Biofilm as a redox conductor: a systematic study of moisture and temperature dependence of its electrical properties


Sample preparations
Commercially available IDAs were used in this study (Fig. S1a; ALS IDA electrodes model 012125) and consisted of 65 pairs of parallel gold rectangular bands, each 2 mm long x 10 µm wide x 90 nm thick, patterned onto quartz substrates. Electrode bands were separated by a 5 µm gap and alternating electrodes were attached to large electrode contacts on opposite sides of the array.

Geobacter sulfurreducens samples were prepared as reported previously. Briefly, G. sulfurreducens biofilms were grown on electrodes poised at +0.5 V (vs. SHE) in freshwater medium (ATCC 2260) with 10 mM acetate and excluding fumarate until the current output stabilized. The water jacketed reactor (Pine Instruments, USA) was stirred and the temperature was maintained at 30°C throughout growth using a temperature controlled water bath. The system was maintained under anaerobic conditions by continuously sparging with a 80% N₂/ 20% CO₂ gas mixture. Electrochemical gating measurements and cyclic voltammetry were performed on the electrodes with a bipotentiostat (Pine Instruments, USA) using the provided software to ensure that the system was performing similarly to samples previously generated. Data is shown in Figure S1. Before the electrode was removed from the reactor, the gate potential, defined as the average of the potentials applied to the two electrodes while maintaining a fixed voltage offset of 0.1 V (source-drain voltage) between the electrodes, was set to the potential where maximum conducted current was observed (-0.19 V vs. SHE) for 3 minutes. This was done with the intention of preparing biofilms with equal concentrations of reduced and oxidized redox cofactors for maximum rate of electron transport. However, it is unclear if this state remained intact after removing the biofilm from the electrolyte prior to analysis. Further characterization of the concentrations of reduced and oxidized cofactors during electrochemical tests was beyond the scope of this study. The electrode was then removed, rinsed 3 times in DI water and placed in an anaerobic chamber.
(relative humidity ~15%) overnight to facilitate drying of the biofilm. The biofilm was dried in order to preserve the state of the biofilm.

Figure S1. Source-drain current vs. gate potential dependencies generated by electrochemical gating experiments of biofilms used in this study under in situ conditions prior to being removed from medium for subsequent ex situ measurements. The current magnitudes are consistent to what has been previously observed for the same experimental parameters. The variation in current magnitude among biological replicates is not uncommon under in situ conditions. However, the difference in conducted current in the reactor medium did not affect results during hydration tests (Fig S5b).

Shewanella oneidensis MR-1 biofilms were grown in microbial three-electrode electrochemical cells similar to those previously reported, with the IDA serving as the working electrode poised at 0.3 V vs. Ag/AgCl (0.5 V vs. SHE). In order to culture the bacteria for these reactors, S. oneidensis MR-1 was struck out on LB agar plates from frozen bacterial stock and incubated at 30 °C overnight to isolate single colonies. Biological replicate cultures were grown by selecting morphologically similar colonies with a sterile loop to inoculate anaerobic (100% N2 atmosphere) modified M1 medium containing 20 mM Na-(L)-lactate as donor and 20 mM Na-fumarate as acceptor. After 24 hours of incubation with shaking in a temperature controlled chamber at 30 °C, a consistent OD of ~0.16 was reached (i.e. 1.6 × 10^8 cfu/mL using the previously determined conversion factor ~1 × 10^9 cfu/mL/OD^6). These stationary phase cultures (fumarate completely consumed) may then be used to inoculate replicate devices as desired.
Poly(N-vinylimidazole [Os(bipyridine)$_2$Cl])$^{+2+}$ (referred to here as PVI-Os(bipy)$_2$Cl) was formed on IDAs using established literature methods in which a 1:0.22:0.44 solution of PVI-Os(bipy)$_2$Cl (10 mg/L):PEGDE crosslinker (3 mg/mL): ethanol (100%) was mixed together and drop cast to cover the electrode surface. The electrode was then allowed to dry overnight in a dessicator. Polyaniline (PANI) was electropolymerized on IDAs using established literature procedures in which 50 mM of aniline monomer in 0.5 M H$_2$SO$_4$ was electrodeposited by first sweeping the potential from open circuit potential (0.57 V) to 1 V vs. SHE and then holding the potential until 2.5 C/cm$^2$ of charge had passed through the electrode to obtain a film ~25 µm thick. The electrode was then rinsed in clean 0.5 M H$_2$SO$_4$ before use. A BioLogic potentiostat Model VMP3 (BioLogic, Inc.) and platinum counter electrode were used to electropolymerize PANI. At pH=0, the electropolymerized PANI films were expected to be fully protonated (doped) and metallic, whose conductivity negligibly depends on humidity (as we observed in our experiments). It should be noted that PANI film’s conductivity can change with humidity depending on the polymer synthesis and the film preparation.

Two-point probe measurements and conductivity calculation

In a typical steady state current voltage measurement, biases from 0 to 2 V were swept between two contacts of the IDA (Fig. S1a) and currents were measured. The conductance of the films, G, was calculated from the slope of current vs. voltage (Fig. S1b). Conductivity was then calculated from the conductance and the geometrical factor S, which was calculated in our previous work to be 20.4.
**Figure S2.** (a) IDA electrode (http://www.als-japan.com/1379.html#defaultTab13) dimension; (b) a typical current-voltage (I-V) curve measured for a *G. sulfurreducens* biofilm or Os-complex film showing the linear relationship between current and applied bias; (c, d) Chemical structures of the osmium redox complex and doped polyaniline, respectively.
Figure S3. Film conductivity as a function of water content at 20°C controlled by changing the relative humidity in the measuring chamber. The conductivity measurements were reproduced for two independent films and two replicates for each film.

Figure S4. (a) Current-voltage (I-V) curves of \textit{G. sulfurreducens} biofilm (T=25°C, RH=45%), Osmium redox polymer film (T=25°C, RH=35%) and polyaniline film (T=25°C, RH=35%); (b) I-V curves of \textit{S. oneidensis} MR-1 biofilm measured at 25°C and relative humidity from 45% to 85%; and bare IDA electrode at T=25°C, RH=38.5%. The current level of bare IDA-electrode (noise current) does not discernably change at different temperature and humidity.
(a) *G. sulfurreducens* Biofilm (25 °C, RH = 95.0%)

(b) 25 °C, RH = 95.0%

(c) 25 °C, RH = 38.7%

(d) 12 °C, RH = 86.8%

**Figure S5.** Two-point probe transient current measurements. (a) *G. sulfurreducens* biofilm at T = 25 °C, RH = 95.0% at two different biases with opposite polarity. (b, c, d) *G. sulfurreducens* biofilm, Os-complex film and *S. oneidensis* biofilm at different temperature.
and humidity conditions: (b) $T = 25 \, ^\circ\text{C}$, RH = 95.0%, (c) $T = 25 \, ^\circ\text{C}$, RH = 38.7% and (d) $T = 12 \, ^\circ\text{C}$, RH = 86.8%. The lines connecting the data points are plotted for visual aid.

![Figure S6](image)

**Figure S6.** (a) Conductivity of Os-complex film in ambient air at constant RH = 60% and temperatures from 15 °C to 35 °C. Water content was calculated from relative humidity and temperature using this website: http://www.OWLstonenanotech.com/humidity/calculator. (b) Conductivity of Os-complex film in ambient air at fixed water content 12,000 ppm and temperatures from 12 °C to 35 °C.

### Reference

### Electronic supplementary information (ESI)


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