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SUPPORTING INFORMATION

A membraneless air-breathing hydrogen biofuel cell based on direct wiring of thermostable enzymes on carbon nanotube electrodes

Noémie Lalaoui,[a] Anne de Poulpiquet,[b] Raoudha Haddad,[a] Alan Le Goff,[a*] Michael Holzinger,[a] Sébastien Gounel,[c,d] Michel Mermoux,[e] N, Pascale Infossi,[b] Nicolas Mano,[c,d*] Elisabeth Lojou,[b*] and Serge Cosnier[a]

Experimental

Methods and instrumentation

Multi-walled carbon nanotubes (MWCNT) (9.5 nm diameter, purity >95%) and SWCNT (produced by HIPCO®, purified) were obtained from Nanocyl and Unidym Inc. respectively, and were used as received without any purification step. Sodium phosphate monobasic, sodium phosphate dibasic, 1-Methyl-2-pyrrolidinone (NMP), isopentyl nitrite (96%), 6-Amino-2-naphtoic acid were purchased from Sigma-Aldrich. All the reagents were used without further purification. All solvents were of analytical grade. Distilled water was passed through a Milli-Q water purification system. The bilirubin oxidase (BOD) from *B. pumilus* and hydrogenase from *A. aeolicus* (MbH1) were produced and purified as described earlier [26,28].

The electrochemical characterization of the bioelectrodes were carried out in a three-electrode electrochemical cell using a Biologic potentiostat. A platinum wire was used as the counter electrode and all potentials were referred to Ag/AgCl electrode. Unless otherwise specified, the experiments were conducted at 45°C in 0.2 mol.L^{-1} phosphate buffer solution (pH 7.2) as the supporting electrolyte. All currents are normalized with the geometric surface of the electrodes.

The morphology of the electrodes was investigated by SEM using an ULTRA 55 FESEM based on the GEMENI FESEM column with beam booster (Nanotechnology Systems Division, Carl Zeiss NTS GmbH, Germany) and tungsten gun. Raman spectra were recorded using a Renishaw inVia spectrometer. 3D and profile images were taken using a Keyence VK-X200 laser microscope.

Synthesis of naphtoic-acid-functionalized SWCNT (f-SWCNT) and MWCNT (f-MWCNT)

50 mg (4 mmol of carbon) of CNT were sonicated in dimethylformamide (DMF) (150 mL) during 30 min. The mixture was stirred under argon and heated to 80°C. Then 6-Amino-2-naphtoic acid (1.5 g, 8 mmol) and isopentyl nitrite (1.1 ml, 8 mmol) were added to the reaction medium which is left under argon and heated to 80°C overnight. The reaction mixture was filtered through 0.45 µm PTFE membrane filters (OMNIPORE membrane filters) and the obtained f-MWCNTs and f-SWCNTs were extensively washed with hot DMF, after vigorous sonication, next with acetone and finally dried under reduced pressure.

Solubility measurements: 3 mg of f-SWCNT and f-MWCNT were dispersed in 3mL of water using an ultrasonic bath for 5 min, followed by centrifugation for 15 min at 3200 rpm. 2 mL aliquot of each supernatant was taken and precipitated in acetone and filtered, and the filter cake was washed with acetone, dried, and weighed. The solubility of pristine MWCNT and SWCNT is taken as near zero, while the solubility of f-SWCNT and f-MWCNT is 12 and 37 µg mL^{-1} respectively.

Preparation of the air-breathing biocathode

The working electrodes were non-treated carbon cloth electrodes purchased from PAXITECH. NMP dispersions of MWCNT were prepared by 30 min sonication of 2.5 mg nanotubes in 1 ml NMP until homogenous black suspension was obtained. Then 20 µl of

the MWCNT solution was drop-casted on a carbon cloth electrode, and NMP was removed under vacuum leaving a homogenous MWCNT film. Electrodes were modified with BOD by drop-coating 10 µL of the enzyme solution (10 µM BOD in 50 mM phosphate buffer pH 7.5) on the pre-modified electrodes followed by drying at room temperature. The resulting electrodes were then washed with phosphate buffer solution before the electrochemical characterization to remove the non-adsorbed enzyme. The electrodes were then sealed in a cylindrical plastic chamber with a gasket joint allowing the electrodes to be in contact with both the electrolyte and air with an active surface of 0.07 cm^2. Both the hydrophobicity of carbon cloth and MWCNT film prevent from any electrolyte leakage.

Preparation of the bioanode

The deposition of f-MWCNT and f-SWCNT film was carried out by drop-coating of 20 µL of CNT dispersion in water on glassy carbon electrodes (3 mm diameter) followed by drying of the solvent under vacuum. To test the influence of the amount of the deposited carbon nanotubes on the performance of the anode, several layers of f-MWCNTs were elaborated by successive depositions, each followed by a drying step under vacuum. The modified electrodes were then incubated in 20 µL of *Aa* MbH1 (5 µM in phosphate buffer 200 mM pH 7.2) for 4 hours at 4°C. The resulting electrodes were then washed with phosphate buffer solution.

Characterization of the H_2/O_2 biofuel cell

Bioanode and biocathode were connected and investigated in phosphate buffer solution (pH 7.2) without adding any supplementary membrane. The anode was set as working electrode while the cathode was plugged as counter-reference electrode. Power curves were plotted from successive thirty seconds discharges at constant voltages. Measurements were performed under high purity hydrogen flow at 45°C.

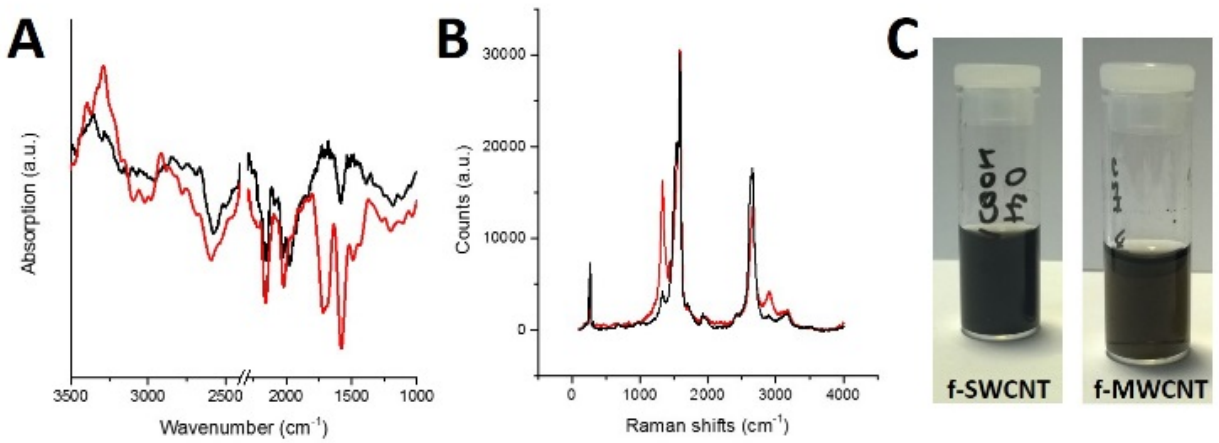

Figure S1. (A) Infra-Red (IR) spectrum for MWCNT (black) and f-MWCNT (red) between 1000 and 3500 cm^{-1}; (B) Raman spectrum of SWCNT (black) and f-SWCNT (red) between 0 and 4000cm^{-1}; (C) Solution of f-SWCNT and f-MWCNT obtained after 5-min ultrasonication followed by centrifugation for 15 min at 3200 rpm

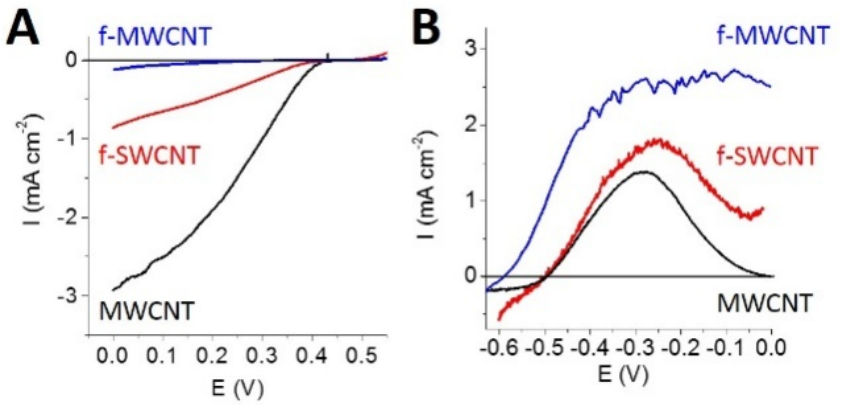

Figure S2. (A) CV for air-breathing electrode under air based on BOD adsorbed on MWCNT, f-SWCNT and f-MWCNT film (10 mV s^{-1}, deaerated phosphate buffer pH 7.2, 45°C, background was subtracted to remove capacitive current contribution) ; (B) CV for MbH1 adsorbed on MWCNT, f-SWCNT and f-MWCNT GC electrodes (v = 5 mV s^{-1}, pH 7.2, 60°C, under H_2, background was subtracted to remove capacitive current contribution)

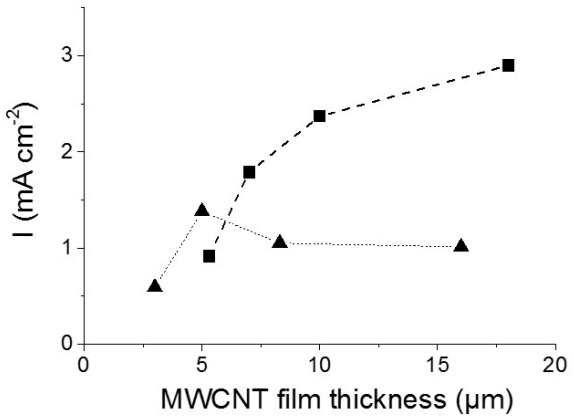

Figure S3. Maximum current densities for bioelectrocatalytic oxidation of H_2 towards CNT film thickness for (▲) MWCNT and (O) f-MWCNT GC modified electrodes (pH 7.2, 60°C, under H_2).