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Short communication

A quinhydrone biofuel cell based on an enzyme-induced pH gradient

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ABSTRACT

We report on an alternative concept of biofuel cell functioning based on the unconventional use of enzymes to create a pH difference generating a potential difference between electrodes soaked in quinhydrone solutions. The electrode and quinhydrone solution were confined in a dialysis bag placed into a compartment containing either glucose oxidase and catalase for the biocathode or urease for the bioanode. In presence of 0.4 mol L^{-1} glucose and urea, the enzyme reactions generate a pH difference of 3.55, both compartments being separated by an agar–agar wall. The resulting biofuel cell exhibits an open-circuit voltage and maximum power of 208 mV and 30.6 μW, respectively, without immobilization and electrical connection of the involved enzymes. In addition, this biofuel cell was able to provide continuously10 μA during 23 h, producing 0.133 J and 0.828 C. A similar biofuel cell configuration based only on dialysis bags was also developed. A graphite disk electrode elaborated by mechanical compression of graphite particles and quinhydrone, was placed in a dialysis bag itself confined into another dialysis bag containing enzyme solution. The resulting power and open-circuit voltage at saturating substrate conditions are 7.6 μW and 157 mV, respectively.

1. Introduction

The ever-increasing depletion of fossil fuels and the need for clean methods of producing electricity have stimulated the emergence of new sources of sustainable and renewable energy without greenhouse gas emissions or environmental pollution. Among the clean alternative sources, the energy production thanks to electrochemical means is seriously considered. An attractive alternative lies in the development of biofuel cells that convert chemical energy into electrical energy, by reactions catalyzed by enzymes [1,2]. In particular, electricity is generated efficiently from the oxidation of saccharides (mainly glucose) coupled to the reduction of oxygen to water. The potential applications of biofuel cells extend from "green powering" of nomadic devices such as mobile phone or GPS to medical applications as implantable power sources for cardiac pacemakers, neurological stimulators, cochlear implants, drug micropumps, etc. [3]. A vast majority of these biofuel cells produce electrical energy from glucose and oxygen via enzymes electrically wired by redox mediators [4–11]. Conventional biofuel cells are based on enzymes and redox mediators immobilized on the electrodes, glucose being oxidised into gluconolactone and oxygen being reduced into water. Since the redox centers of enzymes are deeply embedded in the protein structure, preventing thus a direct electron transfer with the electrode, the electrons are conveyed from the prosthetic sites of enzymes to the electrodes and vice versa by the redox mediators. However, these biofuel cells suffer from various pitfalls leading to a low generated power and a weak stability over time. These limitations may be ascribed to the possible denaturation of enzymes during their immobilization and the poor efficiency of the enzyme connection. Moreover, the presence of oxygen constitutes a shortcut of the electron transfer between redox mediators and glucose oxidase at the bioanode.

In this context, we report here a new concept of biofuel cell based on the potential difference generated between redox species by enzymically induced pH changes. This biofuel cell functioning which does not require the immobilization and electrical connection of enzymes was illustrated with glucose oxidase and urease as pH modifiers for the cathode and anode, respectively. Quinhydrone, an equimolar mixture of quinone and hydroquinone, was chosen as pH-sensitive redox species to fix the electrode potential and exchange electrons. The performance of a biofuel cell based on the same redox species quinhydrone at the anode and cathode whose potentials depend on the pH were thus investigated.

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2. Materials and methods

2.1. Chemicals

Glucose oxidase (GOX) (EC 1.1.3.4 type VII, from *Aspergillus niger*, 179 U mg^{-1}), catalase (EC 1.11.1.6, from Bovine Liver, 1610 U mg^{-1}), urease (EC 3.5.1.5, type IX, from Jack Beans, 70.4 U mg^{-1}), quinhydrone (equimolar mixture of quinone and hydroquinone) and graphite particle were purchased from Sigma–Aldrich.

2.2. Instrumentation

Electrochemical experiments were performed using an Autolab potentiostat 100 (Eco Chemie, Utrecht, The Netherlands), using biocathode as the working electrode and bioanode as the counter electrode. Dialysis membranes were purchased from Spectrumlabs: Spectra/Por® Dialysis membrane, MWCO 6–8000 g mol^{-1}, flat width 32 mm, diameter 20.4 mm, vol/length 3.3 mL cm^{-1}; Spectra/Por® CE, cellulose ester membrane, MWCO 100 g mol^{-1}, flat width 31 mm, diameter 20 mm, vol/length 3.1 mL cm^{-1}.

2.3. Electrodes fabrication

The electrodes were constituted of cubic carbon felt (9 mm × 9 mm × 9 mm) supplied by The Carbone Lorraine. Quinhydrone electrodes were prepared as follows: graphite particles (350 mg), 170 mg of quinhydrone, 0.3 mL water and glycerol (50 μL) were thoroughly mixed in ceramic mortar. The resulting graphite–quinhydrone paste was pressed at 10,000 kg cm^{-2} to form a disk. The surface area and thickness of disk are 1.33 cm^2 and 0.1 cm, respectively. A platinum wire was fixed by a conductive carbon glue on one side of the disk, which was then covered by an insulating silicon film to reinforce the mechanical strength of the biocoating.

2.4. Demonstrator for quinhydrone pH-gradient biofuel cell

Both carbon felt electrodes were immersed in an aqueous 0.15 mol L^{-1} NaCl solution (1 mL) containing quinhydrone (3.2×10^{-3} mol L^{-1}) and enclosed in a dialysis membrane with a molecular weight cut-off of 100 g mol^{-1}. The biofuel cell was constituted by two compartments (6 mL each) separated by a hydrogel wall (agar–agar; thickness: 4 mm). The anode and the cathode bags containing carbon felt electrode and quinhydrone were immersed in 0.15 mol L^{-1} NaCl containing different concentrations of D-glucose and urea. In addition to the electrolyte, substrates and electrode bag, the bioanode compartment contained urease (57.4 mg) while the biocathode compartment contained GOX (22.5 mg) and catalase (3.8 mg). The electrochemical measurements of the biofuel cell performance were carried out after a 1-h waiting whatever the concentration of glucose and urea.

2.5. Compact pH biofuel cell

The quinhydrone electrodes obtained by mechanical compression were immersed in an aqueous 0.15 mol L^{-1} NaCl solution (1 mL) and enclosed in a dialysis membrane with a molecular weight cut-off of 100 g mol^{-1}. Each dialysis bag (anode and cathode) was placed in another dialysis bag with a molecular weight cut-off of 6–8000 g mol^{-1} containing 0.15 mol L^{-1} NaCl (2 mL) and urease (57.4 mg) for the anode or GOX (22.5 mg) and catalase (3.8 mg) for the cathode. The two resulting bags were soaked in 25 mL of 0.15 mol L^{-1} NaCl containing different concentrations of D-glucose and urea.

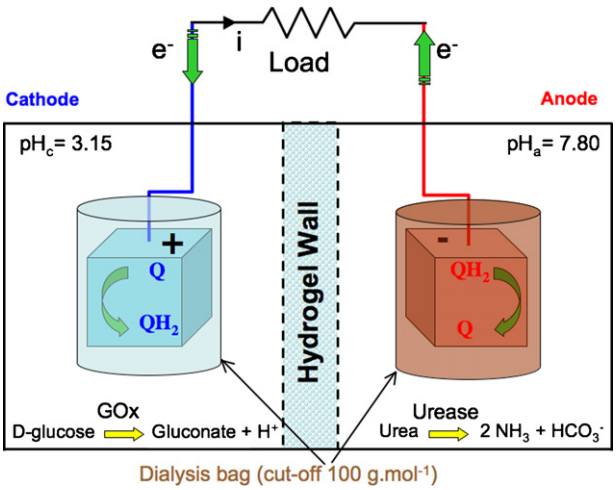

Fig. 1. Schematic representation of a "quinhydrone pH-based glucose and urea biofuel cell" in the demonstrator. Each electrode contains redox species, quinone (Q) and hydroquinone (QH_2) and is confined in a first dialysis bag (nominal cut-off of 100 g mol^{-1}). This bag separates the redox species from the enzymes contained in an anodic and a cathodic compartment. Their compartments contain GOX and catalase for the anode, and urease for the cathode. The driving force result from the enzymatic formation of a pH gradient. External dimensions: 50 mm × 48 mm × 38 mm.

2.6. Stability of the quinhydrone electrodes

The possible leakage of quinhydrone from the composite electrode over time was determined spectrophotometrically at 25 °C. A composite graphite electrode containing quinhydrone was soaked in an aqueous 0.15 mol L^{-1} NaCl solution (20 mL). Samples were taken every 20 min and the absorbance was monitored at 420 nm.

3. Results and discussion

3.1. Demonstrator for quinhydrone pH-gradient biofuel cell

The first realization of a "quinhydrone pH-based biofuel cell" was constituted by two compartments separated by a hydrogel wall (agar–agar). The electroactive part of the biofuel cell consists in carbon felt electrodes immersed in a cellulose acetate dialysis bag containing quinhydrone (3.2×10^{-3} mol L^{-1}) in an aqueous 0.15 mol L^{-1} NaCl solution (1 mL). The dialysis membrane with a molecular weight cut-off of 100 g mol^{-1} allows the permeation of hydroxide and hydronium ions but retains quinhydrone. Each bag is then immersed in a compartment containing GOX and catalase for the biocathode, and urease for the bioanode (Fig. 1).

In the cathodic compartment, glucose oxidase catalyzes the aerobic oxidation of glucose into gluconate and protons with the concomitant production of H_2O_2 inducing thus a decrease in the pH value. Catalase catalyzes the dismutation of the enzymically generated H_2O_2 into H_2O and O_2. The presence of catalase prevents thus the accumulation of H_2O_2 and hence the deactivation of GOX by high concentrations of H_2O_2.

In the presence of urea, urease catalyzes its degradation and generates hydroxyl ions increasing thus the pH in the bioanode compartment. As a consequence of the following enzymatic reactions, a pH gradient is biologically created:

$$\text{cathode :} \quad \text{glucose} + (1/2)O_2 \rightarrow \text{gluconate} + H^+$$

$$\text{anode :} \quad CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-$$

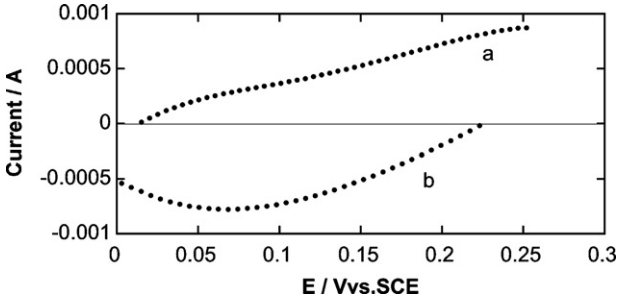

Fig. 2. Polarization curves of the carbon felt electrodes immersed in an aqueous 0.15 mol L^{-1} NaCl solution (1 mL) and enclosed in a dialysis membrane with a molecular weight cut-off of 100 g mol^{-1}. Each bag is then immersed in an aqueous 0.15 mol L^{-1} NaCl solution containing 5.0×10^{-3} mol L^{-1} D-glucose, 3.5×10^{-3} mol L^{-1} urea and (a) urease (bioanode) or (b) GOX and catalase (biocathode).

The electromotive force of the pH gradient-based biofuel cell is given by:

$$E_{th} = E_c - E_a = \frac{RT \ln 10}{F}\left(pH_a - pH_c + \frac{1}{2}\log\left(\frac{[Q]_c[QH_2]_a}{[QH_2]_c[Q]_a}\right)\right)$$

where subscript "a" identifies anode pH and concentrations, subscript "c" corresponding to the cathode, Q and QH_2 representing quinone and hydroquinone, respectively.

For identical concentrations of the redox species, the difference in pH provides a potential difference between the cathode and the anode. According to the Nernst equation, the gradient of pH between the two electrodes, which contain the same pH-sensitive redox couple, modifies the electrical potential of each electrode and generates electron exchanges. The pH gradient-based BFC will discharge according to the following reactions:

cathode : $Q + 2H^+ + 2e^- \rightarrow QH_2$

anode : $QH_2 \rightarrow Q + 2H^+ + 2e^-$

Fig. 2 shows, the polarization curves of the bioanode and biocathode, recorded separately, at 25 °C in an aqueous 0.15 mol L^{-1} NaCl solution containing 5.0×10^{-3} mol L^{-1} D-glucose and 3.5×10^{-3} mol L^{-1} urea. After an incubation period of 9 h, the enzymatic reaction catalyzed by glucose oxidase leads to a pH stabilization at 3.15 in the biocathode compartment. Thus, it appears that the reduction of quinone starts at +223 mV vs. SCE. In contrast, the urease reaction provides an increase of pH until 7.80 in the bioanode compartment while the oxidation of hydroquinone starts at +12 mV. The recorded difference of pH between the two compartments of the biofuel cell (ΔpH = 4.65) should generate a potential difference of 275 mV. It appears that both polarization curves exhibit an open-circuit potential of 210 mV in good agreement with the theoretical value. Moreover, the presence of a semi-plateau on each polarization curve seems to indicate that the reaction mechanisms are controlled by the mass transport diffusion.

The performance of the resulting assembled glucose/urea biofuel cell was investigated for various concentrations of enzyme substrates, namely, glucose and urea. Fig. 3A depicts the power of the biofuel cell as a function of the operating cell voltage for the different substrate conditions. At physiological concentration values of D-glucose and urea, namely 5.0 and 3.5×10^{-3} mol L^{-1}, respectively, the maximum power is 1.04 μW (at 38 mV) and the open-circuit potential value is 73 mV, the biofuel cell exhibiting a ΔpH of 2.94. By doubling the substrate concentration, an improvement of the biofuel cell performance was achieved: the maximum power and the open-circuit potential values reaching 5.03 μW at 65 and 112 mV (ΔpH of 3.07), respectively. At saturating substrate conditions (0.4 mol L^{-1}), the strong enhancement of the maximum power (30.6 μW at 133 mV) and open-circuit potential (208 mV–ΔpH of 3.55) corroborates the beneficial influence of the increase in concentration of glucose and urea on the biofuel cell characteristics. It should be noted that the performances of three different biofuel cells led to a relative standard deviation (RSD) of 4% illustrating the reproducibility of the fabrication process.

The operational stability of the biofuel cell was investigated for a discharge at constant current (10 μA) for several hours in the presence of 0.4 mol L^{-1} urea and glucose. A weak decrease in potential was recorded for the first hour (16 mV h^{-1}) leading to a decrease in the cell power to ca. 8% of the initial value over 1 h. (Fig. 3B). A pseudo-stabilization of the potential was observed after 15 h (slope of 2 mV h^{-1}), the cell voltage being 107 mV after 23 h. This experiment confirms the capacity of this pH-based biofuel cell to operate continuously delivering 10 μA during 23 h, the energy and charge produced by the biofuel cell being 0.133 J and 0.828 C, respectively.

3.2. Development of a compact quinhydrone pH-gradient biofuel cell

With the aim to design a softer device and to reduce its size, dialysis bags with a molecular weight cut-off of 6–8000 g mol^{-1} replace the solid compartments containing the enzyme and its substrate. The electroactive part of the biofuel cell based on a cubic carbon felt electrode soaked into an aqueous solution of quinhydrone was also replaced by a solid composite electrode. The latter were prepared by the easy mechanical compression of a graphite–quinhydrone paste. To investigate the mechanical stability of these redox disk electrodes, the release of quinhydrone was examined by spectrophotometry. It appears that only 0.8% of the initially immobilized amount of quinhydrone was released in solution after 3 h. The good stability of the incorporated redox species may be ascribed to specific π interactions between graphite and quinhydrone complexes.

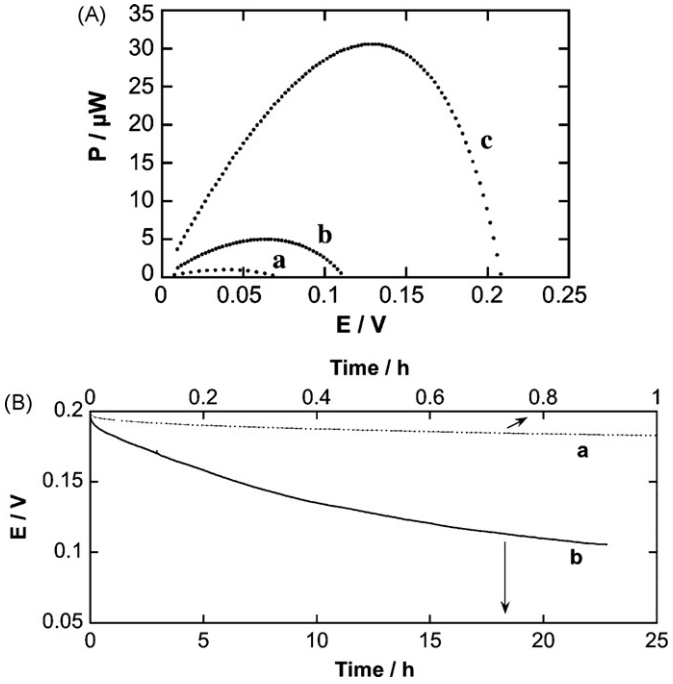

Fig. 3. (A) Dependence of the power output on the operating cell voltage plot for the quinhydrone pH-based biofuel cell in presence of different concentrations of glucose and urea: (a) 5.0 and 3.5×10^{-3} mol L^{-1}, (b) 10 and 7.0×10^{-3} mol L^{-1}, (c) 0.4 and 0.4 mol L^{-1}, respectively. (B) Discharge curve for the quinhydrone pH-based biofuel cell at constant current (10 μA) in presence of 0.4 mol L^{-1} glucose and urea: (a) first hour, (b) 23 h.

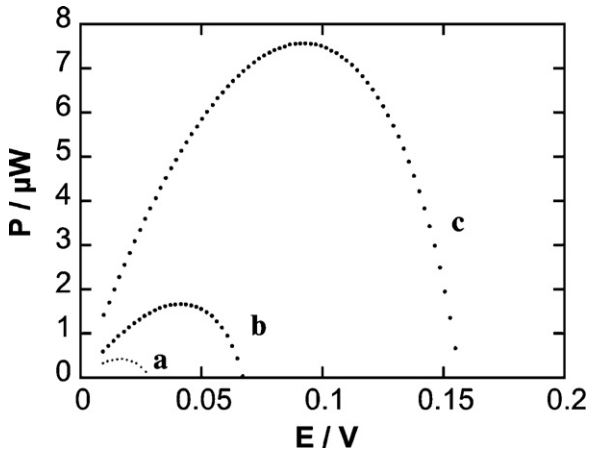

Fig. 4. Dependence of the power output on the operating cell voltage plot for the compact quinhydrone pH-based biofuel cell in presence of different concentrations of D-glucose and urea: (a) 5.0 and 3.5×10^{-3} mol L^{-1}, (b) 10 and 7.0×10^{-3} mol L^{-1}, (c) 0.4 and 0.4 mol L^{-1}, respectively.

Nevertheless, the resulting disk electrodes were inserted in a dialysis bag with a nominal cut-off of 100 g mol^{-1}, to prevent diffusion of quinhydrone. After the insertion of each bag in a second dialysis bag containing GOX and catalase for the bioanode, and urease for the biocathode, the two bags were immersed in the electrolyte 0.15 mol L^{-1} NaCl solution containing different concentrations of D-glucose and urea. Fig. 4 shows the power versus the cell voltage potential of the compact pH biofuel cell for different concentrations of D-glucose and urea. As previously observed with the demonstrator, the power and open-circuit voltage increase with the increase in concentration of glucose and urea. At saturating substrate conditions, the optimum performances are 7.6 μW at 93 and 157 mV for the maximum power and the open-circuit voltage, respectively (RSD for three different biofuel cells: 6%).

The operational stability of this soft biofuel cell was also evaluated. A discharge of 10 μA was achieved for more than 10 h; the energy and charge produced by the biofuel cell being 0.022 J and 0.36 C, respectively. These characteristics are inferior to those recorded with the demonstrator (0.133 J and 0.828 C) due to the absence of a separation by an agar–agar wall between both bioelectrodes that facilitates the establishment of a pH difference. Nevertheless, these results demonstrate the possibility to develop a pH biofuel cell based on enzyme confinements by dialysis bags.

4. Conclusions

In this preliminary study, we have demonstrated the possibility to elaborate a biofuel cell using enzyme reactions to create a difference of pH instead to transfer electrons. This enzymically induced potential difference combined with redox couples whose potential are dependent on the pH is at the origin of biofuel cell operation. In contrast to the majority of biofuel cell, such pH-based biofuel cell does not require the immobilization and electrical wiring of enzymes. Therefore, the enzyme part of the cell can be easily replaced improving thus the lifetime of the biofuel cell. Moreover, the present work reports on the innovative production of biofuel cell associating dialysis bags and redox disk electrodes obtained by mechanical compression of a mixture of graphite particles and redox mediator. It is expected that this new functioning principle of biofuel cell will be helpful for the development of a new generation of enzyme fuel cells.

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References

[1] J.A. Cracknell, K.A. Vincent, F.A. Armstrong, Chem. Rev. 108 (2008) 2439–2461.
[2] M.J. Moehlenbrock, S.D. Minteer, Chem. Soc. Rev. 37 (2008) 1188–1196.
[3] P. Cinquin, C. Gondran, F. Giroud, S. Mazabrard, A. Pellissier, F. Boucher, J.-P. Alcaraz, K. Gorgy, F. Lenouvel, S. Mathé, P. Porcu, S. Cosnier, PLoS One, 5 (5) (2010) e10476. http://dx.plos.org/10.1371/journal.pone.0010476.
[4] K. Szot, J. Niedziolka, J. Rogalski, F. Marken, M. Opallo, J. Electroanal. Chem. 612 (2008) 1–8.
[5] S. Boland, P. Jenkins, P. Kavanagh, D. Leech, J. Electroanal. Chem. 626 (2009) 111–115.
[6] S. Fishilevich, L. Amir, Y. Fridman, A. Aharoni, L. Alfonta, J. Am. Chem. Soc. 131 (2009) 12052–12053.
[7] G. Merle, A. Habrioux, K. Servat, M. Rolland, C. Innocent, K.B. Kokoh, S. Tingry, Electrochim. Acta 54 (2009) 2998–3003.
[8] I. Willner, Y.-M. Yan, B. Willner, R. Tel-Vered, Fuel Cells 09 (2009) 7–24.
[9] M. Zhou, Y. Du, C. Chen, B. Li, D. Wen, S. Dong, E. Wang, J. Am. Chem. Soc. 132 (2010) 2172–2174.
[10] Y. Tan, W. Deng, Y. Li, Z. Huang, Y. Meng, Q. Xie, M. Ma, S. Yao, J. Phys. Chem. B 114 (2010) 5016–5024.
[11] F. Gao, L. Viry, M. Maugey, P. Poulin, N. Mano, Nat. Commun. (2010), doi:10.1038/ncomms1000.