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ABSTRACT

We report the first example of an H$_2$/O$_2$ enzymatic fuel cell able to power a wireless transmission system. Oxygen-tolerant hydrogenase from *Aquifex aeolicus* and bilirubin oxidase from *Myrothecium verrucaria* were incorporated from diluted solutions in carbon felt-based material, allowing mediatorless catalytic currents more than 1 mA to be reached. The enzymatic fuel cell open circuit voltage was 1.12 V, and short circuit current was 767 μA. It delivered a maximum power of 410 μW, sufficient to power the electronic device that measured in real time the anodic/cathodic compartments and room temperatures, the voltage of the capacitor and voltage output of the enzymatic fuel cell itself. Notably, data were sent every 25 s during 7 hours of continuous operation which constitute the highest performances ever reported for a realistic environmental application fully powered with an enzymatic fuel cell.

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1. Introduction

Monitoring gas concentrations, pH, or temperature from industrial or homeland locations is a crucial environmental issue, which raises the question of the required sustainable power sources. Because they use totally biodegradable and bioavailable biocatalysts, enzymatic fuel cells (EFC) can be considered as sustainable energy sources for low-power-consuming applications, including wireless sensors [1]. Similarly to chemical fuel cells, EFC rejects no greenhouse gases. Moreover, the substrates are selectively transformed at high rates and with low overvoltages due to enzyme specificity.

Although many studies have been devoted to EFCs during the last 20 years [2–5], viable applications are rare because the current delivered at a required voltage is still too low for most electronic devices. Energy harvesters able to overcome the voltage issue were recently used in conjunction to carbohydrate/O$_2$ EFCs allowing wireless powering implantable medical devices [6,7]. One report targeted environmental measurements and showed that a sugar/O$_2$ EFC implanted in an orange was able to wirelessly transmit measurements every 20 min [8].

Replacing carbohydrates by hydrogen should in principle lead to more powerful EFCs. Actually, H$_2$/O$_2$ enzymatic fuel cells are a new generation of EFCs that have increasingly drawn researchers' attention [4], essentially because of the identification of O$_2$-tolerant hydrogenases [9–13], the key

enzyme for H$_2$ oxidation. Combined with nanomaterials-based 3D networks, H$_2$/O$_2$ EFCs delivering mW/cm^2 power densities were recently reported [14–17]. However, we also demonstrated that the enzymatic currents were limited by gas transport in the porous nanomaterials and by mechanical stability of the nanomaterials itself, thus limiting the current to a maximum value less than 300 μA [14].

In this paper, we push the limits of our device by using carbon felts modified with carbon nanotubes as host matrixes for both enzyme immobilization and electron collector. Electroactive surface can be easily changed while preserving high porosity for mass transport, and tuned with suitable chemical modifications. We show that currents are consequently increased to a value sufficient to power a wireless system composed of a microcontroller, electronic and radio transmission system.

2. Experimental

2.1. Chemicals and materials

All chemicals were used as received from Sigma. 50 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) pH 7.2 and 100 mM sodium phosphate pH 6 were used as buffers for hydrogenase and bilirubin oxidase, respectively. Carbon felt (CF) RVC 2000 (BET surface area 0.7 m^2/g) was purchased by Mersen. Multi-walled carbon nanotubes functionalized with naphtoic acid functions (CNT-COOH) were synthesized as in [18]. Solutions with a final concentration of 1 mg.mL^{-1} were sonicated until full dispersion. *Aquifex aeolicus* hydrogenase (Aa Hase) was purified as described in Luo et al. [11].

Myrothecium verracuria bilirubin oxidase (Mv BOD) was a gift from Amano Enzyme Inc. (Nagoya, Japan).

2.2. Instrumentation and measurement procedures

Electrochemical experiments were performed using a potentiostat from Autolab with Nova software. The Ag/AgCl (NaCl sat.) reference electrode was separated from the electrolyte using a side junction maintained at room temperature. The fuel cell performances were examined in gas-saturated solutions obtained from a continuous supply of pure H_2 and O_2 for anode and cathode, respectively. A Nafion® membrane (Nafion® 117 from DUPONT-USA) separated the compartments. 100 mM phosphate buffer pH 6 was chosen as the best electrolyte. Temperature was maintained at 25°C at the cathodic side to avoid Mv BOD denaturation and at 60°C at the anodic side to take profit of the hyperthermophilicity of Aa Hase. The polarization curves were obtained by recording the voltage/current responses at a scan rate of 3 mV.s^{-1} and through several external variable resistance loads. All measurements were performed on four different electrodes. Scanning electron microscopy (SEM) was performed with the high scanning resolution microscope JSM 6320F (JEOL FEGSEM).

2.3. Electrode preparation

A CF cylinder (diameter 5 mm, height 7 mm) was cut with a punch, which corresponds to an average mass of 13.70 ± 0.95 mg. Before use, the CFs were treated in 96% ethanol, rinsed with water, fully dried at 60°C, then soaked into 0.1 M HCl, and rinsed and dried again. Ready-to-use CFs were immersed in the CNT-COOH dispersion, sonicated for 30 min, and dried at 60°C. The electrical contact within the CF was achieved using a gold hook. Enzymes were incorporated by running CVs in diluted enzyme solutions (in the range 20–420 nM).

2.4. Wireless electronic device

To illustrate the usability of the EFC, a wireless system was built. It consists in a Zigbee remote device (Maxstream Xbee2.4) powered by the EFC through an energy harvester, built around a bq25504 from Texas Instrument. It acquires 6 voltages and periodically transmits them to the Zigbee device connected to a PC. A specific application was developed for the PC to store a timestamp and all 6 raw measurements for each sample sent by the remote device.

3. Results and discussion

3.1. Characterization of the CF materials

CNT-COOHs were chosen for CF modification because they were previously shown to be efficient for Aa Hase and Mv BOD immobilizations [18]. After deposition of CNT-COOHs, the CF surface is recovered by a dense carpet of nanotubes (Fig. 1A and B), resembling morphology of carbon composite fabricated through CVD procedure [19]. The double layer capacitance increased from 170 ± 20 µF to 800 ± 160 µF, suggesting that the surface area of CF/CNT-COOH was 5 times higher than unmodified CF. Taken a value of 10 µF.cm^{-2} for the carbon tissue [20,21], this leads to an electroactive surface area of 17 ± 2 and 80 ± 16 cm^2 for CF and CF/CNT-COOH, respectively. Based on the CF BET surface, this means that around 20% of the total surface is available.

3.2. Electrocatalytic reaction by enzymes in CF-based materials

Unlike most previous works, incorporation of enzymes in the carbon materials was successfully realized by cyclic voltametry in diluted enzyme solutions from 20 to 420 nM. We obtained direct electron transfer catalytic waves with catalytic currents reaching a limiting value after 20 min of cycling (from -0.65 to 0 V and from $+0.65$ to 0 V at 5 mVs^{-1} for Aa Hase and Mv BOD, respectively). Transfer of the electrodes to an enzyme-free solution maintained 80% of the initial catalytic current value.

Fig. 2A depicts the typical catalytic wave for H_2 oxidation obtained from 20 nM Aa Hase solution and measured in an enzyme-free electrolyte. The onset potential for H_2 oxidation is -550 mV in agreement with the expected potential using O_2-tolerant hydrogenases [9]. The catalytic current is notably around 30 times higher than at the enzyme-modified CF, thus further beyond the increase in surface area, showing that the naphtoate functions on CNTs allow more enzymes to be efficiently adsorbed. Chronoamperometry recorded at -300 mV highlighted the possibility of modulating the temperature between 30°C and 60°C
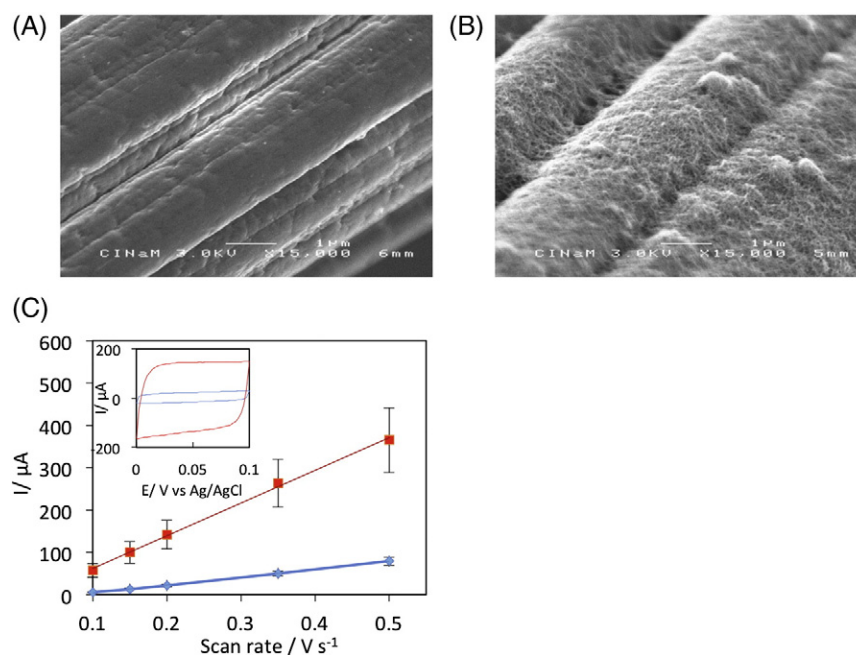

Fig. 1. SEM images of (A) CF, (B) CF/CNT-COOH; (C) Double layer charging current of CF (blue) and CF/CNT-COOH (red) as a function of scan rate. Inset: CV of CF (blue) and CF/CNT-COOH (red), $v = 200$ mV.s^{-1}, 1 M HEPES (pH 7.2).
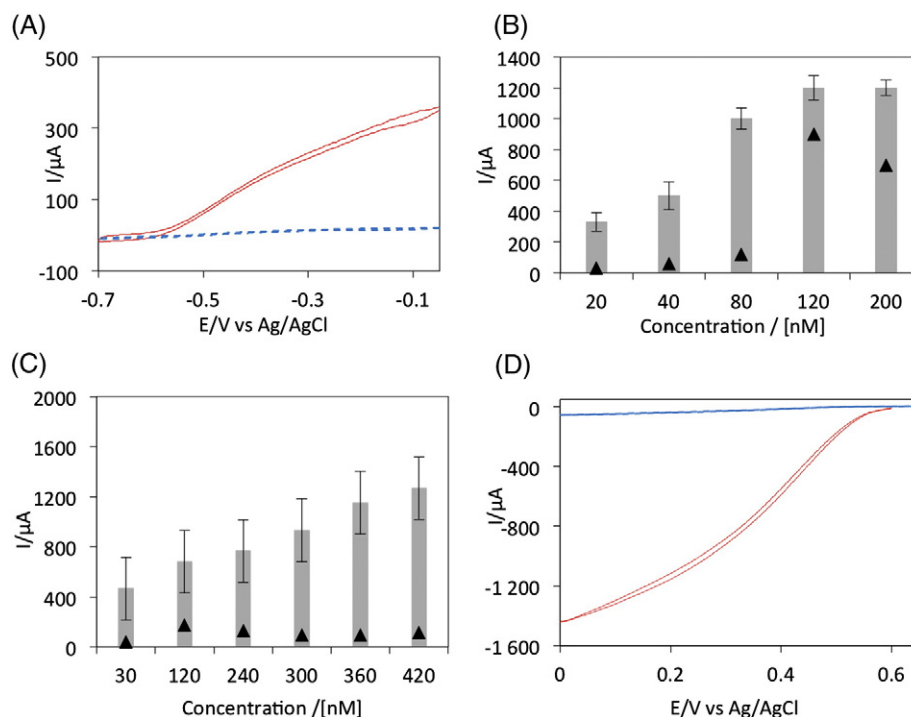
Fig. 2. (A) H_2 oxidation by premodified CF-CNTCOOH/Aa Hase 20 nM (red) and CF/Aa Hase 20 nM (blue) in an enzyme-free electrolyte. (B) Catalytic current dependence on Aa Hase concentrations in solution (grey bars), and remaining catalytic currents recorded with an enzyme-free CF in the pre-used solution (black triangles). (C) Catalytic current dependence on Mv BOD concentrations in solution (grey bars), and remaining catalytic currents recorded at an enzyme-free CF in the pre-used solution (black triangles). (D) O_2 reduction by premodified CF-CNTCOOH/Mv BOD 420 nM (red) and CF/Mv BOD 420 nM (blue) in an enzyme-free electrolyte; scan rate 5 $mV.s^{-1}$.

with less than 20% current loss over more than 1 hour. Increments of enzyme concentration lead to catalytic currents that consequently increase up to 1 mA of net current which is more than 3 times higher than the previous values obtained at carbon nanofiber- [14] or carbon nanotube-based [18,22] electrodes (Fig. 2B). The isotherm profile, however, suggests some saturation of the carbon material since the catalytic current stops increasing although there is enzyme still available in solution as attested by the remaining current recorded with a free-enzyme CF. A turnover frequency k_{cat} of 20 s^{-1} was calculated suggesting that a great amount of enzyme is not available for the catalysis. These interesting issues are currently studied in the laboratory.

The catalytic current for O_2 reduction also increases with increasing Mv BOD concentration in buffer reaching currents more than 1 mA (Fig. 2C). But contrary to Aa Hase, no saturation of the CF/CNT-COOH material is observed. Again, CNT-COOHs are beneficial for the electrocatalysis efficiency since the catalytic current is around 20 times higher when using CF/CNT-COOH rather than CF. The onset potential for O_2 reduction is + 580 mV in agreement with BOD connection through the T1 copper [23]. As expected for Mv BOD, chronoamperometry at + 300 mV revealed that for temperatures higher than 30 °C, progressive denaturation of the protein induced a severe loss in the catalytic current.

3.3. Biofuel cell performances and wireless powering

The performances of the EFC are depicted in Fig. 3A. The open circuit potential and short circuit current are 1.12 V and 765 µA, respectively. From the polarization curves, a maximum power of 410 ± 50 µW is measured. Taken into account the external surface of the carbon felt, the power density falls in the same range than our previous device based on carbon nanotubes deposited on a graphite electrode [24]. The net power was however limited to 20 µW. For another comparison, Xu et al. [17] reported a power of 610 µW using compacted mixtures of graphite and carbon nanotubes assembled in a membrane-less 80/20

H_2/O_2 fuel cell made of 2.5 cm^2 bioanode and 12.3 cm^2 biocathode. Control experiments without enzyme showed a maximum power of 0.18 µW, underlining the preponderance of the enzymatic contribution in the overall process. The EFC was also subjected to a series of external resistance loads (from 1 MΩ to 390 Ω). The internal resistance of about 800 Ω was thus deduced.

For the demonstrator, a highly energy-demanding Zigbee device has been intentionally selected in order to emphasize the good performances of our EFC. Indeed, the energy necessary to wake up the Zigbee device, make an acquisition, and transmit it is about 3 mJ, while the maximum instantaneous required power is about 100 mW (during transmission). Since the EFC is able to provide around 400 µW, the use of an energy harvester is required. This module will store the energy in a 22 mF capacitor, after boosting the voltage to a value usable by the Zigbee device (2.7 V). Once the harvester detects that it has stored enough energy for the process to occur (2.8 V threshold reached at the super-capacitor terminals), it turns on the Zigbee module which will wake up (20 ms cycle), make an acquisition (3 ms), and transmit it (about 17 ms). The device is then turned off after the super-capacitor voltage has reached 2.6 V leading to a delay of about 19 extra ms. Then the charging process continues up to the 2.8 V threshold is reached and a new cycle occurs. The estimated charges removed from the capacitor at each cycle correspond to a little more than 1 mC and the average recycling time is 25 s. The transmitted data are displayed and stored on a remote PC connected to a Zigbee central device.

As an illustration of the capabilities of the system, the EFC as well as the super-capacitor voltage are plotted, in addition to the ambient temperature and the temperature in both EFC compartments (Fig. 3B). The system held operational for more than 7 hours, providing 1032 packets of measurements. CV measurements at the end of the process showed that the bioanode kept 25% and the biocathode 65% from their initial values (data not shown). It is also noticeable that the interval between the measurements increased linearly during the whole process, most
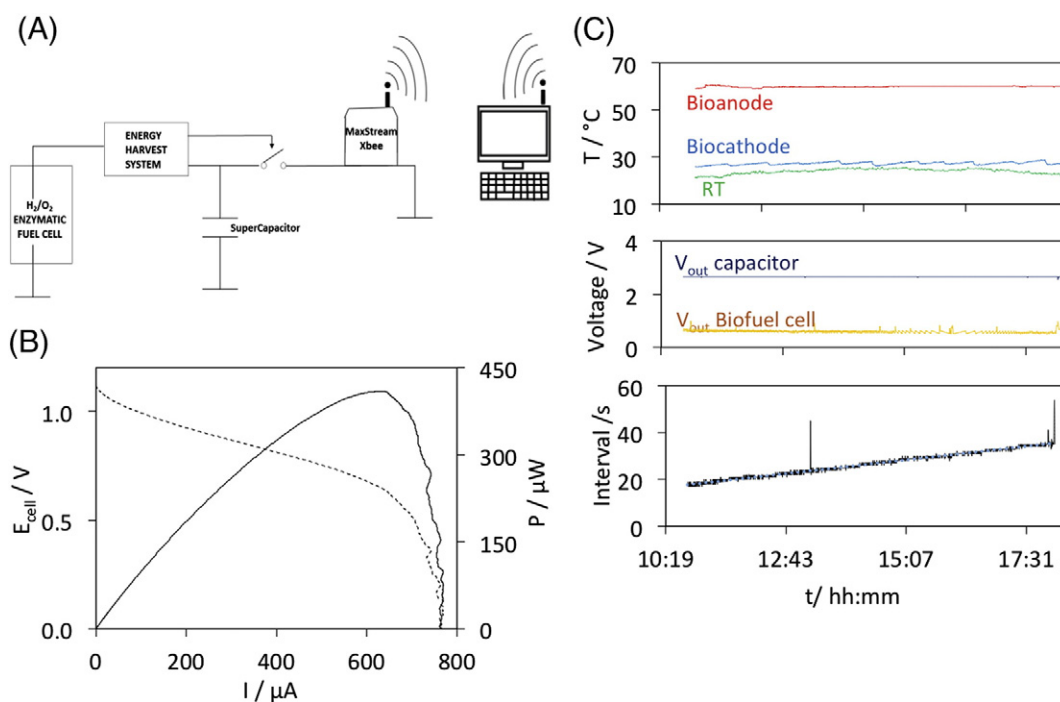
Fig. 3. (A) System block diagram of the electronic device. (B) H$_2$/O$_2$ EFC performances. (C) Wireless measurements: (up) Time series of the measurements, (down) evolution of the interval between the data transmissions during operation.

probably induced by progressive denaturation of the enzymes since no electroactive enzyme could be detected in solution [25].

4. Conclusion

This H$_2$/O$_2$ EFC is the first example of hydrogenase-based EFC able to power a wireless electronic system. The combination of new methods for redox enzymes loading onto surfaces and rational modification of materials provided robust bioelectrodes suitable for practical applications like environmental monitoring. The power harvested from the H$_2$/O$_2$ EFC was large enough to start and maintain a full electronic system (voltage amplifier, capacitor, temperature probes and wireless communication device) with an average of transfer information time of 25 s, for at least 7 hours of continuous operation, which constitute the highest performances ever reported for a realistic environmental application fully powered with an EFC. Design optimization to reduce the internal cell resistance, along with state-of-the-art wireless devices such as Bluetooth Low Energy that require less than 100 µJ, will open new opportunities for autonomous wireless sensing systems fully powered by EFC.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgments

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