

Conjugated oligoelectrolytes increase current response and organic contaminant removal in wastewater microbial fuel cells

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Supporting Information

Methods and Materials

Preparation of 4,4'-bis(4'-(*N,N*-bis(6''-(*N,N,N*-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide (DSSN⁺) was performed according to the synthetic procedure previously reported in the literature.¹

Post-treatment effluent was collected from the discharge area of the water treatment facility housed at the Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. Shredded paper was added and anoxic dark fermentations employing the existing microbial population were run as part of a different study.² The post-fermentation effluent was then used in this study and served as the sole source of nutrients and microorganisms. It is important to note the high cellulose content of this waste stream resulting from addition of shredded paper, which presumably accounts for the high initial total organic carbon and chemical oxygen demand values.

U-tube microbial fuel cells were composed of pairs of 90° ball to plain end and socket to plain end adapters (Adapters, 90 degrees, 28/15 ball to plain end and matching socket to plain end, VWR International) held together with 28/15 pinch clamps (VWR International). Electrodes were composed of carbon felt (carbon felt, 3.18 mm thick, Alpha Aesar). Electrode leads consisted of 0.25 mm diameter titanium wire (Sigma Aldrich). Silicon septa used to seal anode chambers were obtained from Sigma (silicon septa, 18 mm O.D., Sigma Aldrich). Proton exchange membranes employed were composed of Nafion 117 (DuPont, Wilmington, DE, USA). Grease (Dow Corning high-vacuum) was employed to seal the interface between the glass adapters. Syringe filters (Millex[®] GP 0.22 µm) were obtained from Sigma Aldrich.

Microbial Fuel Cell Preparation and Analysis

U-tube MFCs were constructed according to the literature with some modifications.^{3,4} Nafion was cut into circles with ~2 cm diameter and sandwiched between greased ball and socket connections of 90 degree ball and socket adapters and secured via pinch clamp. Each chamber of the glass apparatus was filled with ~20 mL of Millipore water and allowed to sit for ~24 hours to ensure no leaks were observed at the interface between adapters. Electrodes were prepared by first cutting 1/8 inch thick carbon felt to the dimensions of 2 cm by 5 cm. Titanium wire (~25 cm) was then sewn into one end of each carbon felt piece to form the electrode and lead (lead length = ~20 cm). Anode leads were passed through silicon septa that were later used to seal the anode chamber following inoculation. Once the MFCs were leak tested the electrodes were installed by submerging one electrode into the solution within each chamber. Cathode chambers were covered with an inverted, loose fitting vial. Anode chambers were covered with a small piece of aluminum foil to maintain sterility following autoclave sterilization (note that the anode chambers were not sealed via septum prior to sterilization). The MFCs were then sterilized via autoclave and allowed to cool. Under the sterile environment of a biological clean bench, the Millipore water was then poured out of each MFC. Each chamber was then filled with 20 mL of either sterilized (cathodes of abiotic cathode type MFCs) or non-sterile wastewater (anodes and biocathodes) and the desired amount of 1 mM DSSN⁺ (aq.) solution (added to both chambers in all runs which employ this COE). The cathode chambers were then re-capped with an inverted loose fitting vial and the anode chambers were sealed via silicon septa. The MFCs were run in a 30°C incubator for ~30 days. The potential across a 10 kΩ resistor was measured every second via a eDAQ e-corder 1621 data acquisition system coupled with Chart software.

Analysis of Total Organic Carbon and Chemical Oxygen Demand

The as-received wastewater and the MFC effluent from each MFC (following operation) was analyzed to quantify the level of organic contaminants. Samples were filtered via syringe filter and analyzed for total organic carbon and chemical oxygen demand.

Total organic carbon (TOC) analyses were performed with the high range kits and instrumentation from Hach (Loveland, CO) according to Hach procedure #10128.

Chemical oxygen demand (COD) analyses were performed with the high range kits and instrumentation from Hach (Loveland, CO) according to Hach procedure #8000.

Scanning Electron Microscopy Imaging

Following MFC operation the electrodes were removed from the MFCs and the microorganisms on the electrode surfaces were fixed by submerging each electrode into pH 7.2 phosphate buffer containing 2% formaldehyde (by vol.) where they were allowed to soak for 24 hours in the refrigerator. The electrodes were then rinsed with Millipore water and dehydrated by submerging into a 70% ethanol/water solution for 15 minutes (2 dehydration cycles were performed). They were then allowed to dry for 72 hours at room temperature prior to SEM and confocal analysis.

SEM analysis was performed using a FEI XL30 Sirion FEG Digital Electron Scanning Microscope. Whole electrodes (with the titanium wire lead cut off) were mounted on an SEM sample holder and secured via double sided copper tape. The sample and holder was then secured on the sample stage within the instrument and imaged under high vacuum.

Confocal Microscopy Imaging

Confocal images were obtained using an Olympus Fluoview 500 confocal microscope equipped with a 488 nm argon laser excitation source and a 40X oil immersion objective. Samples were analyzed dry by removing a small sample of fibers from the electrode using tweezers. The fiber sample was placed on a traditional slide followed by placement of a cover slip. The slide was then inverted and placed in the microscope stage for analysis. Confocal and brightfield images were collected in tandem and overlaid to generate the final images reported here. Note that the fluorescence response is due to **DSSN+** added prior to MFC operation.

Calculations

Coulombic efficiencies were calculated based upon the number of coulombs harvested as determined by integration of the current vs. time plots. The overall number of electrons available for harvest were calculated based upon COD consumed during MFC operation assuming 4 mol e⁻/mol COD.⁵

Performance of Wastewater Microbial Fuel Cells (Reproduction of results)

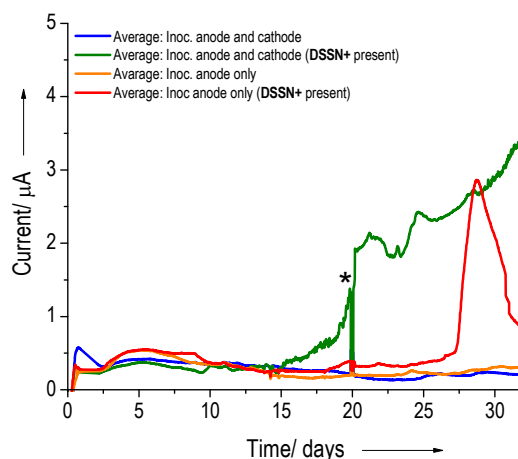


Fig. S1 Performance of a second set of MFCs run using wastewater as a fuel source that demonstrates the reproducibility of this system. MFCs run in the absence of **DSSN+** (blue, orange) yield little current, while those employing **DSSN+** (green, red) yield ~2-8 times greater current. Each trace represents the average of duplicate MFC sets. (*) Indicates a fluctuation in current that occurred upon dislodging gas bubbles formed during operation.

Chemical Oxygen Demand (COD) Data Plot

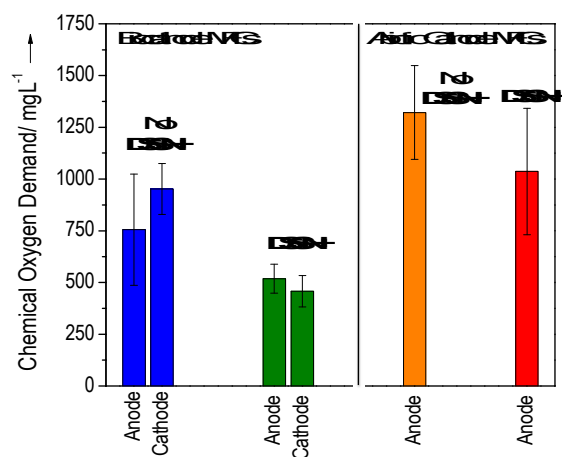


Fig. S2 Comparison of COD remaining after MFC operation. Results are shown as a function of wastewater MFC operating conditions. Error bars show the standard error of the mean calculated from three sets of MFC runs. X-axis labels denote the MFC chamber from which the COD value of the effluent was obtained. Note: $\text{COD}_{\text{initial}} = 3265 \text{ mg L}^{-1}$; presumably high due to additional cellulose content resulting from added paper.²

Confocal analysis of Electrode Biomass

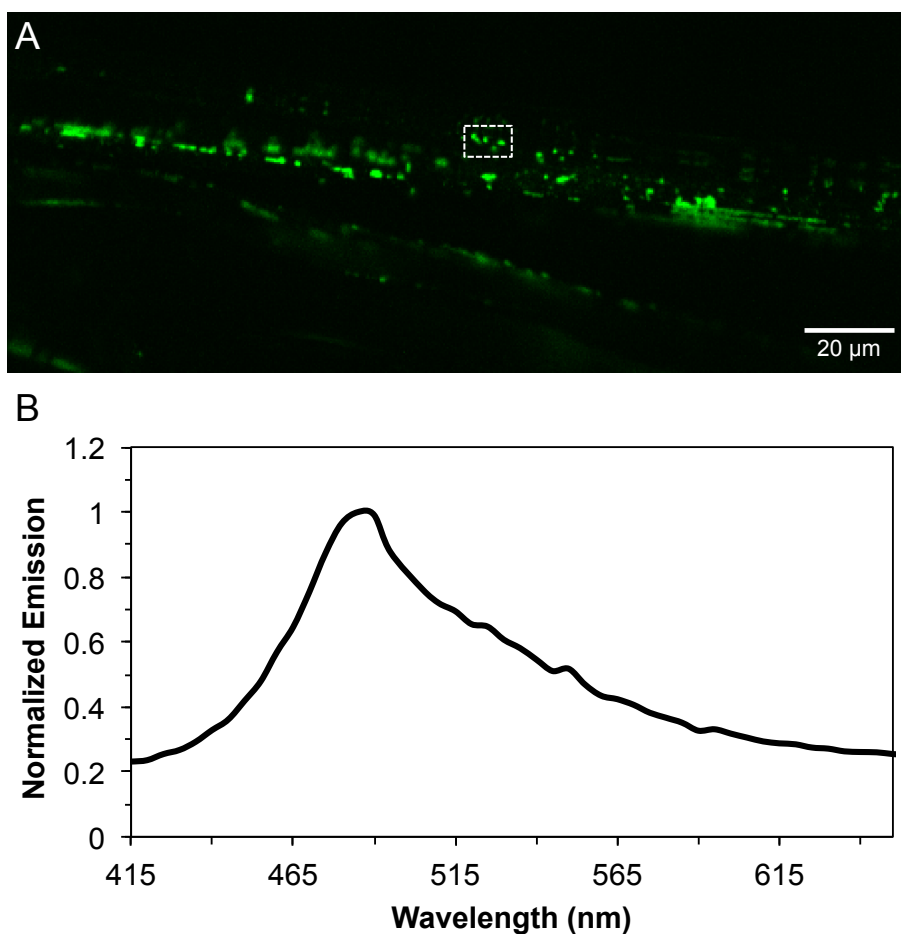


Fig. S3 (A) Confocal microscopy image of biomass on the surface of graphite felt electrode fibers. No additional stain was added and emission is attributed to **DSSN+** contained within the biomass (excitation wavelength = 405 nm). (B) Average emission spectra of **DSSN+** obtained from emissive regions within the dotted rectangle of the confocal image above. That emission maxima of **DSSN+** is observed at 485 nm suggests that the COE exists in non-polar surroundings, presumably positioned within cell membranes of the electrode biomass.

Scanning Electron Microscopy (SEM) Image Comparison

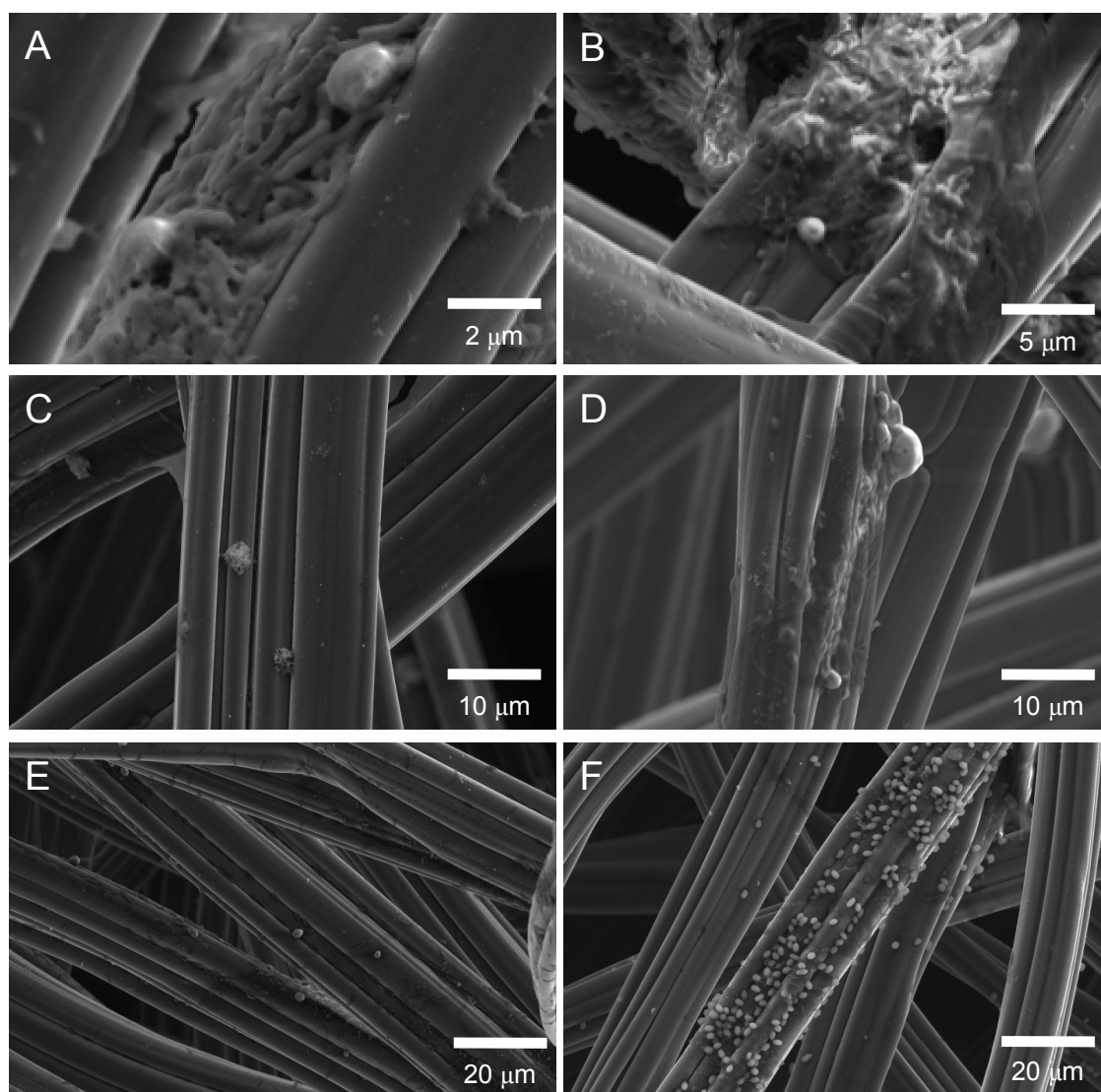


Fig. S4 SEM images of carbon felt electrodes after MFC operation. (A and B) Anodes from biocathode type MFCs run in the absence and presence of 5 μM **DSSN**⁺, respectively. (C and D) Cathodes from biocathode type MFCs run in the absence and presence of 5 μM **DSSN**⁺, respectively. (E and F) Anodes from abiotic cathode type MFCs run in the absence and presence of 5 μM **DSSN**⁺. Note: MFCs run in the presence of COE contained 5 μM **DSSN**⁺ in both chambers in all cases.

References

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