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Electronic Supplementary Information

Bilirubin oxidase bioelectrocatalytic cathodes: the impact of hydrogen peroxide

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Supplementary Figures

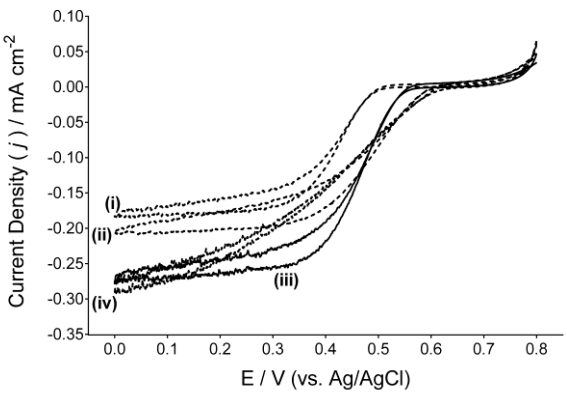

Fig. S1 Cyclic voltammograms of BOd/Ac-MWCNTs Toray-paper electrodes in air-purged hydrodynamic solutions of aqueous citrate/phosphate buffer (0.2 M) at pH 4.5 (iv), 5.5 (ii), 6.5 (iii) and 7.5 (i). The scan rate was 1 mV s^{-1}.

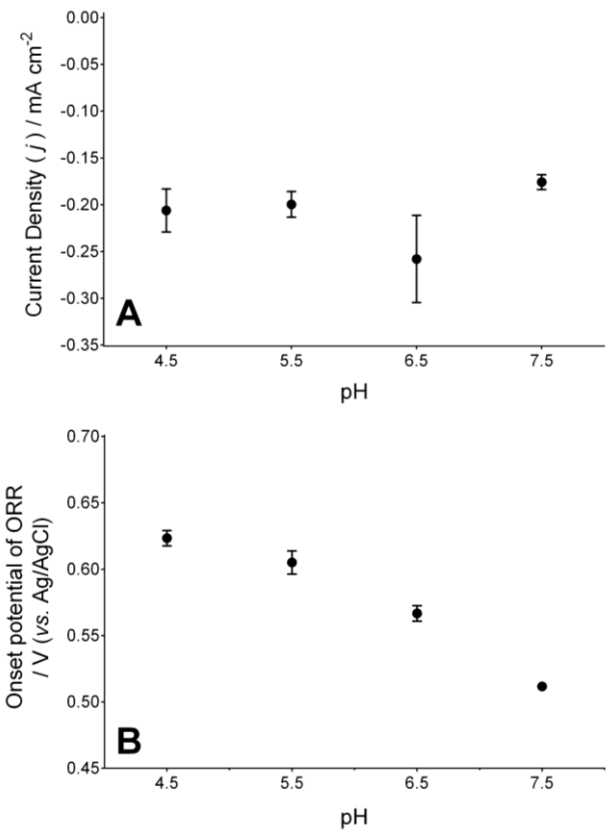

Fig. S2 (A) Catalytic current densities extracted from Fig. S1 at over-potentials of 0.35 V from the onset-potentials of O_2 reduction for each replicate (n = 3, error = SD). (B) Onset-potentials for O_2 reduction extracted from Fig. S1 (n = 3, error = SD).

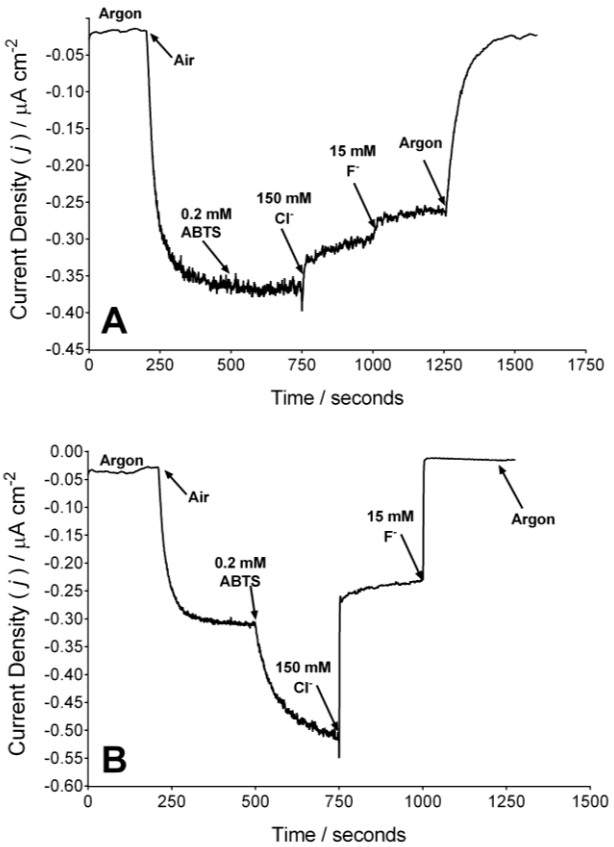

Fig. S3 Chronoamperometric trace for a (A) BOd/Ac-MWCNTs Toray-paper electrode (pH 6.5) and a (B) laccase/Ac-MWCNTs Toray-paper electrode (pH 4.5) in a hydrodynamic aqueous citrate/phosphate buffer solution (0.2 M) poised at 0.2 V (*vs.* Ag/AgCl).

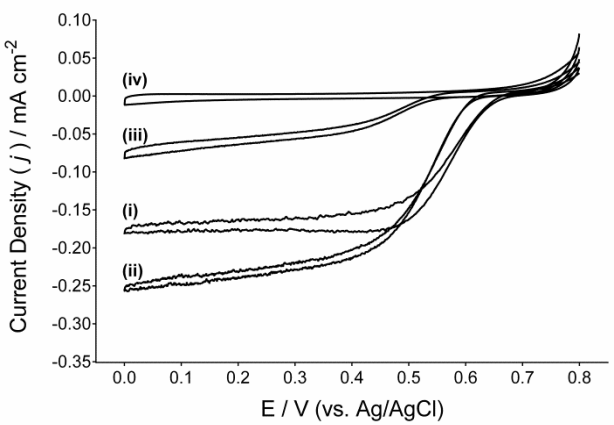

Fig. S4 Average cyclic voltammograms of laccase/Ac-MWCNTs Toray-paper electrodes in air-purged hydrodynamic solutions of aqueous citrate/phosphate buffer (0.2 M) at pH 4.5 (i), 5.5 (ii), 6.5 (iii) and 7.5 (iv). The scan rate was 1 mV s^{-1}.

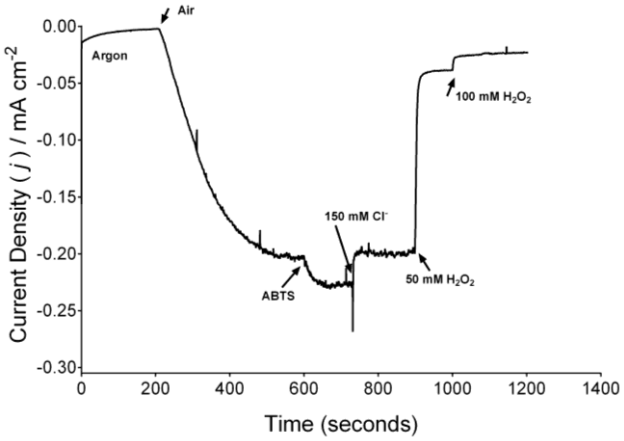

Fig. S5 Chronoamperometric trace for a laccase/Ac-MWCNTs Toray-paper electrode in a hydrodynamic aqueous citrate/phosphate buffer solution (0.2 M, pH 4.5) poised at 0.2 V (*vs.* Ag/AgCl).

Experimental Procedures

Chemicals

Bilirubin oxidase (BOd, *Myrothecium sp.*, ≥1.2 U mg^{-1}, EC: 1.3.3.5) was kindly donated by Sekisui Diagnostics (UK) and used as received. Laccase (*Trametes versicolor*, ≥ 10 U mg^{-1}, EC: 1.10.3.2), catalase (bovine liver, 2000-5000 U mg^{-1}, EC: 1.11.1.6), albumin from bovine serum (BSA), citric acid, sodium citrate, sodium chloride, sodium fluoride, paraffin wax, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide and tetrabutylammonium bromide (TBAB) were purchased from Sigma-Aldrich, and used as received. Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system (18.2 MΩ cm). Anthracene-modified MWCNTs (Ac-MWCNTs) prepared from OH-modified MWCNTs (purchased from www.cheaptubes.com), and TBAB-modified Nafion® were prepared as previously reported.[1] Toray carbon paper (TGP-H-060, non-wet-proofed) was purchased from Alfa Aesar (UK), and used as received.

Instrumentation

Electrochemical analyses were conducted using an AutoLab PGSTAT302N instrument (EcoChemie, Netherlands). Pt-wire and Pt-mesh electrodes were used as counter-electrodes in three-electrode electrochemical tests. Ag/AgCl (3 M KCl) reference electrodes were used. Toray paper electrodes were cut and wax-coated to give an untreated surface area of 1 cm^2. Cyclic voltammetry and chronoamperometry were used to analyse the performance of the enzymatic cathodes. Analysis was performed at room temperature (22 ±1°C). Citrate buffer (pH 4.5/6.5, 0.2 M) was used as the background electrolyte. Current densities are reported as a function of geometric

electrode area. Enzymatic activity assays were performed using a Biochrom Instruments Libra S60 double-beam UV-Visible Spectrophotometer.

Bilirubin Oxidase/Laccase Cathode Fabrication

A stock solution of tetrabutylammonium bromide-modified Nafion® (TBAB-Nafion®) in ethanol and anthracene-modified MWCNTs (Ac-MWCNTS) were prepared as previously reported.[1] Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system.

1.5 mg of crude enzyme was added to 75 µL of citrate/phosphate buffer (0.2 M, at the pH to be investigated) and gently mixed until dissolved. This solution was then added to 7.5 mg of Ac-MWCNTs and mixed by a combination of vortex-mixing and gentle sonication for a total of 4 minutes and 1 minute, respectively. TBAB-Nafion® in ethanol (25 µL) was then added to the mixture, followed by vortex-mixed for a further minute. This mixture was then evenly painted onto Toray paper electrodes (geometric surface area of 1 cm^2) with approximately 30 µL being deposited on each electrode. The electrodes were then gently dried under a positive air-flow and stored at 4°C until use.

Bilirubin Oxidase/Laccase Activity Assays

The specific enzymatic activity of bilirubin oxidase and crude laccase was calculated using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the substrate, at room temperature and at either pH 6.5 (BOd) or 4.5 (laccase). Spectrophotometric analysis was performed on a Biochrom Instruments Libra S60 double-beam UV-Visible Spectrophotometer. Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system.

Stock solutions of ABTS (0.54 mM in water) and enzyme (0.1 mg/mL in water) were prepared, prior to use. 1.36 mL of citrate/phosphate buffer (50 mM), 12.5 µL of the enzyme solution and 0.125 mL of the ABTS solution were added to a cuvette. The solutions were mixed by inversion of the cuvette and the absorbance was recorded at a wavelength of 420 nm for 3 minutes. Control experiments consisted of substituting the 125 µL enzyme fraction with 12.5 µL of water.

For activity assays incorporating catalase (with an incubation time of 5 minutes), 0.125 mL of catalase (0.2 mg/mL in 50 mM citrate/phosphate buffer) replaced 0.125 mL of buffer, and was added at either t = 0 or t = 5 minutes (to allow compensation for the concomitant increase in dissolved O_2 concentration due to H_2O_2 decomposition by catalase).

Acknowledgements

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References

1. M. T. Meredith, M. Minson, D. Hickey, K. Artyushkova, D. T. Glatzhofer and S. D. Minteer, *ACS Catal.*, 2011, **1**, 1683-1690.