Supplementary Information

A Biofuel Cell in Nonaqueous Solution

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Experimental section

1.1. Materials

Sulfuric acid (95–98 %), nitric acid (70%), potassium phosphate monobasic (≥99 %) and dibasic (≥98 %), d-(+)-glucose (99.5 %), ethanol (EtOH, 96%), acetonitrile (ACN, ≥99.9%), acetone (AC, ≥99.8%), 1-propanol (PrOH, ≥99.5%), methanol (MeOH, ≥99.9%), 1-butanol(BuOH, ≥99.7%), 1-pentanol (PeOH, ≥99%), tetraethylammonium *p*-toluenesulfonate (TEATS), and poly(ethylene glycol)diglycidyl ether (PEGDGE) were obtained from Sigma-Aldrich Ireland, Ltd. Absolute ethanol was obtained from Lennox Ltd., Ireland. All solutions were prepared with deionised water (resistivity of 18.2 MΩ cm) from an Elgastat maxima-HPLC (Elga, UK). All experiments were carried out at room temperature (20±2 °C).

The complexes, [Os(2,2′-bipyridine)$_2$(polyvinylimidazole)Cl]$^{+/2+}$(Os(bpy)$_2$PVI, E^o: 0.22 V vs. Ag/AgCl) and [Os(4,4′-dimethyl-2,2′-bipyridine)$_2$(polyvinylimidazole) Cl]$^{+/2+}$(Os(dmbpy)$_2$PVI, E^o: 0.12 V vs. Ag/AgCl) were synthesized using published procedures[1]. GOx from *Aspergillus niger* (EC 1.1.3.4, type II, ≥15,000 U g^{-1}) and BOD from *Myrothecium verrucaria* (EC 1.3.3.5, 2.63 U mg^{-1}) were purchased from Sigma-Aldrich, Ireland, Ltd. and Amano Enzyme Inc. (Nagoya, Japan), respectively.

NPG sheets were prepared by dealloying 100-nm-thick Au/Ag leaves (12-carat, Eytzinger, Germany) in concentrated HNO_3 for 30 min at 30℃, then placed on the surface of glassy carbon electrodes (GCE)[2]. Prior to using, NPG electrodes were electrochemically cleaned by scanning potential in 1 M H_2SO_4.

1.2. Enzyme immobilization procedures

A 5.3 μl aliquot of a 6 mg ml^{-1} aqueous suspension of osmium-based redox polymer, $Os(dmbpy)_2PVI$ or $Os(bpy)_2PVI$, was combined with 1.3 μl of a 15 mg ml^{-1} aqueous solution of PEGDGE and, either 3.2 μl of a 10 mg ml^{-1} solution of GOx or BOD. All the components were homogenously mixed by vortexing. The surface of the NPG electrode was fully covered by a drop of the solution, and immediately placed in a vacuum desiccator connected to a vacuum pump for 10 min. The electrodes were then transferred into the fridge, allowed to dry overnight in the dark at 4°C. To elucidate the role of the enzymes on the catalytic response, NPG electrodes modified only with redox polymer were prepared by the same procedure.

1.3. Electrochemical measurements

Electrochemical studies were performed using a CHI802 potentiostat (CH Instruments, Austin, Texas) in a standard three-electrode electrochemical cell. Platinum wire and saturated calomel electrodes (SCE) were used as the counter and reference electrodes, respectively. Enzyme-modified electrodes were immersed in 0.1 M pH 7.0 phosphate buffer solution (PBS) for at least 20 min prior to electrochemical measurements to allow for film swelling.

The biofuel cell consists of a bioanode made of NPG/$Os(dmbpy)_2PVI$/GOx and a NPG/$Os(bpy)_2PVI$/BOD biocathode. The power density of biofuel cells was measured in different oxygen-bubbled organic solvents containing 5 mM glucose using the bioanode as working electrode and the biocathode as a combined counter/reference electrode. The potential was scannedat a scan rate of 1 mV s^{-1}, while recording the current in the circuit. Storage stability was determined by storing the BFC in buffer solution at 4°C and measuring the response for the required period of time. Nonaqueous solutions were prepared by the addition of the desired volume of buffer solution (4.4 mM phosphate, pH 7.0) to the organic solvent. 0.1 M TEATS was used as the electrolyte. Operational stability tests (for 5 hours in 95% ACN) were

performed by continuously recording the currentat aconstant potential of 0.15 V, with O_2 bubbling of the solution.

Supporting Figures

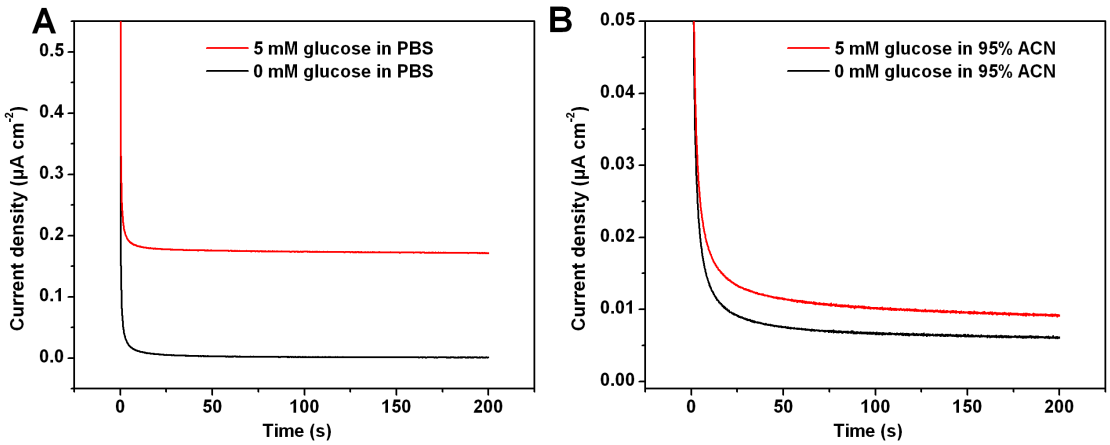

Fig. S1 Chronoamperometry response of the NPG/Os(dmbpy)$_2$PVI/GOx bioanode at +0.2 V vs. SCE in PBS (A) and 95% ACN (B).

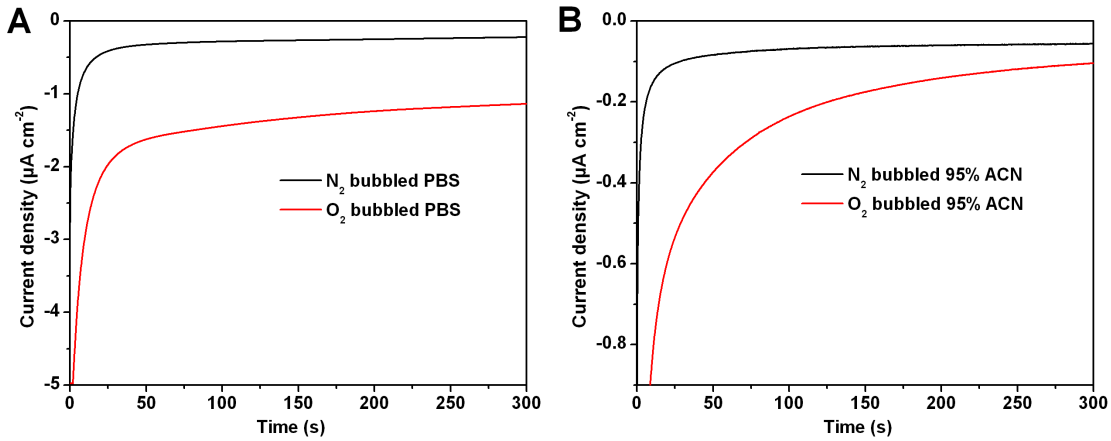

Fig. S2 Chronoamperometry response of the NPG/Os(bpy)$_2$PVI/BOD biocathode at +0.1 V vs. SCE in PBS (A) and 95%ACN (B).

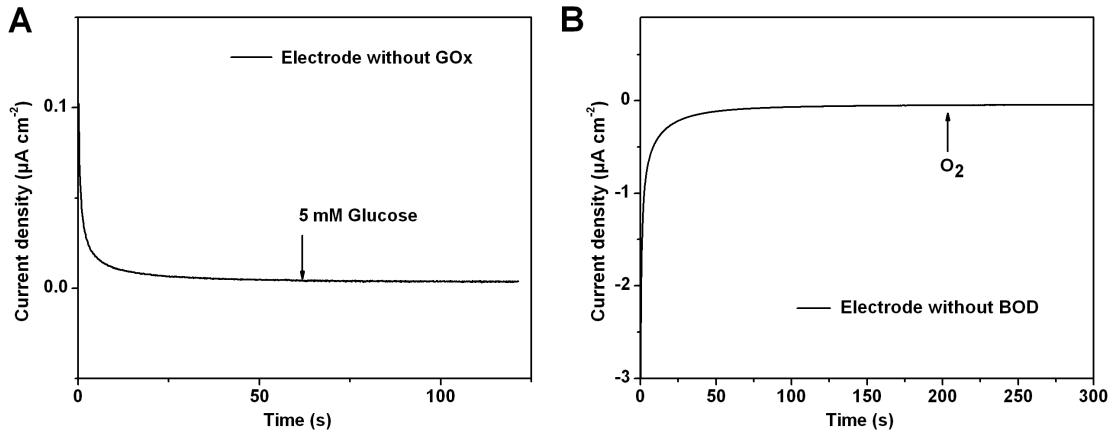

Fig. S3 Chronoamperometric response of blank electrodes without enzymes in PBS: NPG/Os(dmbpy)$_2$PVI at +0.2 V vs. SCE (A); NPG/Os(bpy)$_2$PVI at +0.1 V vs. SCE(B).

Note: No amperometric response was observed upon the addition of glucose or O_2 to modified electrodes without GOx or BOD, indicating that the enzymes were catalytically active.

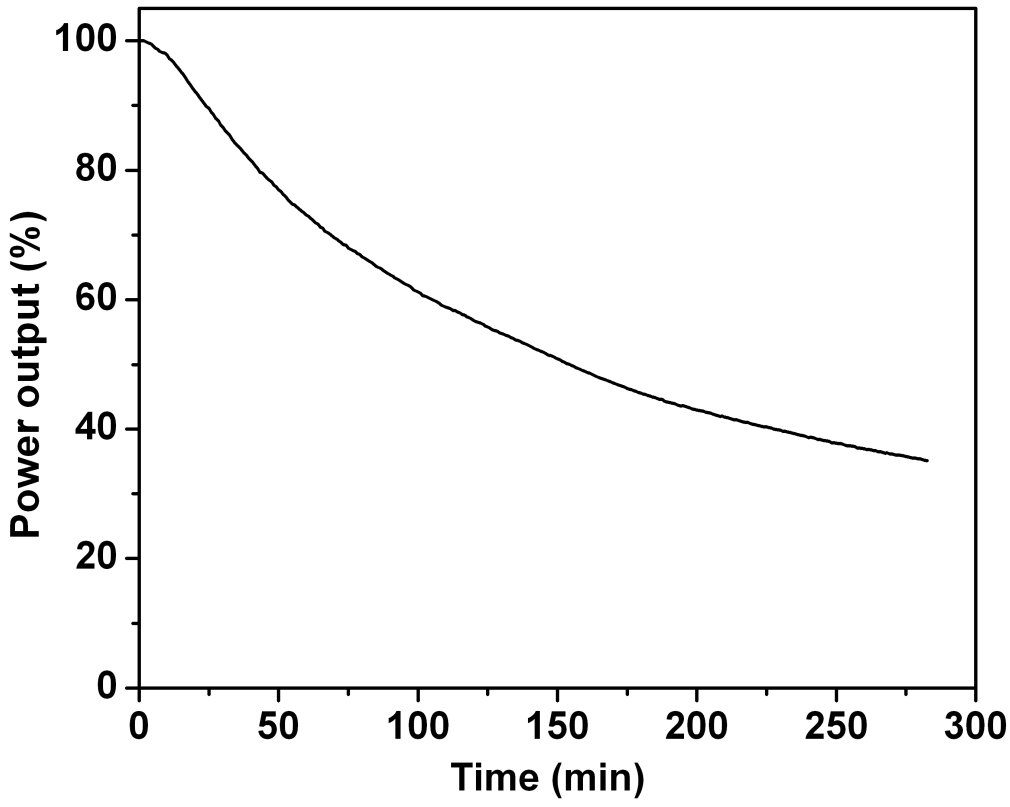

Fig.S4. Operational stability of the BFC in 95% ACN.

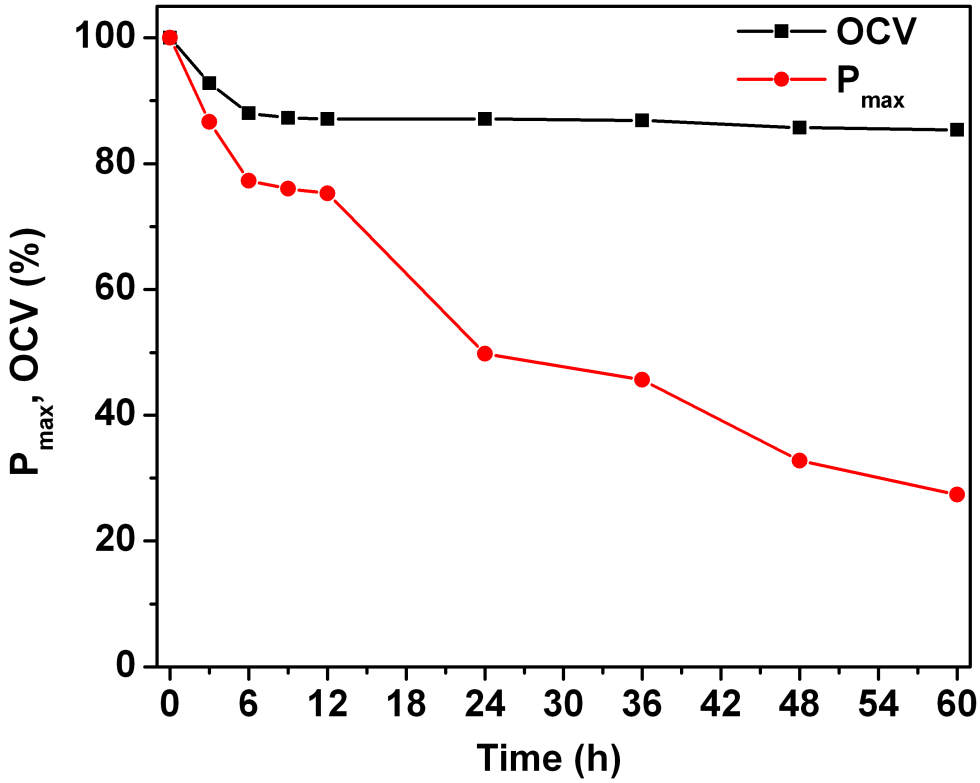

Fig.S5 Storage stability of the BFC.

References

1. P. A. Jenkins, S. Boland, P. Kavanagh and D. Leech, *Bioelectrochem.*, 2009, **76**, 162-168.
2. X. Xiao, J. Ulstrup, H. Li, J. Zhang and P. Si, *Electrochim. Acta*, 2014, **130**, 559-567.