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Biosupercapacitors for powering oxygen sensing devices

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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].

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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