Electronic Supplementary Information (ESI)[†]

Improved microbial electrocatalysis with osmium polymer modified electrodes

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1. Detailed experimental section

1.1 Chemicals and general conditions

 $[Os(2,2'-bipyridine)_2-poly(N-vinylimidazole)_{10}Cl]^{2+/+}/$ (poly $[Os(bpy)_2(PVI)_{10}Cl]^{2+/+})$ with an E° equal to +221 mV vs. Ag|AgCl (sat. KCl) was synthesized as reported.¹ The weight-average molecular weight of the poly(vinylimidazole) as determined by viscometry in ethanol was 10,000 g/mol.² All chemicals were purchased from Sigma-Aldrich/Merck and were of either research or analytical grade. All aqueous solutions were prepared by using water purified and deionized (18 M Ω) with a Milli-Q system (Millipore, Bedford, MA). If not stated otherwise, all potentials provided in this article refer to the Ag|AgCl (sat. KCl) reference electrode. All electrochemical experiments were performed under strictly sterile and anoxic conditions.

1.2. Microbial growth conditions and inoculum preparation

Shewanella oneidensis MR-1 (LMG 19005/ATCC 700550) was obtained from Collections Belgian Coordinated of Microorganisms/Laboratorium voor Microbiologie (BCCMTM/LMG, Ghent University, Belgium). The strain was grown and maintained on tryptone soya agar. For inoculum preparation, a single, wellisolated colony was transferred to 15 mL of LB broth (in 50 mL tubes) and incubated aerobically at 30±2° C while shaking at 150 rpm (Universal shaker SM 30 A, Edmund Bühler GmbH, Germany) for 24 h. Then the cells grown in LB broth were harvested by centrifuging the broth at 4000 rpm for 10 min. Further, the cells were washed once in 50 mM PIPES buffer (pH 7.4) and once in minimal (M1) medium by centrifuging as before. Subsequently, the cells were transferred to 200 mL of minimal growth medium containing sodium lactate 18 mM/L, PIPES buffer 15.1 g/L; NaOH 3.0 g/L; NH₄Cl 1.5 g/L; KCl 0.1 g/L; NaH₂PO₄·H₂O 0.6 g/L; NaCl 5.8 g/L; mineral solution 10 mL/L; vitamin solution 10 mL/L and amino acid solution 10 mL/L.^{3, 4} After incubation at 30±2° C (500 mL baffled E-flasks, aerobically while shaking at 150 rpm for 72 h) the cells were harvested and used either as an inoculum in potentiostatic half-cell set ups hosting a similar minimal growth medium or for further inoculum preparation.

1.3. Preparation of the osmium polymer-modified electrodes

Spectrographic graphite rods (Ringsdorff Werke GmbH, Bonn, Germany) were used as working electrodes. The procedure explained by Coman et al.⁵ was followed to prepare the osmium (Os) polymer modified working electrodes. The material was polished on wet emery paper (Tufbak, Durite, P1200) and subsequently carefully

rinsed with Milli-Q water and dried. Then 20 μ L of Os polymer solution (10 mg/mL in Milli-Q water) was spread onto the entire active surface of the electrode (projected surface area; 0.282 cm²). The electrode was dried at room temperature for 10-15 min and was used for further bioelectrochemical experiments.

1.4. Measurement set-ups and bioelectrochemical experiments

All experiments were conducted using either AUTOLAB PGSTAT 30 (EcoChemie, Utrecht, The Netherlands) or PalmSens (Palm Instruments BV, The Netherlands) with a three-electrode configuration—an Ag|AgCl reference electrode (sat. KCl, Sensortechnik Meinsberg, Germany), a bare/polymer modified graphite as a working and bare graphite as a counter electrode. The sealed, water-jacketed glass vessels connected to a thermostat (Paratherm U2 electronic, Julabo, Schwarzwald, Germany) were employed as electrochemical cells that allowed maintenance of temperature control. All experiments were performed under anoxic conditions, which were achieved by sparging argon for 20 min in the buffer solution/growth medium before use. Furthermore, the headspace of the solution was also sparged with argon during the CV measurements.

With polymer-modified anodes (PMAs), two different approaches for microbial culture addition were investigated. In the first approach (PMA-1), the microbial inoculum was added to the growth medium and natural electrochemical growth of electrogens at the anode was allowed. While in the second approach (PMA-2) the microbial inoculum was directly adsorbed on to the polymer-modified electrode and trapped with the use of a dialysis membrane (Spectrum Laboratories Inc., Rancho Dominguez, CA, molecular mass cutoff: 6000-8000) to prepare a permselective membrane electrode⁶ (referred to as artificial immobilization). The semi-batch chronoamperometric (CA) experiments were performed with PMAs (both approaches) at an applied potential of +0.350 V (vs. Ag|AgCl) with regular medium replenishments. Similar experiments were also performed with unmodified anodes as controls. In this case, a bare graphite electrode was used as the anode and the cells were directly inoculated to the medium. All reported data are based on at least three independent replicates of each experiment. In all cases, cyclic voltammetry (CV) was recorded during turnover conditions, i.e. at the bioelectrocatalytic substrate consumption, and during non-turnover, i.e. substrate deprived conditions at a scan rate of 2 mV s^{-1} .

1.5. Scanning electron microscopy

Clean and bare graphite, Os polymer-modified graphite, enriched biofilm at bare graphite and at polymer-modified graphite (both PMA-1 and PMA-2) were analyzed with SEM (JEOL JSM-6700F). The samples with bacterial cells were fixed overnight using 2.5% glutaraldehyde (prepared in 0.1 M phosphate buffer, pH 7.0) and then washed with phosphate buffer (pH 7.0). Before SEM observation, all samples were dried and coated with Au/Pd.



Fig. S1 Representative CA runs showing bioelectrocatalytic current generation at A) bare electrode and B) PMA-1 in semi-batch experiments. The arrows indicate M1 medium replenishment.



Fig. S2 Cyclic voltammograms of *S. oneidensis* MR-1 recorded during substrate turnover and non-turnover conditions in control experiments (bare electrode). The substrate was 18 mM lactate. The scan rate was 2 mVs⁻¹.



Fig. S3 CV curves of *S. oneidensis* MR-1 with PMA-1 recorded after the second (A) and the third (B) fed batch cycles during substrate (18 mM lactate) turnover conditions. The scan rate was 2 mVs^{-1} .



Fig. S4 SEM images of enriched electroactive biofilms at (A) bare graphite (control) and (B) at PMA-1.

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