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Short communication

Pseudocapacitive polypyrrole–nanocellulose composite for sugar-air enzymatic fuel cells

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ABSTRACT

Efficient, new combination of a bioelectrocatalytic and a pseudocapacitive cellulose-based composite material is reported. The anode comprising *Gluconobacter* sp. fructose dehydrogenase physically adsorbed on *Cladophora* sp. Algae nanocellulose/polypyrrole composite provides large catalytic oxidation currents due to large effective surface area of the composite material, and enables storing of the charge. Supercapacitor properties are useful for larger current demands e.g. during switching on–off the devices. Mediatorless catalytic oxidation current densities as high as 14 mA cm^{-2} at potentials as negative as −0.17 V vs. Ag/AgCl constitute the best anode performance without using mediators reported to date. The fuel cell with GCE cathode covered with laccase adsorbed on naphthylated multiwalled carbon nanotubes, exhibits improved parameters: open circuit voltage of 0.76 V, and maximum power density 1.6 mW cm^{-2}.

1. Introduction

The preparation of biocathodes for enzymatic biofuel cells based on laccases or bilirubin oxidase in combination with nanostructured materials is well described in the literature [1–5]. Biocathodes prepared using arylated carbon nanotubes and adsorbed laccase have been the subject of our recent reports [6–12]. We have likewise reported on the construction of a hybrid biofuel cell (biobattery), comprising a zinc anode covered with hopeite, the potential of which did not change during the operation of the biobattery [8,9].

A real challenge is now to create an efficient bioanode working at more negative potentials than those achieved with mediators and glucose oxidase or glucose dehydrogenases, and not sensitive towards oxygen since this would eliminate formation of hydrogen peroxide at the anode. In this work, an efficient new bioanode has been prepared based on a nanostructured cellulose/polypyrrole composite [13–15] and fructose dehydrogenase (FDH) physically adsorbed on this material. FDH oxidizes D-fructose to 5-keto-D-fructose via direct electron transfer to the electrode and does hence not require any mediator [16–18]. During this direct electron transfer (DET) process, the fructose oxidation and reduction of the flavin-containing subunit are followed by electron transfer to the heme-c containing subunit [19]. Due to its insensitivity to oxygen, FDH is ideal for biofuel cell applications [20]. The application of FDH as anode catalyst in DET-type biofuel cells was first reported by Kamitaka et al. [21] and since then, various strategies e.g. encapsulation, organometallic-catalyzed immobilization, polymer-grafting and screen printing have been explored to optimize the enzyme performance [16,19,22]. Miyake et al. [23] reported a carbon nanotube forest ensemble (CNTF) with fructose dehydrogenase (FDH) showing the oxidation current density of 16 mA cm^{-2} in stirred 200 mM fructose solution. The power density of a biofuel cell using the FDH–CNTF anode and the laccase–CNTF cathode reached 1.8 mW cm^{-2} (at 0.45 V), however, in the stirred oxygenated fructose solution [23]. All these approaches are difficult in combination with low-cost quality-controlled mass production.

The unique approach presented in this paper is based on the use of a readily available eco-friendly cellulose/polypyrrole composite as the anode material on which FDH was immobilized. This combination of a bioelectrocatalytic system and a paper-based supercapacitor-like composite resulted in highly improved performance of the anode, suitable for the biofuel cell. As the previously described biocathode, covered with carbon nanotubes and laccase was employed, the device can be considered a hybrid–supercapacitive and bioelectrocatalytic system.

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2. Experimental

2.1. Materials and chemicals

Fructose dehydrogenase (FDH) from *Gluconobacter* sp. was purchased from Sorachim. The inorganic reagents from POCh (Gliwice, Poland) and the organic reagents from Aldrich were used without further purification. Water was distilled and passed through a Milli-Q purification system.

Laccase *Cerrena unicolor* C-139 was obtained from the culture collection of Regensberg University and deposited in the fungal collection of the Department of Biochemistry (Maria Curie-Sklodowska University, Poland) under the strain number 139. The laccase activity dissolved in 1 ml of water was 262 U g^{-1} [6].

Pyrrole (Merck), $FeCl_3 \cdot 6H_2O$ (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 [24].

2.2. Material preparation and characterization

The PPy/cellulose composite has been characterized extensively in our previous work [13–15,25,26]. For 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 of Tween-80 were dissolved in 50 ml of 0.5 M HCl and mixed with the cellulose dispersion. 12.857 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 100 ml 0.5 M HCl. To initiate the polymerization the $FeCl_3$ solution was added dropwise to the pyrrole and cellulose mixture. 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 pressed and dried under ambient conditions. To obtain the bioanode for the biofuel cell, the composite was dispersed in 96% ethanol (8 mg ml^{-1}) via high-energy ultrasonication and dropped to 0.75 ml in few steps on the surface of a glassy carbon electrode (GCE, BAS) with a surface area of 0.071 cm^2. Subsequently 60 μl of FDH solution containing 20 mg ml^{-1} of enzyme was applied and the electrode was kept in a fridge overnight to evaporate the solvent during the enzyme adsorption on GCE.

The voltammetric experiments were performed in a three-electrode arrangement employing an Ag/AgCl (KCl sat.) reference electrode, a platinum foil counter electrode and the bioanode as the working electrode (see Fig. 3A). All cyclic voltammetry experiments were carried out using an ECO Chemie Autolab potentiostat at 22 ± 2 °C and all current densities were calculated using the geometric electrode area.

For calculations of electrode capacitance, cyclic voltammograms in three-electrode system were recorded at different scan rates from 1 to 100 mV s^{-1} [27]. The capacitance was calculated from the obtained pseudocapacitive current as $C = i\upsilon^{-1}$ where i is the average current and υ the scan rate of voltage. The reported specific capacitance values are expressed with respect to the active material present on one electrode.

The biocathode with naphthylated carbon nanotubes and laccase in a layer of Nafion (MWCNT–NAPH–Lac) was prepared using a laccase solution containing 1 mg of the enzyme in 0.64 ml of McIlvaine buffer, pH 5.3. A 1% Nafion solution was prepared by dilution of a 5% Nafion solution with ethanol. A mixture of laccase and Nafion was prepared by adding 50 μl Nafion to 50 μl laccase solution. Modified multiwalled carbon nanotubes were prepared according to the procedures described in our previous paper [28] and 10 μl of the MWCNT–NAPH in ethanol (4 mg ml^{-1}) suspension was placed on the GCE surface. After drying, 20 μl of a mixture of laccase and Nafion was applied to the electrode and allowed to dry.

The biofuel cell parameters, i.e. the open circuit voltage (OCV) and the cell voltage (V_{cell}) were measured in an oxygenated McIlvaine buffer solution (pH 5.3) containing 0.1 M of fructose with various external resistances applied. The resistance applied to the circuit ranged from 10 MΩ to 1 kΩ. Moreover, the anode potential (V_a) and the cathode potential (V_c) were measured vs. a reference Ag/AgCl electrode inserted in the system (Fig. 3A). To minimize power loss caused by fuel depletion, the duration of each measurement should be restricted to 5 s after each load application, but to investigate the dependence of bioelectrode response on this time, the measurements done 60 and 600 s after applying each resistance are also shown.

3. Results and discussion

3.1. Capacitance studies of bioanode

The cyclic voltammograms for a three-electrode system depicted in Fig. 1A were recorded in a pH 5.3 McIlvaine buffer using scan rates of 4 and 20 mV s^{-1}, respectively. The shapes of the voltammograms for the devices 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 the higher scan rates deviated from the rectangular shape, it is still evident that the device could be reversibly charged and discharged even at the highest scan rates. Absence of redox peak typical for polypyrrole film can be explained with high thickness (ca. 2 mm) and porosity of modification. The specific capacitance (SC) obtained for a scan rate of 1 mV s^{-1} was 164 F g^{-1} and the dependence of the SC value on the scan rate is shown in Fig. 1B. The decrease in the SC value seen for increasing scan rates can most likely be explained by the RC time constant of the device [25,26] as the current (and hence also the iR drop due to the cell resistance) increased with increasing scan rate. These results, nevertheless, demonstrate the usefulness of the present composite electrode for bioelectrocatalytic systems since mA currents typically are obtained in the biofuel cells.

3.2. Studies of catalytic properties of the bioanode

As seen in Fig. 2A, the voltammogram for the bioanode in a three-electrode set-up exhibited a typical capacitive shape in the absence of fructose in good agreement with the results in Fig. 1A. Following the addition of fructose to the solution, no additional peaks were observed in the voltammograms in studied potential range without enzyme present in film. After overnight FDH adsorption, catalytic fructose oxidation waves, however, appeared at −0.17 V indicating mediatorless FDH catalysis of the D-fructose oxidation. The current onset potential, ca. −0.1 V vs. Ag/AgCl is more negative than those achieved for other enzymes and substrates so far [29]. As a linear increase in the current was seen for fructose concentrations up to 50 mM (see Fig. 2B), this bioanode may also be considered for use in fructose sensing systems. The current density at +0.5 V was 14.1 mA for a scan rate of 1 mV s^{-1} and a fructose concentration of 110 mM.

To examine the useable pH range of the composite electrode, the pH dependence of the catalytic fructose oxidation current at +0.4 V was studied in McIlvaine buffer solutions (see Fig. 2C). The optimum pH was found to be ca. 5 which is about one pH unit lower than the values reported for highly oriented pyrolytic graphite based electrodes [19] and SAMs of thiols on gold nanoparticles [29]. The obtained optimum pH is in good agreement with the optimum pH for the enzymatic reaction in solution [30]. The loss in activity for pH values higher than about 5 is not unexpected as it has been reported that the FDH complex decomposes and loses activity in neutral and alkaline solutions [16]. The present results suggest that the adsorption of FDH on the porous nanostructure of the composite might prevent denaturation.