Electronic Supplementary Material (ESI) for Energy & Environmental Science

A Lipid Membrane Intercalating Conjugated Oligoelectrolyte Enables Electrode Driven Succinate Production in *Shewanella*

Alexander W. Thomas, Logan E. Garner, Kelly P. Nevin, Trevor L. Woodard, Ashley E. Franks, Derek R. Lovley, James J. Sumner, Christian J. Sund, and Guillermo C. Bazan

Supplementary Information

Control Experiments

Two controls, a sterile device containing no *S. oneidensis* cells in which the current at a poised cathode was measured in the presence of 5 μM **DSSN+** and an *S. oneidensis* control in which the current at a poised biocathode is measured in the absence of **DSSN+** provide key evidence of the role of the COE. That a sterile control run in the presence of **DSSN+** gives little-to-no current response indicates that this COE does not undergo electrochemical reduction at the cathode under the conditions of this study and that it does not catalyze the reduction of fumarate to succinate. Additionally, that little-to-no current response is observed in *S. oneidensis* controls to which no COE was added confirm that this microbe cannot utilize the graphite electrode as the sole electron donor under these conditions.

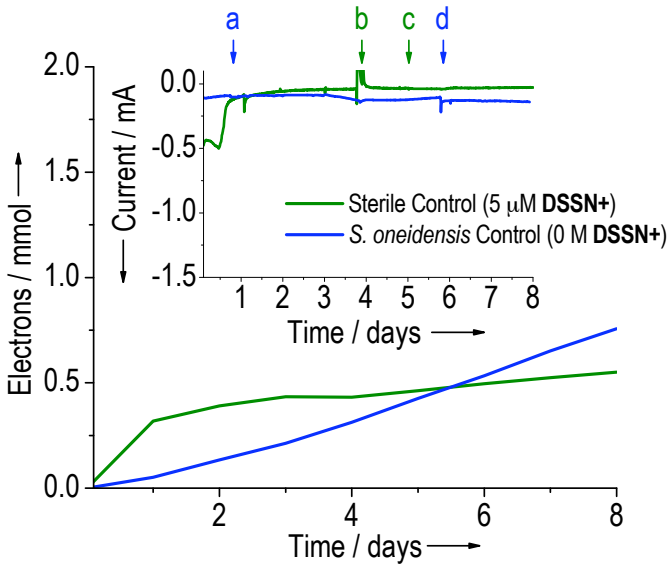

Fig. S1 Cumulative electrons transferred and current vs. time (inset) data corresponding to a sterile control to which 5 μM DSSN+ was added (green) and a S. oneidensis control, run in the presence of fumarate (40 mM) and absence of DSSN+ (blue). Inset labels: a) inoculation of S. oneidensis control; b) addition of 40 mM fumarate to sterile control; c) addition of 5 μM DSSN+ to sterile control; d) injection of additional fumarate into S. oneidensis control. Note: color of inset labels denotes the control to which the label corresponds.

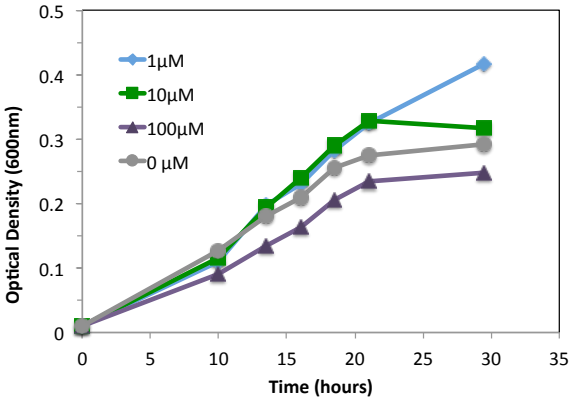

Fig. S2 Growth curves at 30°C of *S. Oneidensis* in anaerobic freshwater medium supplemented with 40mM lactate and 40mM fumarate. Culture tubes contained indicated concentrations of **DSSN+** at the time of inoculation. Optical density was monitored at 600nm. Points are averages of 4 replicate cultures.

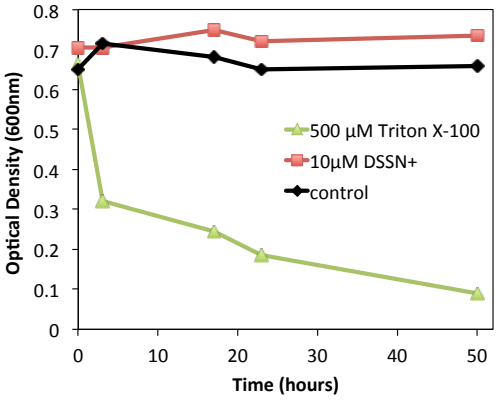

Fig. S3 Change in optical density of stationary phase *S. Oneidensis* cells monitored at 30°C in freshwater medium supplemented with 40mM fumarate but without an electron donor, amino acids, or vitamins to mimic conditions in current consumption experiments. Cells were initially grown according to the experimental section below. Culture tubes contained indicated concentrations of additives at the time of inoculation. Points are averages of 2 replicates. The path length of the culture tubes was 1.6 cm.

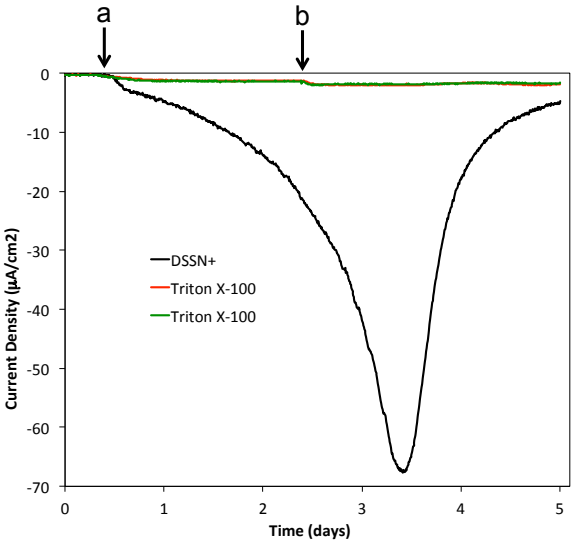

Fig. S4 Current density vs. time of *S. Oneidensis* H-cells run with 40mM fumarate electron acceptor. H-cells were inoculated at time 0. a) Addition of **DSSN+** and Triton X-100 to 5µM. b) Addition of Triton X-100 to 500µM.

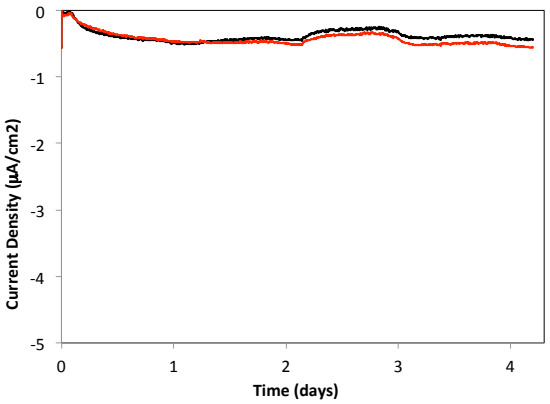

Fig. S5 Current density vs. time of replicate *S. Oneidensis* H-cells poised at -360mV vs. SHE with 40mM fumarate electron acceptor. H-cells were inoculated at 0.1 days.

Experimental

Chemicals and Reagents

All chemicals and reagents were purchased from Aldrich, Fisher, Acros, VWR, or Alpha Aesar and used as received. The conjugated oligoelectrolyte, **DSSN+**, was synthesized as previously described.[1]

Cell Cultures

Shewanella oneidensis MR-1 (ATCC 700550) was cultured anaerobically using N_2-CO_2 (80:20) in the freshwater medium[2] described below at 30°C supplemented with sodium L-lactate (40 mM) electron donor and sodium fumarate (40 mM) electron acceptor. Casamino acids (0.1% w/v) were added as sources of essential amino acids.

The freshwater medium employed consisted of the following: $HaHCO_3$, 2.5 g L^{-1}; NH_4Cl, 0.25 g L^{-1}; NaH_2PO_4, 0.06 g L^{-1}; KCl, 0.1 g L^{-1}; vitamin mix, 10 mL L^{-1}; mineral mix, 10 mL L^{-1}. The vitamin mix employed was composed of: biotin, 0.002 g L^{-1}; pantothenic acid, 0.005 g L^{-1}; B-12, 0.0001 g L^{-1}; p-aminobenzoic acid, 0.005 g L^{-1}; thioctic acid (alpha lipoic), 0.005 g L^{-1}; nicotinic acid, 0.005 g L^{-1}; thiamine, 0.005 g L^{-1}; riboflavin, 0.005 g L^{-1}; pyridoxine HCl, 0.01 g L^{-1}; folic acid, 0.002 g L^{-1}. The mineral mix employed was composed of: NTA trisodium salt, 1.5 g L^{-1}; $MgSO_4$, 3 g L^{-1}; $MnSO_4$, 0.5 g L^{-1}; NaCl, 1 g L^{-1}; $FeSO_4 \cdot 7\ H_2O$, 0.1 g L^{-1}; $CaCl_2 \cdot 2\ H_2O$, 0.1 g L^{-1}; $CoCl_2 \cdot 6\ H_2O$, 0.1 g L^{-1}; $ZnCl_2$, 0.13 g L^{-1}; $CuSO_4 \cdot 5\ H_2O$, 0.01 g L^{-1}; $AlK(SO_4)_2 \cdot 12\ H_2O$, 0.01 g L^{-1}; H_3BO_3, 0.01 g L^{-1}; $Na_2MoO_4 \cdot 2\ H_2O$, 0.025 g L^{-1}; $NiCl_2 \cdot 6\ H_2O$, 0.024 g L^{-1}; $Na_2WO_4 \cdot 2\ H_2O$, 0.025 g L^{-1}.

Upon reaching an optical density (600nm) of ~0.5, cells were concentrated via centrifuge at ~8000 x *g* for 10 minutes, re-suspended in freshwater media and used to inoculate H-cell devices (~5mL of concentrated cells injected). The media used in the H-cells and in re-suspending the bacteria after growth were devoid of lactate, casamino acids and vitamins.

H-cell setup and monitoring

H-cells were prepared and constructed as previously described.[3] Working electrodes were 0.5" x 1" x 3" graphite blocks (Mersen USA) with the top 1" x 0.5" face covered in epoxy in order to insulate the lead connection, giving a total working surface area of 9.5 in^2 or 61.3 cm^2. The working electrode chambers were equipped with Ag/AgCl reference electrodes (Edaq ET072) that were interfaced via embedding within one of the butyl rubber septa that seal each of the three utility ports on each H-cell chamber. Poised potential and current monitoring at the cathode-working electrodes was achieved using a Gamry potentiostat (Reference 600, Series G 300 or Series G 750 models) and multiplexer (model ECM8) setups. Gamry software (Framework Version 5.65, 2011) was used to measure and record the current response at 10 min intervals throughout each experiment. Both chambers were continuously bubbled with N_2-CO_2 (80:20) throughout the experiment.

High performance liquid chromatography (HPLC) analysis

HPLC analysis of organic acid metabolites was performed using an Aminex HPX-87H column (Bio-rad), a 0.008 N H_2SO_4 mobile phase (0.5 mL min^{-1}) and UV detection at 210 nm.

Calculations

Cumulative electrons drawn were determined via integration of current vs. time traces. The electrons appearing in succinate were calculated based on a 2-electron reduction process from values obtained by HPLC analysis of aliquots removed from H-cell cathode chambers on daily intervals.

Confocal Microscopy Analysis

Following device operation the cathodes were removed without touching the surface (by holding the protruding electrical connection). Confocal microscopy analysis of cathode biomass was performed by first dipping the cathodes in freshwater medium to remove any unattached biomass. The electrode surface was then imaged based upon excitation of **DSSN+** via 488 nm argon laser. No additional fluorescent stain was employed. The instrumentation employed was a Leica TCS SP5 confocal microscope with HCX PL APO CS 20, HCX APO 63 and HCX PL APO CS 100X objectives.

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[1] L. E. Garner, J. Park, S. M. Dyar, A. Chworos, J. J. Sumner, G. C. Bazan, *J. Am. Chem. Soc.,* 2010, **132**, 10042–10052.
[2] D. R. Lovley, S. J. Giovannoni, D. C. White, J. E. Champine, E. J. P. Phillips, Y. A. Gorby, S. Goodwin, *Arch. Microbiol.,* 1993, **159**, 336-344.
[3] K. P. Nevin, T. L. Woodard, A. E. Franks, Z. M. Summers, D. R. Lovley, *mBio,* 2010, **1**, e00103-e00110.