Nuñez et al, the equilibrium constant K_{eq} was estimated:

$$K_{eq} = \frac{Mic_{ox(eq)}}{Mic_{red(eq)}} = \frac{Mic_{tot} - Mic_{red(eq)}}{Mic_{red(eq)}} = \frac{F_{red} - F_{eq}}{F_{eq} - F_{ox}} = \frac{14x10^4 - 3x10^4}{3x10^4 - 2x10^4} = 11$$

As the equilibrium constant is also the ratio in between forward ($k_{mic}.Omc_{tot}$) and backward (k_{ac}) rate constants representing the oxidation and the reduction of cells, respectively:

$$k_{mic}.Omc_{tot} = 11\,k_{ac}$$

With this estimation, the rate constants can be estimated from fitting parameter t_3.
The distribution of charge in the electrogenic biofilm can be estimated from the fitting parameters. The total amount of charge stored in interfacial cytochromes haems Omc^{*}_{red0}, is easily found by dividing A by t_1. The total amount of charge stored in matrix cytochromes is found by dividing B by t_2. The charge stored in cell internal cytochromes haems (Mic_{red0}) is

estimated using fitting parameter C and the rate constant value estimated combining parameter t_3 and fluorescence analysis results.

Abiotic background experiments

The intrinsic double-layer capacitance of the electrode surface may also contribute to the current transients. To verify that this contribution was negligible compared to the current produced by biofilm discharge, current transients were measured in media for same electrodes used in the presented experiments but without biofilms, for discharge potentials from -400 to 200mV vs Ag/AgCl stepped in 100mV. Current was measured every 0.1ms. Open circuit time for all discharges was 20 minutes. In figure S2, the initial current density of each discharge is plotted against the discharge potential.

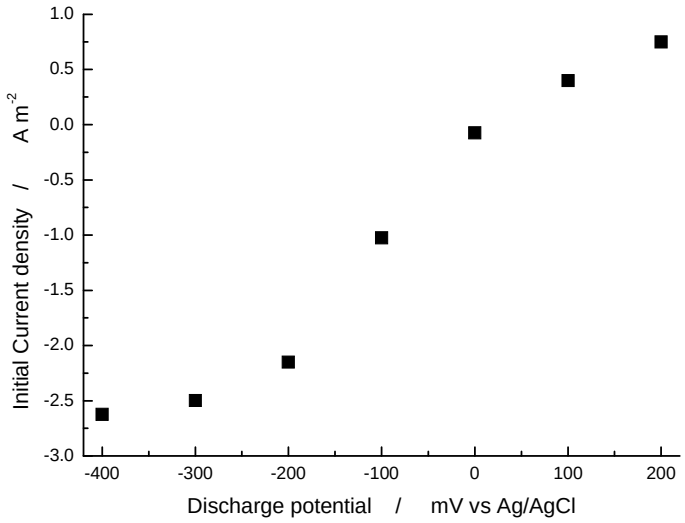

Figure S1 – Initial current density against discharge potential for electrodes in the growing media but without biofilm.

As it can be seen, the initial current density for the abiotic control of 200mV vs Ag/AgCl is aproximately 1% of the current measured for electrodes with biofilms discharging at the same potential. Thus, the contribution of the double layer capacity of the electrode surface was considered negligible to the discharge current obtained on the presence of biofilm.

Confocal laser microscopy

At the end of the experiments a piece of electrode with the biofilm was stained by immersion in a solution of Acridine Orange 0.01% in growth media and incubated at room temperature for 20 minutes. After gently washing to eliminate loosely adhered cells the sample was observed in a Nikon Eclipse C1plus confocal microscope at an excitation wavelength of 488nm using a 60X water dipping objective. Images were acquired every 10um along the Z axis (Figure S2) and analyzed using the EZ-C1 software (Nikon, USA) to determine the biofilm thickness.

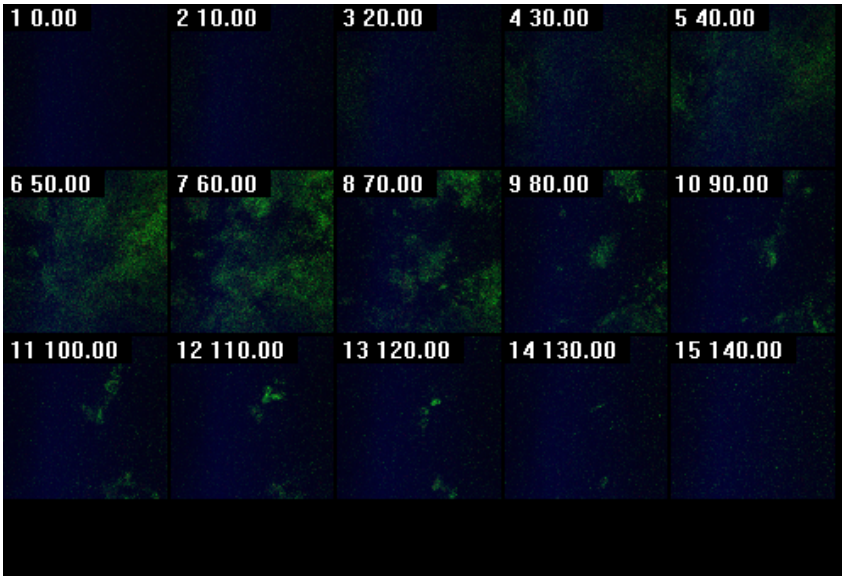

Figure S2 - Confocal images of the biofilm in top view

References

1. J. J. Carberry, *Chemical and catalytic reaction engineering* New York, 1976.
2. J. M. Smith, *Chemical Engineering kinetics*, McGraw-Hill Kogakusa, LTD, Tokyo, 1970.
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