Bioelectrochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Biosupercapacitors for powering oxygen sensing devices

Michal Kizling [a], Sylwia Draminska [a], Krzysztof Stolarczyk [a], Petter Tammela [b], Zhaohui Wang [c], Leif Nyholm [c], Renata Bilewicz [a,*]

[a] Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
[b] Nanotechnology and Functional Materials, Department of Engineering — The Ångström Laboratory, Uppsala University, Box 534, 751 21 Uppsala, Sweden
[c] Department of Chemistry — The Ångström Laboratory, Uppsala University, Box 538, 751 21 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 6 December 2014
Received in revised form 13 April 2015
Accepted 19 April 2015
Available online xxxx

Keywords:
Supercapacitor
Biofuel cell
Laccase
Fructose dehydrogenase
Polypyrrole
Nanocellulose
Oxygen biosensor

ABSTRACT

A biofuel cell comprising electrodes based on supercapacitive materials — carbon nanotubes and nanocellulose/polypyrrole composite was utilized to power an oxygen biosensor. Laccase *Trametes versicolor*, immobilized on naphthylated multi walled carbon nanotubes, and fructose dehydrogenase, adsorbed on a porous polypyrrole matrix, were used as the cathode and anode bioelectrocatalysts, respectively. The nanomaterials employed as the supports for the enzymes increased the surface area of the electrodes and provide direct contact with the active sites of the enzymes. The anode modified with the conducting polymer layer exhibited significant pseudocapacitive properties providing superior performance also in the high energy mode, e.g., when switching on/off the powered device. Three air–fructose biofuel cells connected in a series converted chemical energy into electrical giving 2 mW power and open circuit potential of 2 V. The biofuel cell system was tested under various externally applied resistances and used as a powering unit for a laboratory designed two-electrode minipotentiostat and a laccase based sensor for oxygen sensing. Best results in terms of long time measurement of oxygen levels were obtained in the pulse mode — 45 s for measurement and 15 min for self-recharging of the powering unit.

1. Introduction

The increasing industrialization and associated pollution of the environment call for fast and cost-effective analytical techniques to be used in different monitoring programs. The need for specific systems and tools for environmental applications, in particular for environmental monitoring and medical use, has encouraged the development of new technologies and more suitable methodologies [1–5]. In this context, electrochemical biosensors and biofuel cells based on redox enzymes have emerged as suitable alternatives or complementary analytical tools.

The procedure of enzyme immobilization is an important aspect which needs to be considered when trying to enhance the overall operational performance of biosensors and biofuel cells [6–18]. Multicopper oxidases such as laccase or bilirubin oxidase have been intensively investigated as bioelectrocatalysts of oxygen reduction to water. A range of methods of binding enzymes by means of carbon nanotubes have been proposed [17–21]. Pyrene functionalization of multi walled carbon nanotube (MWCNT) for oriented immobilization of laccases led to high-performance biocathodes for oxygen reduction exhibiting a maximum current densities over 1 mA cm^{-2} [21]. We have also recently shown that covalent modification of single wall carbon nanotubes with different analogs of laccase natural substrates (such as syringic or veratric acid) can lead to significantly enhanced electrocatalytic reduction of oxygen [22].

Efficient power generating devices based on enzymatic catalysis is a rapidly developing research area and one of the significant difficulties to be overcome is the rapid voltage drop often seen when turning the powered devices on. As the latter is associated with the power limitations of the systems development of devices also containing capacitive components has recently been suggested [23]. The latter types of systems are of particular importance when high power has to be delivered or stored within a very short time. Skunik-Nuckowska et al. [24] thus demonstrated that a more stable power output could be obtained by connecting the power biofuel cell to a supercapacitor. The combination of catalytic properties of enzymes with a charge storing matrix to obtain efficient powering system has consequently attracted a large interest during the last few years [24]. Electrochemical supercapacitors can be seen as the bridge between batteries and classic capacitors due to their high energy densities and ability to undergo rapid charge and discharge [25,26]. Pankratov et al. [27] described a self-charging device consisting of bioelectrodes comprising gold electrodes connected to a catalytic and capacitive system consisting of carbon nanotubes and conducting polymer. The device gave rise to a stable power output for charge/discharge cycles and was, therefore, considered an efficient power source for pulsed current generation [27].

* Corresponding author at: Faculty of Chemistry, University of Warsaw, ul. Pasteura 1, 02093 Warsaw, Poland.
E-mail address: bilewicz@chem.uw.edu.pl (R. Bilewicz).

http://dx.doi.org/10.1016/j.bioelechem.2015.04.012
1567-5394/

In a recent communication we presented a pseudocapacitive polypyrrole-nanocellulose composite useful for the anode of a sugar–air enzymatic fuel cell [28]. The combination of the fructose dehydrogenase direct electron transfer-type bioelectrocatalytic system with this paper-based supercapacitor-like composite resulted in unique anode performance and durability without the need of using mediators. We likewise proved that high specific capacitance can prevent rapid potential loss during the operation of the fuel cell.

Especially for medical applications, a vital issue is to provide a stable power source which ideally should not be an enclosed battery since this prevents straightforward miniaturization and also limits the lifetime as the battery would need to be eventually replaced. Amsel et al. [29] recently designed a prototype self-powered light therapeutic device to be implanted inside a blood vessel to perform blood irradiation therapy which was powered by capturing the energy associated with the hydraulic movement in the blood flow. Borton et al. [30] designed and implanted a wireless neural recording device housed in a titanium enclosure powered by a Li-ion battery, which could be recharged via an inductive transcutaneous power link. Furthermore, an implantable wireless blood flow sensor, powered through an inductive link, was described by Cheong et al. [31]. Recently Falk at al. [32] presented a device including a wireless electronic unit, radio transmitter and a separate sensing bioelectrode for oxygen and carbohydrate determination. The system was supplied with electrical energy from an enzymatic fuel cell based on bilirubin oxidase and cellobiose dehydrogenase. A wireless electronic unit, consisting of a micropotentiostat, an energy harvesting module and a radio microchip was employed to enable sensing of lactose.

In this paper, we describe a biofuel cell used for powering an oxygen biosensor as a perspective prototype for medical or environmental usage. The power generation was achieved by employing fructose dehydrogenase based fructose oxidation and laccase for dioxygen reduction to water. Fructose dehydrogenase from *Gluconobacter* sp. the membrane-bound enzyme was selected since it shows high DET type bioelectrocatalytic activity [33–38] and undergoes stable adsorption on pseudocapacitive polypyrrole–nanocellulose composites [28, 39–41]. The matrices used for enzyme immobilization enabled charge accumulation which improves the long-term performance of the biofuel cell (BFC). In order to be able to utilize the BFC as a power supply for sensors, a suitable minipotentiostat with appropriate current sensitivity was designed to take apply potential and provide the analytical signal from the biosensor. The operation of the developed biosensor was investigated in buffers containing various concentrations of oxygen to assess the applicability of the biosensor-biofuel cell integrated device.

2. Experimental

2.1. Materials and chemicals

Citric acid ($C_6H_8O_7$), disodium hydrogen phosphate (Na_2HPO_4), ethanol (C_2H_5OH) and fructose ($C_6H_{12}O_6$) were purchased from POCh. Laccase *Trametes versicolor* was obtained from the Sigma Aldrich while multi-walled carbon nanotubes (MWCNTs) were purchased from Nanocyl (Belgium) and modified with naphthalene groups as previously described [42]. Toray teflon treated carbon paper (CP) (i.e., TGP–H–120 Fuel Cell Store) was used for the electrode preparation. The inorganic reagents from POCh (Gliwice, Poland) and the organic reagents from Aldrich were used without further purification. The water was distilled and had passed through a Milli-Q purification system.

Fructose dehydrogenase (FDH) from *Gluconobacter* sp. was purchased from Sorachim whereas Laccase *Trametes versicolor* was obtained from Sigma Aldrich (activity ≥ 10U/mg). The enzymes were used without further purification.

Pyrrole (Merck), $FeCl_3 \cdot 6\,H_2O$ (BDH Prolabo), Tween-80 (Merck), 37% HCl (Merck), and NaCl (BDH Prolabo) were used as received and were mixed with deionized water to the desired concentrations. The *Cladophora* sp. algae were collected, and the cellulose was prepared as previously described [19,32–34].

2.2. Material preparation and characterization

The PPy/cellulose composite has been thoroughly characterized in our previous work [39–41]. In the cellulose/polypyrrole composite (CCPPy) preparation, a dispersion of cellulose was prepared by ultrasonication (VibraCell 750 W, Sonics, U.S.) of 300 mg cellulose disposed in 60 ml of deionized water. 1.5 ml of pyrrole and a drop Tween-80 were dissolved in 50 ml of 0.5 M HCl and mixed with the cellulose dispersion. 12.857 g of $FeCl_3 \cdot 6\,H_2O$ was dissolved in 100 ml 0.5 M HCl. To start the polymerization the $FeCl_3$ solution was added drop-wise to the mixture of pyrrole and cellulose. The polymerization was allowed to proceed for 30 min under stirring, after which the product was collected in a Büchner funnel and washed with 5 l of 0.5 M HCl followed by 1 l of 0.1 M NaCl. The collected composite was dried under ambient conditions.

To obtain the bioanode for the biofuel cell, the composite was mixed with acetylene black in proportion 95:5 in an agate mortar. Electrode was processed as cylindrical sheets made of carbon paper modified with 10 mg of electrode material mentioned above under reduced pressure. A composite suspension was obtained by mixing 10 mg electrode material with 5 ml ethanol and further sonification. After drying, 150 μl of FDH solution containing 20 mg ml^{-1} of enzyme was applied and the electrode was kept in a fridge overnight to allow the evaporation of the ethanol.

For cathode preparation, CP was modified with MWCNTs under reduced pressure. A MWCNTs suspension was obtained by adding 8 mg nanotubes to 12 ml ethanol (4 ml of suspension for 3 CP electrodes). Subsequently 1 ml of naphthylated MWCNT was adsorbed on the CP surface by the same technique. The electrode areas were 3.14 cm^2. Laccase was physically adsorbed on the modified CP from 9 ml of McIlvaine buffer solution. After one day of laccase adsorption from a solution containing 24 mg ml^{-1} of laccase, the electrode was washed thoroughly with water and used as biocathode in the biofuel cell.

The fuel cell contained three parts: a cathode, an anode and a reaction compartment which ensured the presence of a flow of electrolyte between the electrodes. The distance between electrodes was 5 mm and the electrode contacts were made from a glassy carbon material. A pH 5.3 McIlvaine buffer solution suitable for the work with the laccase and FDH based electrodes was prepared by mixing 0.1 M citric acid and 0.2 M disodium phosphate. This solution was used in as the electrolyte in the fuel cell. The open circuit voltage (OCV) was measured in all experiments and both electrodes had an area of 3.14 cm^2.

The O_2 sensing electrode was prepared by adsorbing 1 μL of naphthylated MWCNT suspension on the surface of a glassy carbon electrode. After drying, the electrode was kept in 24 mg ml^{-1} laccase solution overnight.

The voltage between the anode and cathode was measured under loads varying from 1 kΩ to 10 MΩ. To minimize power losses due the fuel depletion, the duration of each measurement was generally restricted to 5 s after the application of each load. To investigate the dependence of the responses of the bioelectrodes on time, measurements were, however, also carried out 60 s after the application of each resistance. All measurements were carried out in flowing solutions, flow rate 20 ml/min.

The catalytic performances of the bioelectrodes and the sensing electrode were evaluated employing cyclic voltammetry using a three-electrode arrangement comprising an Ag/AgCl (KCl sat.) reference electrode, a platinum foil counter electrode and the bioelectrode/sensing electrode as the working electrode. All electrochemical experiments were carried out using an Electrochemical Analyzer CHI 400 B potentiostat at 22 ± 2 °C,

The determinations of the capacitances were performed in a pH 5.3 McIlvaine buffer solution using galvanostatic and cyclic voltammetric

Please cite this article as: M. Kizling, et al., Biosupercapacitors for powering oxygen sensing devices, Bioelectrochemistry (2015), http://dx.doi.org/10.1016/j.bioelechem.2015.04.012

experiments. In the galvanostatic charge/discharge cycling, the current density ranged from 100 to 5000 mA g^{-1} and the capacitance was estimated based on the discharge time only. The cyclic voltammetry experiments were carried out using scan rates, υ from 1 to 100 mV s^{-1} and the capacitance was calculated from the obtained pseudocapacitive current based on equation $C = i\upsilon^{-1}$ In the galvanostatic case the equation $C = it\mathrm{dU}^{-1}$ was used (t is the discharge time in the range of voltage dU) [43]. The reported specific capacitances have been normalized with respect to the mass of the active material present on one electrode. The total mass of each electrode was close to 60 mg, mass of active material on anode was 10 mg, on cathode 4 mg. Amount of material was limited by mechanical stability of electrodes during experiments.

The chronoamperometric (CA) experiments performed during the oxygen sensing were carried out in a pH 5.3 McIlvaine buffer using a hand-held minipotentiostat and a two-electrode configuration. An analogous set-up including an Electrochemical Analyzer CHI 400 B instrument was, however, also utilized for comparison. The hand-held minipotentiostat was custom-built by Dr. S. Kalinowski (Warmia and Mazury University in Olsztyn, Department of Chemistry, Poland). As the working electrode substrate a glassy carbon (GC) electrode with an area of 0.008 cm^2 was used while an Ag/AgCl (KCl sat.) electrode was the reference electrode.

Scheme 1 shows the general scheme of the minipotentiostat. Since the latter was supposed to be powered by a single biobattery (with a cell voltage of about 1.6 V), the power supply part within the minipotentiostat contains a DC–DC step-up converter, generating a 4 V DC voltage. This was sufficient to power most of the electronics within this potentiostat, including the current measuring device, the LCD display and the potential control. This scheme in Fig. 1 also contains a double layer supercapacitor Cellergy (50 mF) permanently coupled in parallel to the biobattery as a power back-up, and a switch to close and open the circuitry. In the "open" state, the biofuel cell battery can recharge (due to internal capacitance) itself and the external supercapacitor between the measurements. In the closed position, both the fuel cell and the external supercapacitor power the minipotentiostat for a time span sufficient for the amperometric detection of the analyte (i.e., oxygen). External supercapacitor was used as a power back-up, to prevail depletion of biofuel cell.

3. Results and discussion

3.1. Catalytic activity studies

To study the performance of the enzyme modified electrodes, cyclic voltammetric experiments were performed for each electrode (Fig. 1).

As already described both electrodes were based on carbon paper disks covered with the specific electrode material, i.e., CCPPy with adsorbed FDH for the anode (Fig. 1A) and naphthylated MWCNT with adsorbed laccase for the cathode (Fig. 1B). The electrochemical experiments were carried out in the absence and presence of 100 mM fructose in argon or oxygen saturated solutions.

In the absence of fructose (Fig. 1A) a peak at a potential of about 0.2 V (vs. Ag/AgCl (1 M KCl_{aq})) could be seen due to the oxidation of the polypyrrole. In the presence of 100 mM fructose, the onset of a catalytic fructose oxidation curve was, on the other hand, observed at about −0.15 V yielding a maximum catalytic current plateau of 17.5 mA. These data indicate that the FDH was involved in a direct electron transfer as no mediators were present. In this case, the CCPPy composite also serves as a conductive matrix with a large surface area which probably facilitates the attainment of a proper enzyme orientation as well as sufficient substrate mass transport rates. From the cyclic voltammograms the capacitance of the composite was found to be 0.6 F which means that it can store a significant amount of charge.

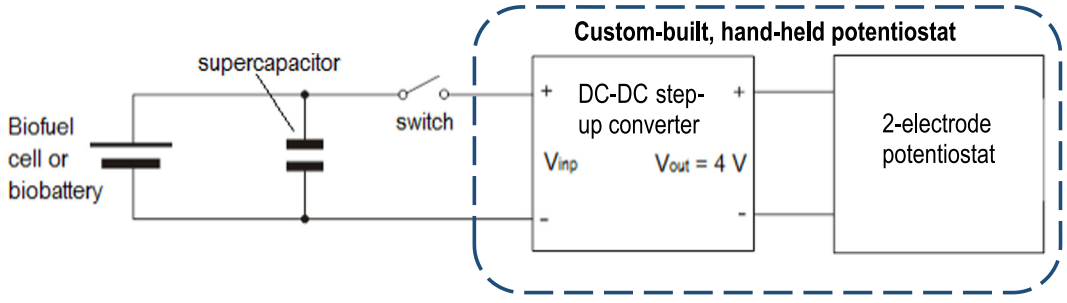
When the buffer solution was saturated with oxygen, catalytic oxygen reduction curves were observed for the negative electrode demonstrating the activity of the adsorbed laccase. The catalytic wave for the 4-electron direct reduction of oxygen to water appeared at the potential of 0.6 V in the absence of any mediators. Naphthylated multiwalled carbon nanotubes give rise to significantly larger oxygen reduction currents than unmodified MWCNTs or naphthylated SWCNTs. The naphthyl groups are hydrophobic and possess conjugated double bonds and are therefore able to get closer to the hydrophobic pocket of the enzyme which gives a more efficient contact with the active sites of the laccase. For the cathode covered with naphthylated MWCNTs, the background current at 0.2 V was ca. −200 μA (Fig. 1) while the catalytic reduction current was −938 μA (capacitance reached 0.05 F). Even though capacitance of cathode was significantly lower than that of the anode, we found it necessary to examine it thoroughly.

3.2. Capacitance studies

To check the charge accumulation ability, the biofuel cell was tested as a supercapacitor and its specific capacitance was measured. The experiments were performed in a two-electrode arrangement in a one-compartment vessel as described above. The voltammograms, which are shown in Fig. 2A, were in all cases symmetric and rectangular, particularly at low scan rates, indicating that the device behaved as a supercapacitor.

Although, the shapes of the voltammograms obtained at higher scan rates deviated from the rectangular shape as a result of an RC time constant effect it is still evident that the device could be reversibly charged and discharged even at relatively high scan rates, e.g., 20 mV s^{-1}.

The charge–discharge (CD) characteristic of the anode composite is shown in Fig. 2B, for various current densities. The CD curves deviate slightly from the expected linear shapes during the charging for each current density. For current densities higher than 1 A g^{-1} the iR drops on electrodes were significant (0.25 V) and caused significant decrease of capacitance. The device was also studied using one hundred charge



Scheme 1. Scheme of the power source system comprising a biofuel cell connected to a custom-built minipotentiostat containing a DC–DC step-up converter. The latter converts the potential of a single biobattery (V_{inp}) to a 4 V output potential (V_{out}) needed to drive the two-electrode sensing potentiostat.

Please cite this article as: M. Kizling, et al., Biosupercapacitors for powering oxygen sensing devices, Bioelectrochemistry (2015), http://dx.doi.org/10.1016/j.bioelechem.2015.04.012

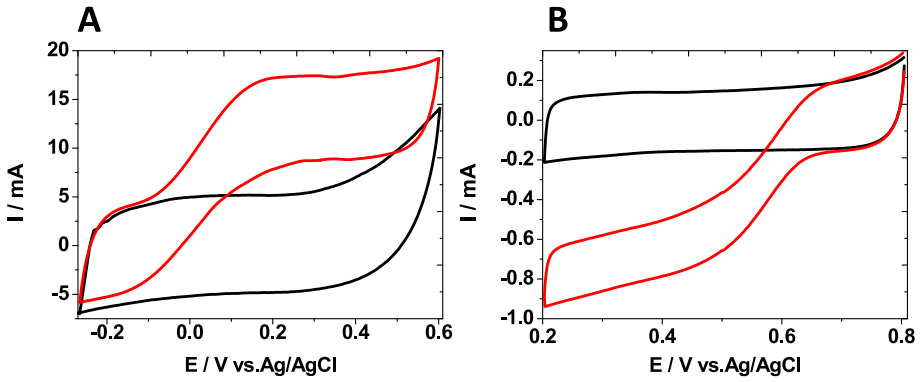


Fig. 1. Cyclic voltammograms recorded in McIlvaine buffer, pH 5.3 at a scan rate of 5 mV s^{-1} for (A) an anode made of carbon paper covered with CCPPy and FDH, (B) a cathode covered with naphthylated MWCNTs and adsorbed laccase. The experiments were conducted in (A) the absence (black line) and presence of 100 mM (red line) fructose; and (B) in O_2 (red line) and argon (black line) saturated solutions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and discharge cycles with constant density 100 mA g^{-1} (due to low current densities generated by enzyme catalysis) and only slight decrease of capacitance −6 F g^{-1} was observed.

The specific device capacities obtained from the CVs and the charge and discharge curves were in good agreement, i.e., 65 and 67 F g^{-1}, respectively, and the dependence of the specific capacity on the current density is shown in Fig. 2C and D. The slight decrease in the specific capacity for increasing scan rates can most likely be explained by the changes of iR drop. It should be noted that the current (and hence also the iR drop due to the cell resistance) increased with increasing scan rate. These results, nevertheless, demonstrate that the device has significant internal capacitance which can influence the power output and resistance to high current loads.

3.3. Device power output studies

To power the minipotentiostat a voltage of at least 1 V was necessary which was why three biofuel cells were connected in series. Even though the OCV of each fuel cell was about 0.7 V, the whole fuel cell battery produced a voltage of about 2 V (Fig. 3A).

The maximum power density was found to be 2 mW in the flow system (20 ml min^{-1}), when a resistance of 1 kΩ was used as the load. The sampling was in this case done 5 s after the start of the measurement. Under stationary solution conditions in the maximum power was 1.5 mW due to a more rapid potential drop caused by mass transport limitations. Due to the fact that especially cathode performance, according to Fig. 1B is not limited by diffusion, constant supply of reagents to the interfacial region reduces this restriction

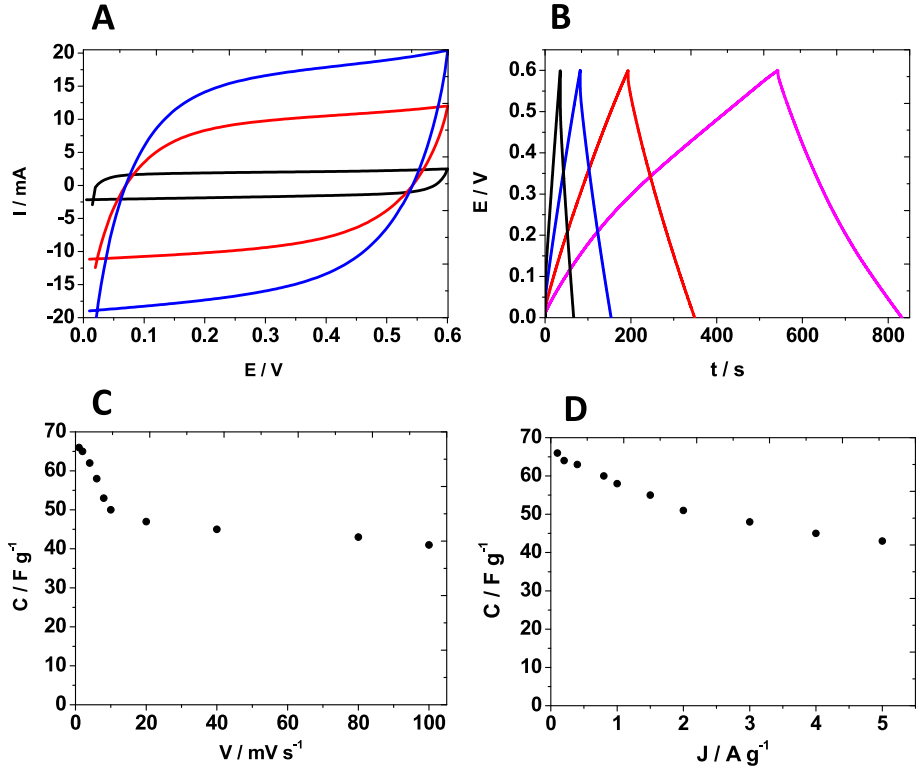


Fig. 2. (A) cyclic voltammograms recorded for the biofuel cell at scan rates of 1, 10, and 20 mV s^{-1}, (B) galvanostatic charge–discharge curves for the biofuel cell recorded at current densities of 0.1, 0.2, 0.4, and 1 A g^{-1} (C and D) specific capacitances of the biofuel cell as a function of the cyclic voltammetric scan rate (C) and the current density (D), respectively.

Please cite this article as: M. Kizling, et al., Biosupercapacitors for powering oxygen sensing devices, Bioelectrochemistry (2015), http://dx.doi.org/10.1016/j.bioelechem.2015.04.012

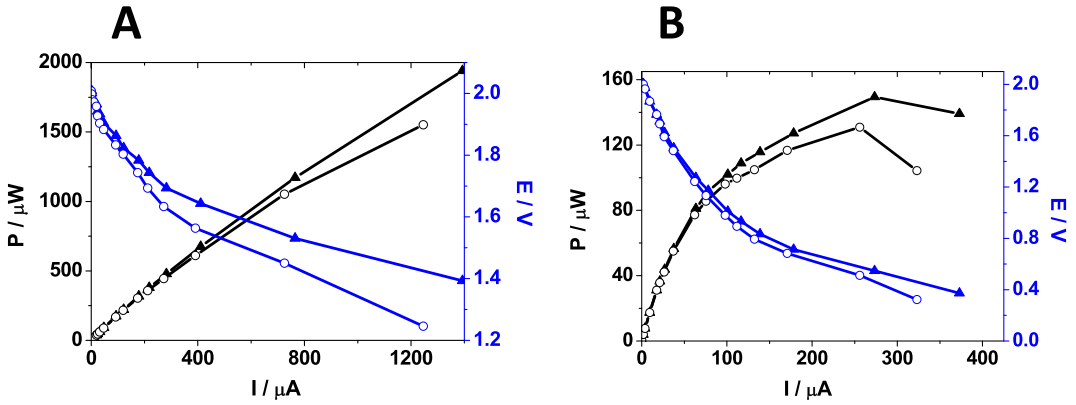


Fig. 3. Power and polarization curves for the fuel cell under stationary (empty circles) and flow conditions 20 ml min^{-1} (triangles) with sampling: (A) 5 s and (B) 60 s after the application of each resistance.

and enables faster regeneration of potential. When the sampling time was set to 60 s the maximum power output was about an order of magnitude lower both in flow and stationary system, which, however, still is satisfactory. In the flow system, the output was higher for low resistances approximately 1.25 times higher in both experiments.

3.4. Fuel cell powering of a sensing unit

In Fig. 4, the voltage–time dependence for a battery consisting of three fuel cells powering the minipotentiostat is shown.

In the experiment shown in Fig. 4A, the fuel battery was powering the sensing device until a complete depletion of the powering device was attained. The switching on the minipotentiostat gave rise to the introduction of a load of about 5 kΩ to the circuit. Under such conditions, the device was able to maintain sufficient power for 100 s, after which voltage decreased below the limit of about 1 V set by the requirements of the minipotentiostat. The influence of the solution flow rate on the output voltage was also studied as is shown in Fig. 4A. Under solution flow conditions the battery was found to be regenerated within 40 min which is significantly faster than the time of about 1 h found under stationary conditions. In Fig. 4B the use of the integrated system for multiple measurements is shown in which the potentiostat was switched on during pulses of 45 s, a time sufficiently long enough to measure the currents. Following the discharge of the battery, the potential was regenerated in about 15 min (Fig. 4B). The maximum battery voltage after each measurement decreased slightly after each measurement and after ten measurements a maximum potential of 1.73 V could be achieved. The decrease can be explained by the fact that each discharge cycle causes a significant load to be applied to the bioelectrodes, which is why some enzyme moieties may undergo denaturation.

3.5. The oxygen sensor

Chronoamperometric experiments were performed both with an Electrochemical Analyzer CHI 400B instrument and the hand-held, custom-built minipotentiostat powered by the biobattery. To determine the suitable constant potential for the oxygen sensing, cyclic voltammograms were recorded for the sensing electrode (Fig. 5A) in an oxygen saturated McIlvaine buffer. Without laccase the reduction of oxygen at bare GCE takes place at −0.6 V while at CNTs covered at approx. −0.2 V relative to Ag/AgCl, hence far from that of the laccase catalyzed process.

The catalytic current did not reach a stable plateau which can be explained by the fact that population of enzyme on the electrode surface was small, due to the limited amount of naphthylated MWCNT. As a consequence, a true limiting current could not be reached due to the overlap with the current due to the oxygen reduction on the bare carbon nanotubes. An arbitrarily constant potential of +0.2 V was therefore was selected for the sensing experiments. In Fig. 5B, the amperometric curves following repeated additions of 2 ml of oxygen saturated solution (with an oxygen concentration of about 1.1 mM)

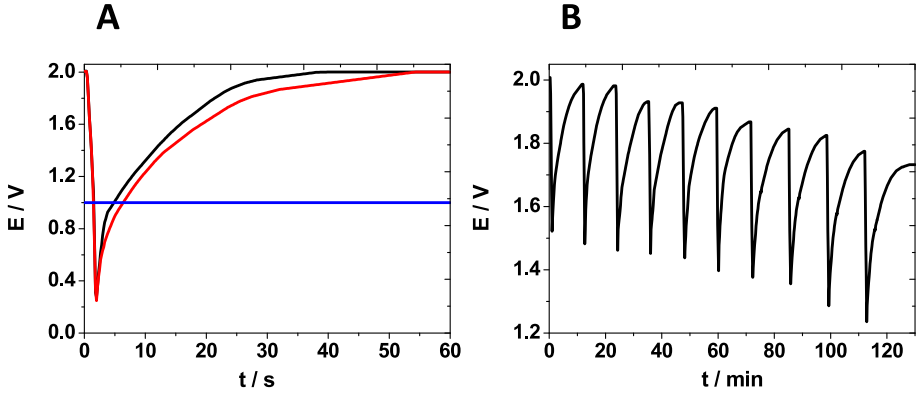


Fig. 4. Voltage–time dependence for 3 biofuel-cells in a series integrated with minipotentiostat (as a load) (A) complete depletion of the battery following which the load was disconnected under stationary (red line) and flow (black line) conditions; (B) during 45 s of measurement pulse after which the load was disconnected and the voltage was allowed to recover to a stable value under flow conditions. The blue line in A represents the threshold voltage of the minipotentiostat. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article as: M. Kizling, et al., Biosupercapacitors for powering oxygen sensing devices, Bioelectrochemistry (2015), http://dx.doi.org/10.1016/j.bioelechem.2015.04.012

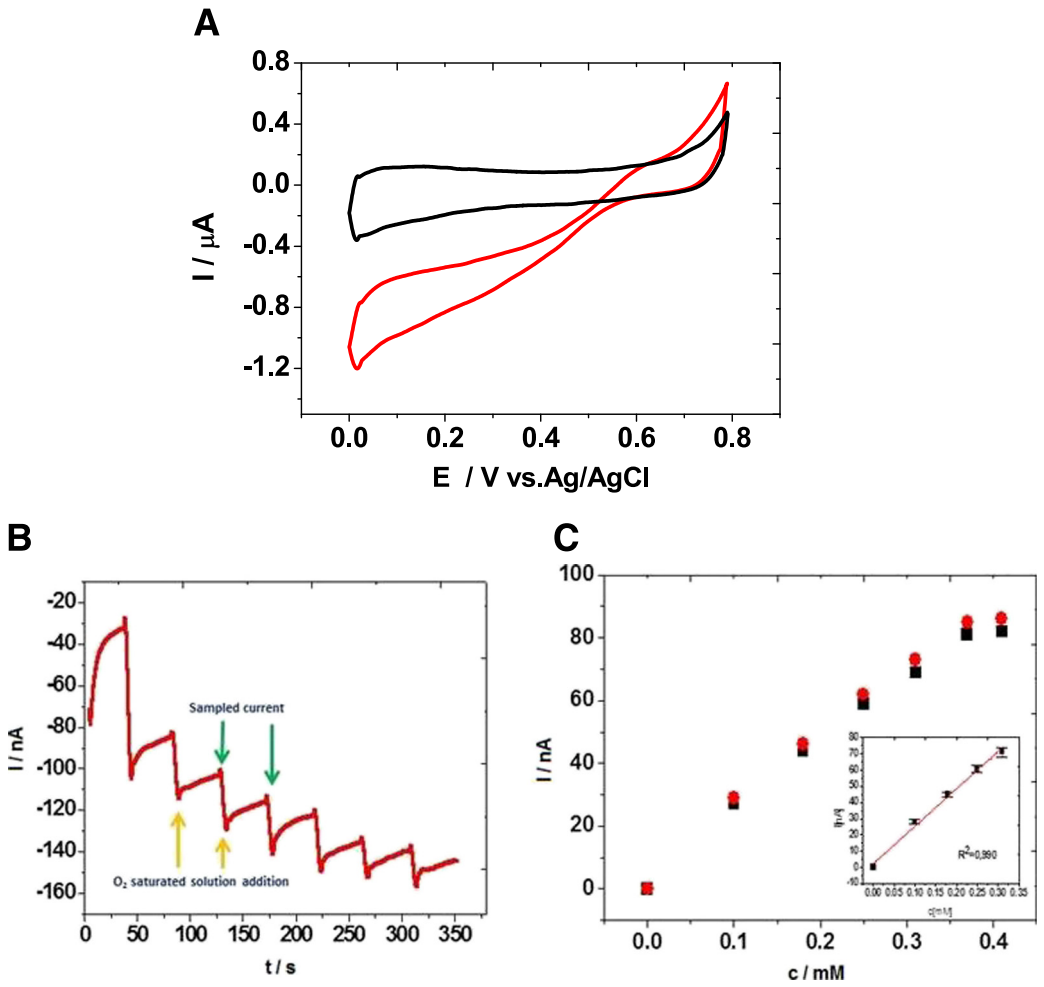


Fig. 5. (A) Cyclic voltammograms recorded in a pH 5.3 McIlvaine buffer for the sensing electrode covered with naphthylated MWCNTs and adsorbed laccase in oxygen (red line) and argon (black line) saturated solutions, respectively. Scan rate: 1 mVs^{-1}. (B) Current-time curves for the two-electrode sensor in McIlvaine buffer, pH 5.3 with different oxygen concentrations. The area of the sensor electrode: 0.008 cm^2. (C) Calibration curves for oxygen recorded in a pH 5.3 McIlvaine buffer solution with (■) Electrochemical Analyzer CHI 400B and (●) hand-held minipotentiostat. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are shown. An approximately constant current was obtained after 45 s (i.e., the measurement time allowed by the fuel cell battery system in the pulsed mode) demonstrating the versatility of the described sensor system. The relative standard deviation of the biosensor response to 0.3 mM oxygen was 3% for 3 measurements. The calibration curves recorded also with the Electrochemical Analyzer CHI 400 B Potentiostat and the minipotentiostat were almost identical and the correlation coefficient was at least 0.990 when the oxygen reduction current was sampled 45 s after the application of the sensing voltage (Fig. 5C) The current was measured as a function of the oxygen concentration for oxygen concentrations up to 0.4 mM and the linear range was found to extend up to 0.3 mM.

4. Conclusions

A battery composed of three enzymatic fuel cells with fructose as the fuel and oxygen as the oxidant was successfully used as the power source for a hand-held potentiostat controlling an oxygen sensing electrode. The fuel cell cathode was composed of carbon paper covered with covalently naphthylated MWCNTs and laccase while the anode was coated with polypyrrole–nanocellulose and fructose dehydrogenase. Both electrodes were found to operate in the mediatorless DET mode. The inherent capacitances of the employed two nanomaterials enable the storage of charge in the fuel cell itself which is advantageous when large currents are required from the system. The power and open circuit potential of the biofuel cell system were evaluated and the power was found to exceed 2 mW while the open circuit potential was about 2 V. It was also demonstrated that the presence of a flow of the fuel solution gave rise to an improved power output.

A system including the biofuel cell battery, a home-made minipotentiostat and an oxygen biosensor was tested for chronoamperometric sensing of oxygen. This type of integrated device was able to measure the oxygen concentration ten times in the pulse mode without any disturbances. As the present biofuel cell system is able to power devices requiring low currents it is evident that the development within bioelectrocatalysis and enzyme engineering make self-powered integrated devices a technologically important research direction and that this type of devices also is very likely to find its own niche on the market.

Acknowledgments

This project was supported by FP7-People-2013-ITN Grant “Bioenergy, Biofuel Cells: From fundamentals to application of bioelectrochemistry” under grant agreement no 607793. MK thanks the Polish Ministry of Sciences and Higher Education for support through the project “Diamond Grant” No. 0154DIA20134 and Warsaw Consortium of Academic Chemistry for KNOW scholarship. We thank Dr. Slawomir Kalinowski for the construction of the minipotentiostat.

Please cite this article as: M. Kizling, et al., Biosupercapacitors for powering oxygen sensing devices, Bioelectrochemistry (2015), http://dx.doi.org/10.1016/j.bioelechem.2015.04.012

References

[1] K.R. Rogers, Recent advances in biosensor techniques for environmental monitoring, Anal. Chim. Acta 568 (2006) 222–231.
[2] W. Gellett, M. Kesmez, J. Schumacher, N. Akers, S.D. Minteer, Biofuel cells for portable power, Electroanalysis 22 (2010) 727–731.
[3] D.R. Thevenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: recommended definition and classification, Biosens. Bioelectron. 16 (2001) 121–131.
[4] Y. Lei, W. Chen, A. Mulchandani, Microbial biosensors, Anal. Chim. Acta 568 (2006) 200–210.
[5] J. Newman, S. Setford, Enzymatic biosensors, Mol. Biotechnol. 32 (2006) 249–268.
[6] D. Leech, P. Kavanagh, W. Schuhmann, Enzymatic fuel cell: recent progress, Electrochim. Acta 84 (2012) 223–234.
[7] C. Spain, S.D. Minteer, Enzyme immobilization in biotechnology, Recent Pat. Eng. 2 (2008) 195–200.
[8] C. Tortolini, S. Rea, E. Carota, S. Cannistraro, F. Mazzei, Influence of the immobilization procedures on the electroanalytical performances of *Trametes versicolor* laccase based bioelectrodev, Microchem. J. 100 (2012) 8–13.
[9] C. Leger, P. Bertrand, Direct electrochemistry of redox enzymes as a tool for mechanistic studies, Chem. Rev. 108 (2008) 2379–2438.
[10] M. Pita, C. Gutierrez-Sanchez, M.D. Toscano, S. Shleev, A.L. De Lacey, Oxygen biosensor based on bilirubin oxidase immobilized on a nanostructured gold electrode, Bioelectrochemistry 94 (2013) 69–74.
[11] Y. Ulyanova, S. Babanova, E. Pinchon, I. Matanovic, S. Singhal, P. Atanassov, Effect of enzymatic orientation through the use of syringaldazine molecules on multiple multi-copper oxidase enzymes, Phys. Chem. Chem. Phys. 16 (2014) 13367–13375.
[12] S. Shleev, J. Tkac, A. Christenson, T. Ruzgas, A.I. Yaropolov, J.W. Whittaker, Direct electron transfer between copper-containing proteins and electrodes, Biosens. Bioelectron. 20 (2005) 2517–2554.
[13] C.F. Blanford, R.S. Heath, F.A. Armstrong, A stable electrode for high-potential, electrocatalytic O_2 reduction based on rational attachment of a blue copper oxidase to a graphite surface, Chem. Commun. 17 (2007) 1710–1712.
[14] L. Dos Santos, V. Climent, C.F. Blanford, F.A. Armstrong, Mechanistic studies of the 'blue' cu enzyme, bilirubin oxidase, as a highly efficient electrocatalyst for the oxygen reduction reaction, Phys. Chem. Chem. Phys. 12 (2010) 13962–13974.
[15] D.J. Kosman, Multicopper oxidases: a workshop on copper coordination chemistry, electron transfer, and metallophysiology, J. Biol. Inorg. Chem. 15 (2010) 15–28.
[16] S. Shleev, Y. Wang, M. Gorbacheva, A. Christenson, D. Haltrich, R. Ludwig, T. Ruzgas, L. Gorton, Direct heterogeneous electron transfer reactions of *Bacillus halodurans* bacterial blue multicopper oxidase, Electroanalysis 20 (2008) 963–969.
[17] R. Bilewicz, M. Opallo, Biocathodes for dioxygen reduction in biofuel cells, in: A. Wieckowski, J.K. Norskov (Eds.), Fuel Cell Science: Theory, Fundamentals and Biocatalysis, John Wiley & Sons, Hoboken, New Jersey 2010, pp. 169–214.
[18] Y. Li, X. Huang, Y. Qu, A strategy for efficient immobilization of laccase and horseradish peroxidase on single-walled carbon nanotubes, J. Chem. Technol. Biotechnol. 88 (2013) 2227–2232.
[19] V. Papper, K. Elouarzaki, K. Gorgy, A. Sukharaharja, S. Cosnier, R.S. Marks, Biofunctionalization of multiwalled carbon nanotubes by electropolymerized poly(pyrole-concavalin A), Chem. Eur. J. 20 (2014) 13561–13564.
[20] N. Lalaoui, K. Elouarzaki, A. Le Goff, M. Holzinger, S. Cosnier, Efficient direct oxygen reduction by laccases attached and oriented on pyrene-functionalized polypyrrole/carbon nanotube electrodes, Chem. Commun. 49 (2013) 9281–9283.
[21] M. Bourourou, K. Elouarzaki, N. Lalaoui, C. Agnes, A. Le Goff, M. Holzinger, A. Maaref, S. Cosnier, Supramolecular immobilization of laccase on carbon nanotube electrodes functionalized with (methylpyrenylaminomethyl) anthraquinone for direct electron reduction of oxygen, Chem. Eur. J. 19 (2013) 9371–9375.
[22] M. Karaśkiewicz, D. Majdecka, A. Więckowska, J.F. Biernat, J. Rogalski, R. Bilewicz, Induced-fit binding of laccase to gold and carbon electrodes for the biological fuel cell applications, Electrochim. Acta 126 (2014) 132–138.
[23] J. Keskinen, E. Sivonen, M. Bergelin, J.-E. Eriksson, P. Sjoberg-Eerola, M. Valkiainen, M. Smolander, A. Vaari, J. Uotila, H. Boer, S. Tuurala, Printed supercapacitor as hybrid device with an enzymatic power source, Adv. Sci. Technol. 72 (2010) 331–336.
[24] M. Skunik-Nuckowska, K. Grzejszczyk, K. Stolarczyk, R. Bilewicz, P.J. Kulesza, Integration of supercapacitors with enzymatic biobatteries toward more effective pulse-powered use in small-scale energy harvesting devices, J. Appl. Electrochem. 44 (2014) 497–507.
[25] C. Agnes, M. Holzinger, A. le Goff, B. Reuillard, K. Elouarzaki, S. Tingry, S. Cosnier, Supercapacitor/biofuel cell hybrids based on wired enzymes on carbon nanotube matrices: autonomous reloading after high power pulses in neutral buffered glucose solutions, Energy Environ. Sci. 7 (2014) 1884–1888.
[26] D. Pankratov, Z. Blum, S. Shleev, Hybrid electric power biodevices, ChemElectroChem 1 (2014) 1798–1807.
[27] D. Pankratov, P. Falkman, Z. Blum, S. Shleev, A hybrid electric power device for simultaneous generation and storage of electric energy, Energy Environ. Sci. 7 (2014) 989–993.
[28] M. Kizling, K. Stolarczyk, J. Sim Sin Kiat, P. Tammela, Z. Wang, L. Nyholm, R. Bilewicz, Pseudocapacitive polypyrrole-nanocellulose composite for sugar–air enzymatic fuel cells, Electrochem. Commun. 50 (2015) 55–59.
[29] A.D. Amsel, A. Rudnitsky, Z. Zalevsky, An implantable self-powered medical device for blood irradiation therapy, J. At. Mol. Opt. Phys. (2012) 963187–963192.
[30] D.A. Borton, M. Yin, J. Aceros, A. Nurmikko, An implantable wireless neural interface for recording cortical circuit dynamics in moving primates, J. Neural Eng. 10 (2013) 16.
[31] J.H. Cheong, S. Sheung Yan Ng, X. Liu, R.-F. Xue, H.J. Lim, P.B. Khannur, K.L. Chan, A.A. Lee, K. Kanh, L. Shiah, W.-T. Park, M. Je, An inductively powered implantable blood flow sensor microsystem for vascular grafts, IEEE Trans. Biomed. Eng. 59 (2012) 2466–2475.
[32] M. Falk, M. Alcalde, P.N. Bartlett, A.L. De Lacey, L. Gorton, C. Gutierrez-Sanchez, R. Haddad, J. Kilburn, D. Leech, R. Ludwig, E. Magner, D.M. Mate, P. Conghaile, R. Ortiz, M. Pita, S. Poller, T. Ruzgas, U. Salaj-Kosla, W. Schuhmann, F. Sebelius, M. Shao, L. Stoica, C. Sygmund, J. Tilly, M.D. Toscano, J. Vivekanthan, E. Wright, S. Shleev, Self-powered wireless carbohydrate/oxygen sensitive biodevice based on radio signal transmission, PLoS ONE 9 (2014) 109104.
[33] M. Tominaga, C. Shiraikihira, I. Taniguchi, Direct heterogeneous electron transfer reactions and moluecular orientation of fructose dehydrogenase adsorbed onto pyrolytic graphite electrodes, J. Electroanal. Chem. 610 (2007) 1–8.
[34] Y. Kamitaka, S. Tsujimura, N. Setoyama, T. Kajino, K. Kano, Fructose/dioxygen biofuel cell based on direct electron transfer-type bioelectrocatalysis, Phys. Chem. Chem. Phys. 9 (2007) 1793–1801.
[35] K. Murata, K. Kajiya, N. Nakamura, H. Ohno, Direct electrochemistry of billirubin oxidase on three-dimensional gold nanoparticle electrodes and its application in biofuel cell, Energy Environ. Sci. 2 (2009) 1280–1285.
[36] T. Miyake, S. Yoshino, T. Yamada, K. Hata, M. Nishizawa, Self-regulating enzyme-nanotube ensemble films and their application as flexible electrodes for biofuel cells, J. Am. Chem. Soc. 133 (2011) 5129–5134.
[37] K. So, S. Kawai, Y. Hamano, Y. Kitazumi, O. Shirai, M. Hibi, J. Ogawaa, K. Kano, Improvement of a direct electron transfer-type fructose/dioxygen biofuel cell with substrate-modified biocathode, Phys. Chem. Chem. Phys. 16 (2014) 4823–4829.
[38] E. Nazaruk, E.M. Landau, R. Bilewicz, Membrane bound enzyme hosted in liquid crystalline cubic phase for sensing and fuel cells, Electrochim. Acta 140 (2014) 108–115.
[39] A. Mihranyan, L. Nyholm, A. Garcia-Bennett, M. Strømme, A novel high specific surface area conducting paper material composed of polypyrrole and Cladophora cellulose, J. Phys. Chem. B 112 (2008) 12249–12255.
[40] G. Nyström, A. Razaq, M. Strømme, L. Nyholm, A. Mihranyan, Ultrafast all-polymer paper-based batteries, Nano Lett. 9 (2009) 3635–3639.
[41] L. Nyholm, G. Nyström, A. Mihranyan, M. Strømme, Toward flexible polymer and paper-based energy storage devices, Adv. Mater. 23 (2011) 3751–3769.
[42] D. Majdecka, S. Draminska, K. Stolarczyk, M. Kizling, P. Krysinski, J. Golimowski, J.F. Biernat, R. Bilewicz, Sandwich biobattery with enzymatic cathode and zinc anode integrated with sensor, J. Electrochem. Soc. 162 (2015) F555–F559.
[43] E. Frackowiak, V. Khomenko, K. Jurewicz, K. Lota, F. Beguin, Supercapacitors based on conducting polymers/nanotubes composites, J. Power Sources 153 (2006) 413–418.

Please cite this article as: M. Kizling, et al., Biosupercapacitors for powering oxygen sensing devices, Bioelectrochemistry (2015), http://dx.doi.org/10.1016/j.bioelechem.2015.04.012