Supplementary information

An oxygen-reducing biocathode with "oxygen tanks"

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1. Experimental section

1.1. Materials

Myrothecium verrucaria BOx (EC 1.3.3.5, 15-65 U mg^{-1}), sodium phosphate (monobasic dehydrate ≥99% and dibasic ≥99%), poly(ethylene glycol)diglycidyl ether (PEGDGE), and polytetrafluoroethylene (PTFE, 60 wt % dispersion in H_2O), were obtained from Sigma-Aldrich, Ltd. [Os(2,2′-bipyridine)$_2$(polyvinylimidazole)$_{10}$Cl]$^{+/2+}$ (Os(bpy)$_2$PVI) was synthesized through an established procedure [1, 2]. Deionized water (18.2 MΩ cm, Sartorius arium® pro, Germany) was used to prepare all the solutions.

1.2. Electrode preparation

For **Scheme 1C** electrodes with PTFE mixed within the redox polymer/BOx layer, a 5.3 µl aliquot of a 6 mg ml^{-1} aqueous suspension of Os(bpy)$_2$PVI, 1.3 µl of a 15 mg ml^{-1} aqueous solution of PEGDGE and 3.2 µl of a 10 mg ml^{-1} solution of BOx, with a certain volume (0, 2, 4 and 6 µl) of 20% PTFE water suspension, were homogenously mixed with the assistance of a vertex mixer for 2 min. The cocktail solutions containing enzymes were drop-cast onto the surface of pre-polished glassy carbon electrodes (GCEs, diameter: 4 mm), which were immediately placed in a vacuum desiccator connected to a vacuum pump for 10 min. The resulted electrodes were labeled as GCE/Os(bpy)$_2$PVI-BOx-PTFE(n), where n=0, 2, 4 and 6, corresponding to the employed volume of 20% PTFE water suspension. The electrodes were then transferred into a fridge, allowed to dry overnight at 4°C. Prior to utilization, the electrodes were gently rinsed with 0.1 M pH 7.0

phosphate buffer solution (PBS) consisting of the mixture of monobasic/dibasic sodium phosphate for 30 min to remove loosely bound enzyme/polymer.

To form **Scheme 1B** electrodes containing a PTFE capping layer, 5 ul of 20% PTFE water suspension was drop-cast onto GCE/Os(bpy)$_2$PVI-BOx-PTFE(0) (**Scheme 1A**), which was immediately placed in a vacuum desiccator connected to a vacuum pump for 10 min and dried in a fridge overnight.

1.3. Material characterisation

Scanning electron microscopy (SEM, Quanta FEG 200 ESEM, 15 kV) was used to characterize PTFE submicro-rods. The average length and diameter of PTFE submicro-rods were obtained by measuring at least 30 times with ImageJ software (National Institutes of Health, Bethesda, Maryland) [3].

1.4. Electrochemical measurements

Electrochemical characterisation was performed using an electrochemical workstation (Autolab PGSTAT12) with a three-electrode system composed of bioelectrode based working electrodes, a saturated calomel electrode (SCE) reference electrode and a platinum wire counter electrode. Cyclic voltammetry (CV) was conducted in 0.1 M pH 7.0 PBS either saturated with Ar or equilibrated with air. All experiments were performed at room temperature (20±2 °C) unless stated otherwise.

Maximum background corrected catalytic current density (Δj_{max}) for oxygen reduction, omitting the contribution from electrode capacitances, was obtained by subtracting the background current density of the electrode in the Ar-saturated solution from that in air-equilibrated solution [4].

Surface coverage (Γ, nmol cm^{-2}) of Os polymer on the electrode is obtained based on the following equation:

$$\Gamma = Q/(n'FA) \quad (S1)$$

where Q (nC) is the integrated area of the reduction peak of the blank CV, n' is the number of electron involved, F is the Faraday constant and A (cm^{-2}) is the geometric area of GCE.

2. Supplementary figures

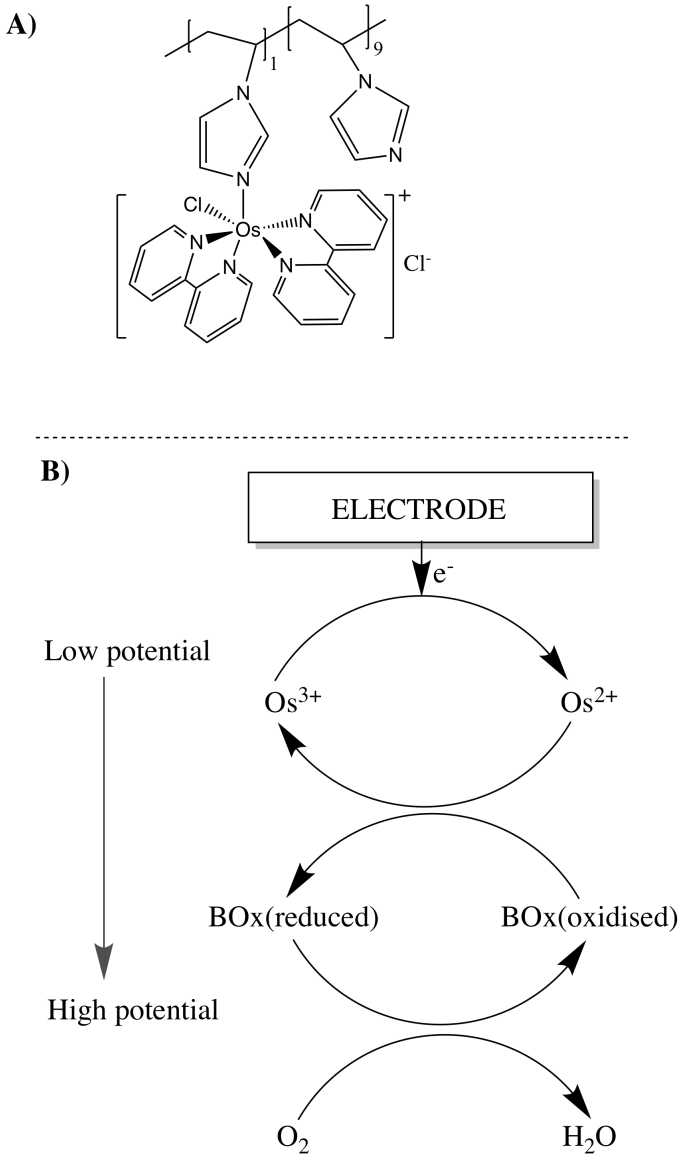

Fig. S1. (A) Structure of $Os(bpy)_2PVI$; (B) the mediated electron transfer between BOx and the electrode surface with the assistance of $Os(bpy)_2PVI$.

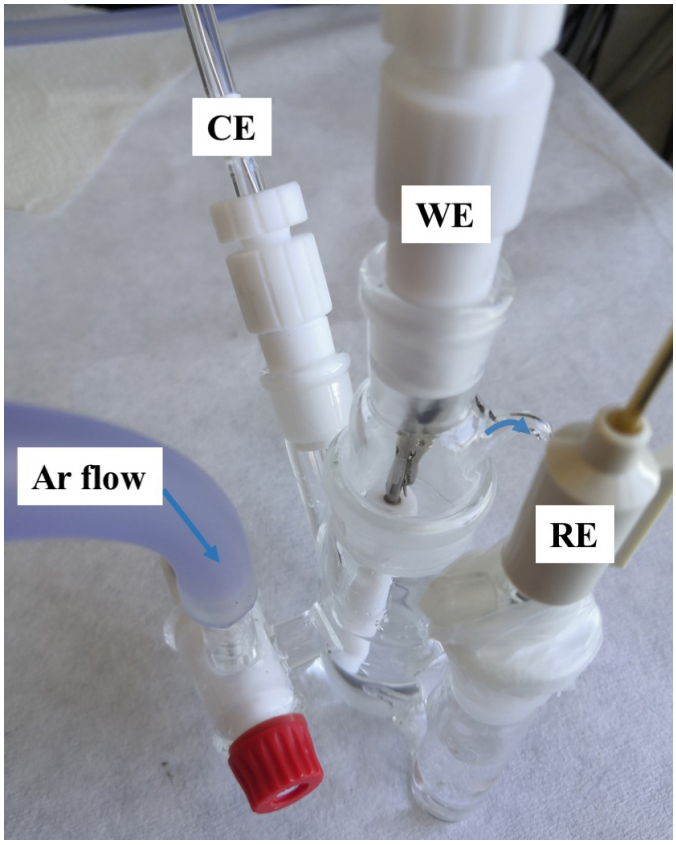

Fig. S2. Digital photo of the well-sealed electrochemical cell used for testing biocathodes in an oxygen-free condition.

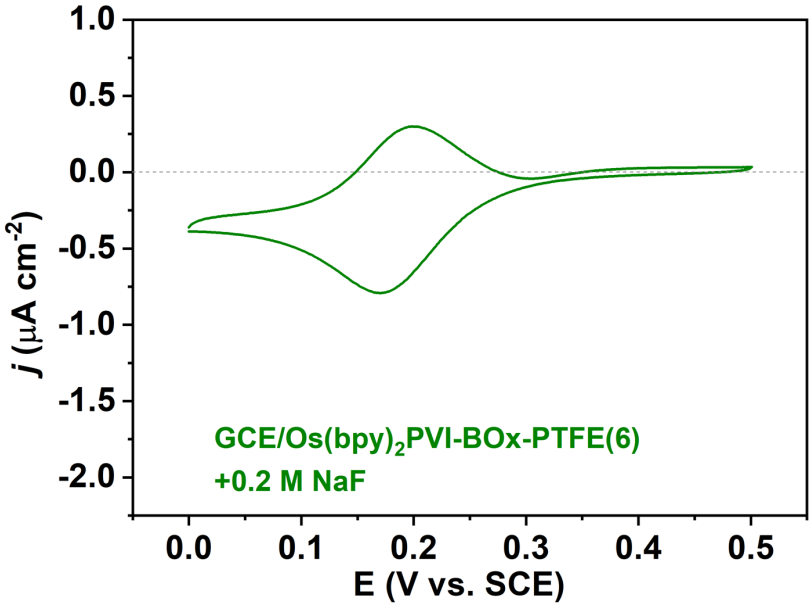

Fig. S3. CVs of GCE/Os(bpy)$_2$PVI-BOx-PTFE(6) in Ar-equilibrated 0.1 M pH 7.0 PBS containing 0.2 M NaF; Scan rate: 5 mV s^{-1}.

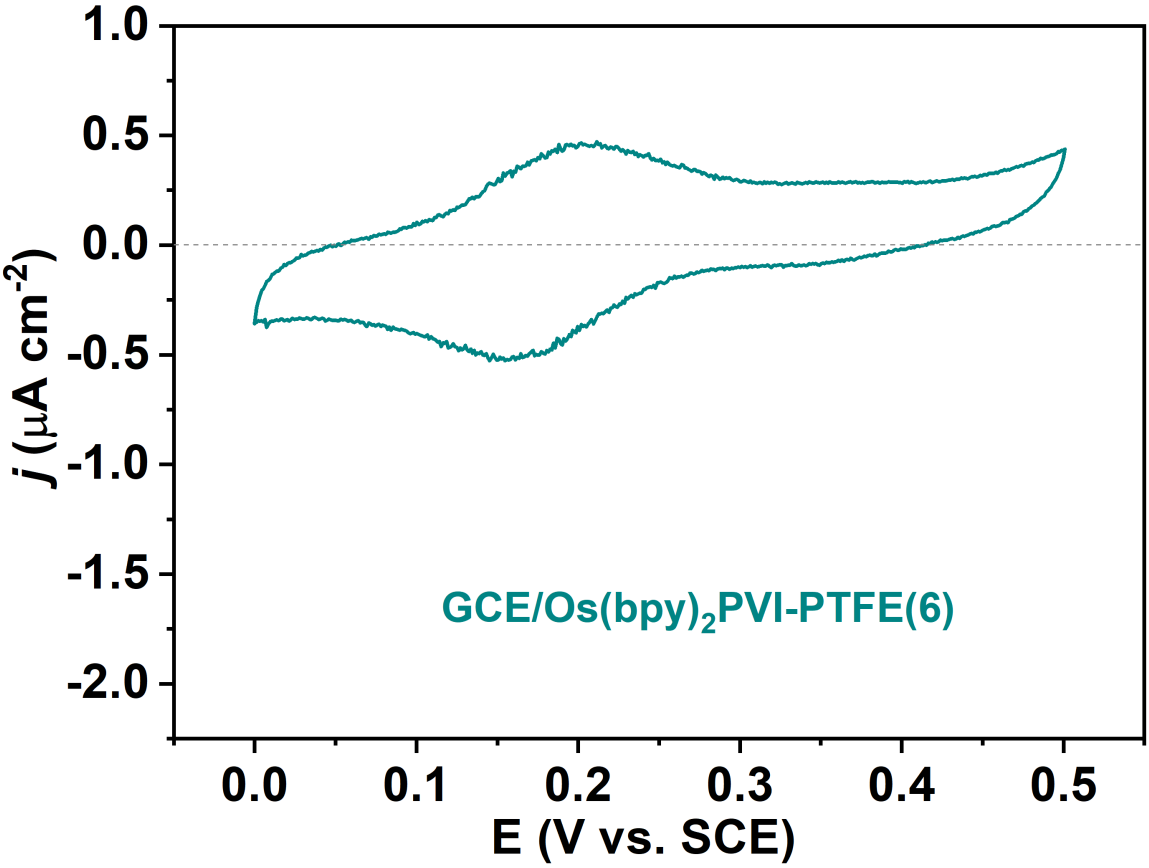

Fig. S4. CVs of GCE/Os(bpy)$_2$PVI-PTFE(6) in Ar-equilibrated 0.1 M pH 7.0 PBS; Scan rate: 5 mV s^{-1}.

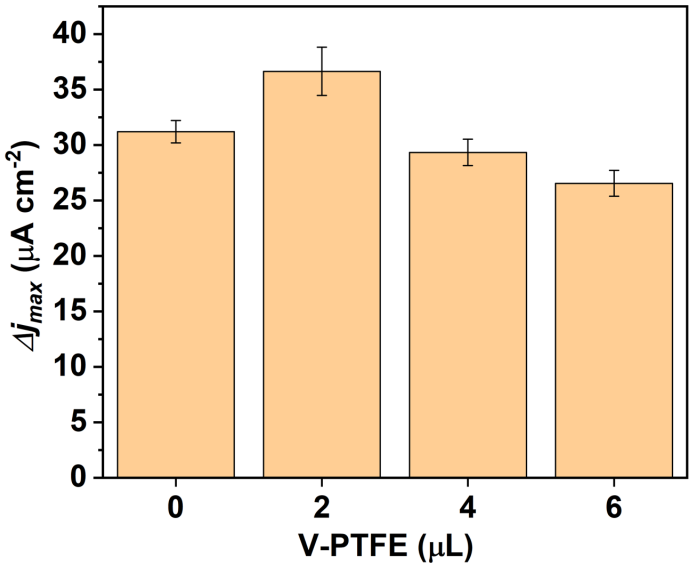

Fig. S5. Δj_{max} derived from **Fig. 3** for mixed-type GCE/Os(bpy)$_2$PVI-BOx-PTFE(n) (n=0, 2, 4, 6).

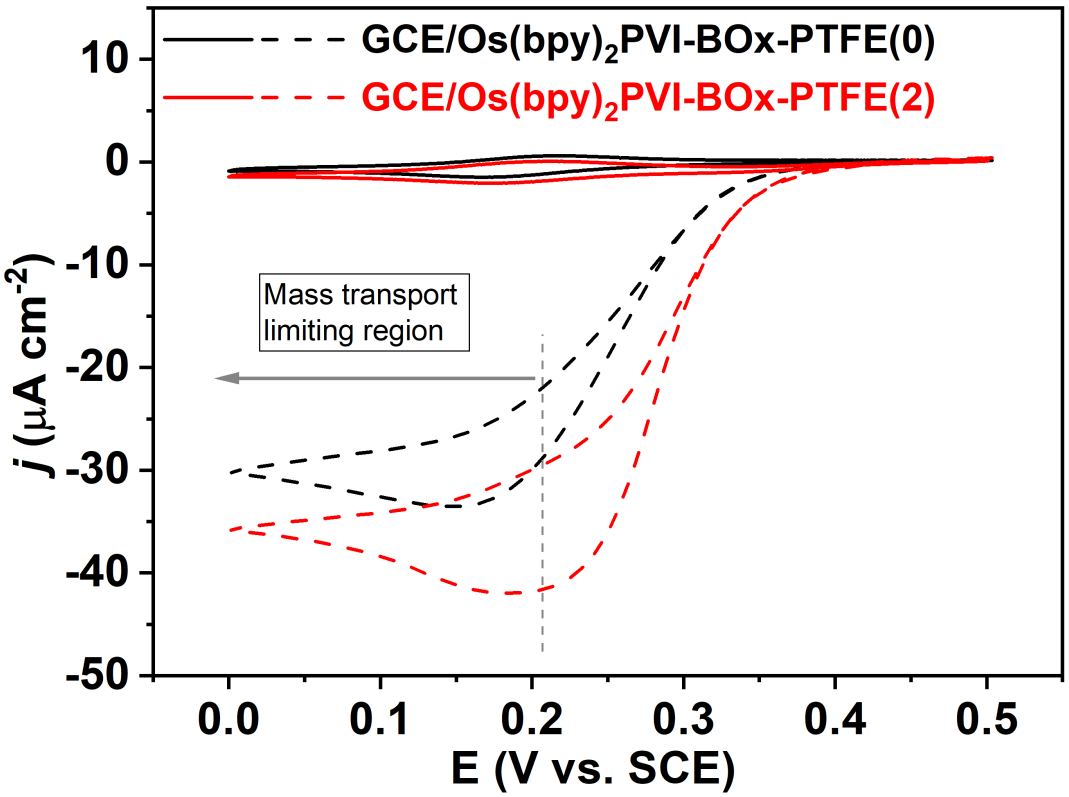

Fig. S6. Cyclic voltammograms (CVs) of GCE/Os(bpy)$_2$PVI-BOx-PTFE(0) (black curve) and two-layered GCE/Os(bpy)$_2$PVI-BOx-PTFE(2) (red curve) in either Ar- (solid curve) or air-equilibrated (dashed curve) 0.1 M pH 7.0 PBS; Scan rate: 5 mV s^{-1}. The data is from **Fig. 3A-B** and **3E-F**, and overlapped for a better comparison.

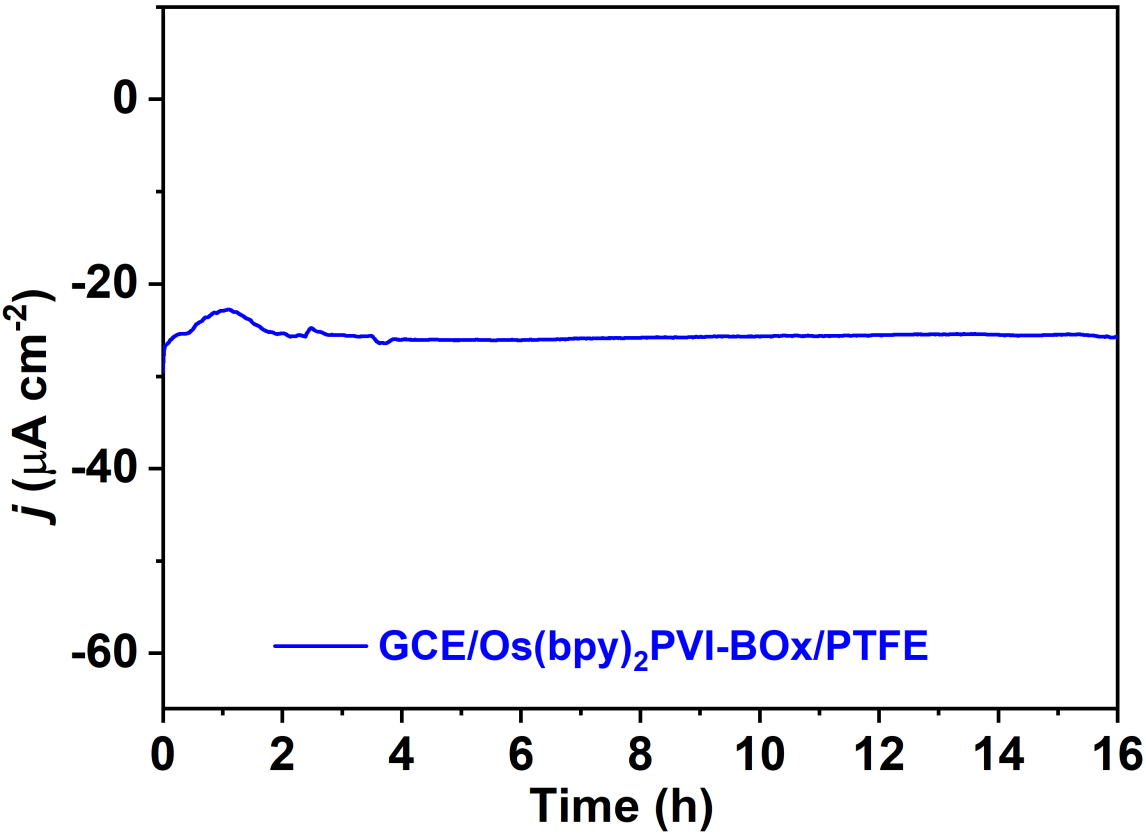

Fig. S7. Operational stability of GCE/Os(bpy)$_2$PVI-BOx/PTFE in the capping configuration (**Scheme 1B**) in air-equilibrated 0.1 M pH 7.0 PBS; applied constant potential: 0.1 V vs. SCE.

References

[1] E.M. Kober, J.V. Caspar, B.P. Sullivan, T.J. Meyer, Synthetic Routes to New Polypyridyl Complexes of Osmium(Ii), Inorg. Chem., 27 (1988) 4587-4598.
[2] R.J. Forster, J.G. Vos, Synthesis, Characterization, and Properties of a Series of Osmium- and Ruthenium-containing Metallopolymers, Macromolecules, 23 (1990) 4372-4377.
[3] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, NIH Image to Imagej: 25 Years of Image Analysis, Nat. Methods, 9 (2012) 671.
[4] A.J. Gross, X. Chen, F. Giroud, C. Abreu, A. Le Goff, M. Holzinger, S. Cosnier, A High Power Buckypaper Biofuel Cell: Exploiting 1,10-Phenanthroline-5,6-dione with FAD-Dependent Dehydrogenase for Catalytically-Powerful Glucose Oxidation, ACS Catal., 7 (2017) 4408-4416.