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A Biofuel Cell in Non-aqueous Solution

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We report the first example of a biofuel cell operating in organic solvents. The cell utilises glucose oxidase and bilirubin oxidase immobilised on nanoporous gold. The power output of the cell decreases with increasing solvent hydrophobicity in the alcohols, methanol (MeOH), ethanol (EtOH), 1-propanol (PrOH), 1-butanol (BuOH) and 1-pentanol (PeOH).

Enzymatic biofuel cells (BFCs) that utilise oxidoreductases to generate electrical energy are of interest due to their potential applications as autonomous power supplies^{1,2}. A wide range of reports have described the development of miniaturized BFCs with extended lifetimes and increased power densities, focussing on screening enzymes from a variety of sources, developing more efficient methods of immobilization, the use of a range of electrode materials and the deployment of enzyme cascades^{3,4}. Due to potential applications in biomedical devices, the properties of BFCs are invariably examined in physiological conditions, with no reports on their use in nonaqueous solvents.

Enzymes can preserve their native structures and retain catalytic activity in nonaqueous solutions⁵. While the catalytic activity of enzymes in such media can be significantly lower than in aqueous solution⁶, the use of enzymes in such media has a number of advantages that include increased substrate solubility, increased thermal stability, suppression of side reactions that can occur in water and changes in enzymatic selectivity^{7,8}. Enzymes are insoluble in nonaqueous media and as a consequence, electrochemical studies of enzymes in such media require that the enzyme be immobilised on the electrode. Studies on enzyme modified electrodes in organic solvents have focussed on their use in biosensors and in probing the kinetics and thermodynamics of the redox process^{9,10,11}. Properties such as log *P* (a quantitative measure of solvent polarity)¹², viscosity¹³ and the dielectric constant^{14,15} can affect the enzymatic activity and specificity¹⁶.

Herein, we describe the properties of a well-studied BFC based on glucose and O₂ using glucose oxidase (GOx, *Aspergillus niger*) and bilirubin oxidase (BOD, *Myrothecium verrucaria*) entrapped with the osmium polymers [Os(4,4'-dimethyl-2,2'-bipyridine)₂(polyvinylimidazole)Cl]⁺²⁺ (Os(dmbpy)₂PVI) and [Os(2,2'-bipyridine)₂(polyvinylimidazole)Cl]⁺²⁺ (Os(bpy)₂PVI) (Fig. 1A)¹⁷. Poly(ethylene glycol)diglycidyl ether (PEGDGE) was used as the cross-linking agent. The enzyme/redox polymer mixtures were immobilised onto dealloyed nanoporous gold (NPG) electrodes (details in ESI†)¹⁸. NPG is a stable and conductive support¹⁹ that enables the polymer to be confined within the porous structure of the support, as well as improved rates of electron transfer between the enzymes and the electrode²⁰. Due to the high selectivity of enzymes, the fully enzymatic BFC can be tested in a one-compartment cell.

The catalytic activities of the NPG/Os(dmbpy)₂PVI/GOx and NPG/Os(bpy)₂PVI/BOD electrodes were separately studied in a three-electrode cell. Cyclic voltammograms (CVs) of NPG/Os(dmbpy)₂PVI/GOx in 0.1 M pH 7.0 phosphate buffer (PBS) exhibited a pair of well-defined redox peaks corresponding to the conversion of Os²⁺/Os³⁺ at a low scan rate of 5 mV s⁻¹ (Fig. 1B, solid line). The peak potential separation, Δ*E*_p, of 15 mV, was indicative of a rapid and reversible electron transfer process. Upon addition of 5 mM glucose, a sigmoidal-shaped curve, characteristic of the bioelectrocatalytic oxidation of glucose, with an onset potential of -0.1 V, was obtained (Fig. 1B, dashed line). The response current density, *j*_{response}, defined as the difference between the catalytic and background current density was 54 μA cm⁻² in PBS. The electrode was then transferred into acetonitrile (ACN) containing 5% added buffer. A reversible redox curve (Fig. 1C, solid line) with a *j*_{response} of 2.2 μA cm⁻² (4% of the original activity in PBS, Fig. 1C, dashed line), as well as a positively shifted onset potential of -0.03 V, was obtained. The catalytical response in both PBS and ACN

was further confirmed by chronoamperometry, with a catalytic current clearly evident (Fig. S1). This agrees with previous reports that enzymes in organic

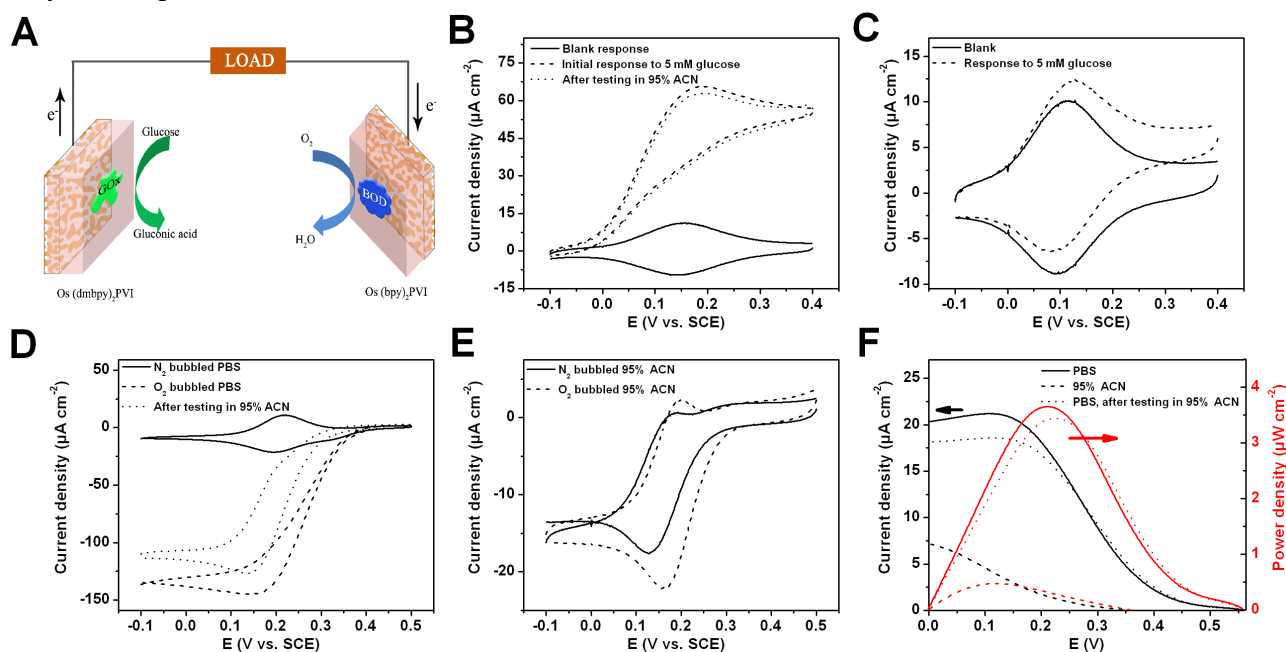


Fig. 1 (A) Schematic diagram of the biofuel cell. Cyclic voltammograms (CVs) of NPG/Os(dmbpy)₂PVI/GOx modified electrodes in PBS (B) and 95% ACN (C) at a scan rate of 5 mVs⁻¹. (C) CVs of NPG/Os(bpy)₂PVI/BOD electrode in PBS (D) and 95% ACN (E) at a scan rate of 5 mVs⁻¹. (F) Polarization and power curves for the BFC in O₂ bubbled PBS (initial curve: solid line; after testing in 95% ACN: dotted line) and 95% ACN (dashed line).

media possessed only a fraction of the catalytic activity observed in water⁶ with the decreased activity arising from a range of effects²¹ (e.g. decrease in molecular flexibility²², reduction in the amount of water bound to the enzyme). There was no perceptible change in the cathodic peak potential. It is noteworthy that the response of the electrode was largely retained on re-immersion in aqueous buffer solution (j_{response} of 52 $\mu\text{A cm}^{-2}$, 96% of initial response) (Fig. 1B, dotted line) indicating that GOx had not been denatured in the organic solution.

NPG/Os(bpy)₂PVI/BOD cathodes also showed a pair of reversible and well-defined redox peaks in N₂ bubbled PBS (Fig. 1D, solid line). An initial j_{response} of 123 $\mu\text{A cm}^{-2}$ and onset potential of ~ 0.43 V in O₂ bubbled aqueous solution were obtained (Fig. 1D, dashed line). On switching to 95% ACN, a significantly lower j_{response} of 5 $\mu\text{A cm}^{-2}$ (Fig. 1E, dashed line) was observed, while the onset potential of O₂ reduction decreased to approximate 0.33 V. Chronoamperometric data confirmed the activity of the enzyme in ACN (Fig. S2). The recovery of activity (j_{response} of 105 $\mu\text{A cm}^{-2}$, 85% of initial response) in PBS indicated that BOD had not been significantly denatured on exposure to 95% ACN (Fig. 1D, dotted line). The indispensable role of enzymes on catalytic response has been proved by investigating blank electrodes (Fig. S3).

Based on the above results, GOx and BOD modified anodes and cathodes were subsequently assembled into BFCs (Fig. 1A), and the response of the cell monitored by linear sweep voltammetry (LSV) at a scan rate of 1 mV s⁻¹. The BFC displayed an open circuit voltage (OCV) of 0.56 V (decided by the difference of the onset potentials of glucose oxidation and O₂ reduction), a maximum current density of 21.2 $\mu\text{A cm}^{-2}$, and a maximum power density of 3.65 $\mu\text{W cm}^{-2}$ at a potential of 0.21 V in O₂ bubbled PBS containing 5 mM glucose (Fig. 1F, solid line). On replacement with 95% ACN, the performance of the BFC decreased, with an OCV of 0.36 V, a maximum current density of 7.11 $\mu\text{A cm}^{-2}$, and a maximum power density of 0.47 $\mu\text{W cm}^{-2}$ at 0.12 V (Fig. 1F, dashed line). The decrease in power arises both from changes in OCV and in current density (enzyme activity). The response of the cell was retained on re-immersion in PBS, (OCV of 0.56 V, the same value as initial one), and a maximum power density of 3.44 $\mu\text{W cm}^{-2}$ (94% of the original response, Fig. 1F, dotted line). Leakage of redox polymer from the electrode surface was mainly responsible for the loss response¹⁷ as evidenced by the decrease in the peak current in a blank buffer solution (data not shown). The operational stability of BFC in 95% CAN was examined, with a half-life time of ca. 3 h (Fig. S4). By storing at 4°C and measuring in PBS, the BFC displayed a half-life time of ~ 20 h (Fig. S5). A minor drop of less than 15% in OCV after 60 h storage was registered.

The response of the BFCs was examined in solutions with varying water content (1-5% (v/v)) in ACN (Fig. 2A). The maximum power density 0.47 $\mu\text{W cm}^{-2}$ was obtained with 5% added buffer decreasing to 0.13 $\mu\text{W cm}^{-2}$ in 99% ACN, indicating as expected, that the enzymes are more active at higher water content. The response of the BFC was examined in a series of solvents with 5% v/v added buffer (Fig. 2B). The maximum power density was obtained in MeOH and decreased in EtOH, PrOH, BuOH and PeOH. The response in these solvents decreased with increasing solvent hydrophobicity in an approximately linear manner (Fig. 2C). The response in ACN and acetone (AC) was lower than in the alcohols and did not follow this linear relationship. This may ascribed to the complexity of the integration of two different enzymes immobilized in the polymer matrix. Generally, the BFC had a higher power density in organic solvents with lower values of log P . Similar trends were reported for single enzyme electrodes^{14, 23}. The trend observed here is likely to arise from solvent based interactions at the enzymes active sites with either the enzymatic substrates or with the redox polymers.

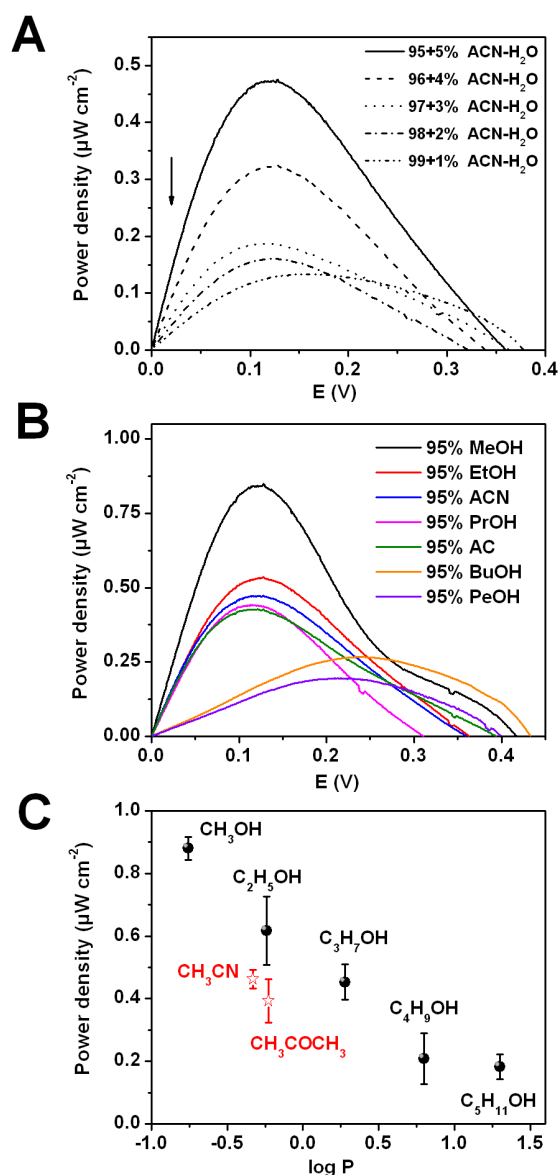


Fig. 2 Power density curve of the BFC in different percentages of O_2 bubbled ACN containing 5 mM glucose (A) and different organic solvents containing 5 mM glucose (B). (C) Plot of the power density versus log P (data points taken from reference¹²). The error bars correspond to the values recorded for three BFCs.

In conclusion, we describe the assembly of a membraneless BFC that coupled an NPG/Os(dmbpy)₂PVI/GOx bioanode with a NPG/Os(bpy)₂PVI/BOD biocathode that operates in organic solvents. More importantly, both bioelectrodes displayed reversible recovery of their initial activities in PBS after operation in organic solutions. A well defined trend that maximum power densities decrease with higher log P was obtained in straight-chain monohydric alcohols (MeOH, EtOH, PrOH, BuOH, PeOH). The use of this BFC is limited to a small range of solvents due to the low solubility of glucose in nonaqueous media (generally not higher than 5 mM glucose in 95 % organic solvent). Possible applications exist for the generation electricity from low water content samples of pharmaceuticals and petrochemicals. Future work will focus on the use of oxidases that utilise substrates (e.g. EtOH) with high solubility in such media.

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