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Fully enzymatic mediatorless fuel cell with efficient naphthylated carbon nanotube - laccase composite cathodes

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Abstract

An efficient, mediator-free enzymatic glucose/O_2 biofuel cell with an oxygen insensitive anode based on glucose dehydrogenase is presented. In the device, the power of the biofuel cell and electrode potentials of each of the enzymatic electrodes were monitored in parallel under the biofuel cell working conditions. The carbon nanotube composite biocathode demonstrates an almost constant electrode potential vs. saturated calomel electrode under changing loads of the biofuel cell, even when relatively high current flows through the circuit. The fuel cell reveals good stability in time and is more powerful than other enzymatic film based biofuel cells; the power densities were 131±4 $\mu W \cdot cm^{-2}$ at 300 mV.

Key words: direct electron transfer, laccase, glucose dehydrogenase, biofuel cell, carbon nanotube.

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1. Introduction

Enzymatic fuel cells converting chemical energy into electrical power are able to work under ambient conditions and at physiological pH in contrary to unfavourable metal catalysts [1-6] Nanostructuring of electrode surface using carbon nanotubes with covalently bonded enzymes eliminates problems caused by leaching or interactions with other components. Bioconjugates of SWCNT with laccase were effective in the dioxygen reduction process; we applied this finding to a Zn/O_2 biobattery, which gave power densities of ca. 1 $mW \cdot cm^{-2}$. [7] By virtue of a hydrophobic pocket around the T1 centre, laccase exhibits affinity towards appropriately sized aromatic moieties. [2] When attached to the electrode surface, these groups may orient laccase on the (conducting) electrode in a way favouring direct electron exchange; it leads to a stable and catalytically efficient biocathode. [8-10] The biocatalyst typically employed in the anode is glucose oxidase (GOx). [11-13] Unfortunately, the main disadvantage of GOx is its sensitivity to dioxygen, which decreases the lifetime and efficiency of devices. [14, 15] Moreover, hydrogen peroxide produced in the GOx catalytic reaction can affect the dioxygen catalytic reduction current. [16-23] NAD^+ dependent glucose dehydrogenase (GDH) is a dioxygen insensitive enzyme that catalyses glucose oxidation and is suitable for the bioanode since the formation of hydrogen peroxide is prevented. [18, 24] Direct NADH oxidation at bare electrodes proceeds with a large overpotential that reaches values close to 1 V. [25] Application of pristine single-walled carbon nanotubes or gold nanoparticles can shift the NADH oxidation potential to ca. 0.2 V. [26] The biofuel cell described by Nishizawa and co-workers using GDH and bilirubin oxidase (BOD) immobilized on Ketjen Black exhibited power density of 52 $\mu W \cdot cm^{-2}$ at 0.3V. [20] Implementation of O_2-insensitive GDH/Au NPs and BOD in biofuel cell leads to power output of 32 $\mu W\ cm^{-2}$. [16] Single-walled carbon nanotube (SWNT)-modified carbon fiber microelectrodes employed in biofuel cell with GDH and BOD as catalysts gave power density of 52 $\mu W\ cm^{-2}$. [31]

In the present work, we describe a fully enzymatic, mediator-free biofuel cell. Laccase bioconjugates [7] or arylated carbon nanotubes are employed to achieve favorable enzyme orientation at the cathode and to extend electron conducting track between enzyme active center and the electrode [8] and dioxygen insensitive, NAD dependent glucose dehydrogenase (GDH) is used for the anode.

2.1 Materials and equipment

Glucose dehydrogenase from *Thermoplasma acidophilum* (100 U mg^{-1}), D-(+)-glucose and monoolein (1-oleoyl-rac-glycerol) (MO) purchased from Sigma were used as received. Na_2HPO_4, KH_2PO_4 and citric acid were from POCh (Polish Chemicals Co.). All solutions were prepared using MilliQ water (18.2 $M\Omega \cdot cm^{-1}$), Millipore, Bedford, MA, USA. Cyclic voltammetry experiments were performed using a CHI 700B bipotentiostat in a three-electrode arrangement with a saturated calomel electrode and a platinum sheet as the counter electrode. All current densities were calculated using geometrical area of the GCE (BAS) (A=0.071 cm^2). The biofuel cell parameters were examined in dioxygen saturated 0.15 M McIlvaine buffer solution, pH 6. The cell voltage (V_{cell}) and potentials of each of the electrodes vs. SCE were measured under varying loading in the range from 1 kΩ to 10 MΩ.

2.2 Electrode modification procedures

Bioanode: A suspension of pristine carbon nanotubes in ethanol (4 mg ml^{-1}) was sonicated for 30 minutes, and then 60 µl of the suspension was dropped onto each GC electrode. Electrodes were then left to dry for at least 1 h and then covered with a cubic phase film incorporating glucose dehydrogenase. The cubic phase preparation was described elsewhere. [27-29, 30]

Biocathode 1: Modification of GC electrode with SWCNTs with covalently bonded laccase was described in our previous paper. [8]

Biocathode 2: Naphthalene modified carbon nanotubes were prepared as follows: a mixture of SWCNTs (40 mg), 2-naphthylamine hydrochloride (940 mg, 5.2 mmol), pyridine (0.45 ml), *o*-dichlorobenzene (5 ml) and acetonitrile (5 ml) was sonicated for 0.5h at room temperature. Then amyl nitrite (0.72 ml) was added. The mixture was maintained at 65°C for 6 h with continuous sonication. TEM images taken always before and after each modification, show that the sizes of CNTs did not change upon sonication. The modified SWCNTs were separated by centrifugation; the sediment was mixed with methanol, sonicated for 10 min and centrifuged again. This procedure was repeated five times. The resulting slurry was dried at 60°C in vacuo.

A suspension of naphthalene functionalized SWCNTs in ethanol (60 µl containing 240 µg of SWCNTs) was dropped onto the electrode surface and left to dry. Then the electrode was kept for 12h in the laccase solution prepared by dissolving 20 mg of enzyme in 3 ml McIlvaine buffer, pH5.3. The electrode rinsed with water was used in the experiments.

2. Result and discussion

Our previous studies show that the electrode modified with SWCNTs with covalently attached laccase is an efficient, simple and very stable cathode and the immobilization of the enzyme eliminates the problems associated with the diffusion of substrates. [7] Such bioelectrode was employed in the current paper for the construction of the enzymatic biofuel cell (Fig. 1A). As shown earlier, due to the presence of hydrophobic pocket laccase exhibits affinity to aromatic groups attached to the carbon nanotubes; it is directed onto the carbon nanotubes covered electrode in favorable orientation to achieve an efficient biocathode. [8] In this paper, SWCNTs with covalently bound naphthalene were used to construct the biocathode 2. Cyclic voltammograms recorded at SWCNTs_naph/laccase modified GC electrode are presented in Fig. 1B. Well developed catalytic curve was observed in the presence of dioxygen. Dioxygen reduction starts at ca. 600 mV *vs.* SCE and confirms favorable laccase orientation with T1 site directed towards the electrode. Such enzyme orientation leads to high current density - 900 µA cm^{-2}.

Oxygen insensitive NAD^+ dependent glucose dehydrogenase (GDH) was employed for glucose oxidation at the anode. Non-toxic, biodegradable liquid-crystalline cubic phase film was used to hold GDH at the electrode surface. (Fig. 1C) Because the oxidation of NADH on common electrodes proceeds with a large overpotential, the pristine SWCNTs were used to facilitate electron transfer. In our system NAD^+ is both in the matrix and in the solution. Placing it only in the matrix would lead to fast removal to the solution and depletion in the matrix resulting in fast decrease of the catalytic current on the anode. The concentration of

NAD^+ is large so there are no depletion effects connected with lack of this compound at the electrode surface. Covalent binding of NAD^+ to the nanotubes retains the labile factor in the matrix but does not lead to equally high catalytic currents f glucose oxidation. With GDH present in the cubic phase and glucose present in the solution an increase of oxidation current was observed. Such an effect was not seen in the absence of the enzyme. NADH produced in the catalytic reaction is oxidized on the pristine carbon nanotube modified electrode at ca. 0 V. The current density depends on the glucose concentration, and maximum of current is reached at 100 mM; this concentration was used in the biofuel cell experiments. The maximum current density was 600±36 μA cm^{-2}.
The GC/SWCNTs electrode covered with cubic phase incorporating GDH as the anode and both types of biocathodes were tested in the biofuel cell. The open circuit potential (OCP) measured for the cell employing cathode with the SWCNTs/laccase bioconjugate (biocathode 1) was 0.58±0.03 V whereas for that with naphthalene functionalized carbon nanotubes on the cathode (biocathode 2) it was slightly lower: 0.52±0.02 V. The maximum power density was 56±5 $\mu W \cdot cm^{-2}$ at 0.2 V for the system with bioelectrode 1 and higher - 131±4 $\mu W \cdot cm^{-2}$ at 0.3 V for the cell that included naphthalene functionalized SWCNTs (Fig. 2). The potentials of each biocathode were measured in parallel vs. the SCE reference electrode under different loads applied to the biofuel cell. When the applied loads changed from 1 MΩ to 1 kΩ, the biocathode potential decreased from 580 to 325 mV for biocathode 1 and only from 545 to 500 mV for biocathode 2 (Fig. 2).

Conclusions

In this paper we presented a fully enzymatic biofuel cell based on mediator-less electron transfer process. Two biofuel cells employing the same oxygen independent anode, containing NAD-dependent GDH embedded in the liquid-crystalline cubic phase, were compared. In the first one, the biocathode containing laccase bioconjugate of SWCNT was employed and the power achieved under loading was ca. 56 μW cm^{-2}. Under 1 MΩ loading it decreases to 40% of initial power after 24h (Fig.2B). SWCNTs/laccase bioconjugates have shown catalytic activity even after several months storage at 4ºC. On the other hand, the power density of the second biofuel cell based on arylated nanotubes and laccase is always larger, 131±4 $\mu W \cdot cm^{-2}$. This, to our knowledge, is the largest value reported for a GDH based device. [16, 18, 20, 31] It should also be noted that the covalent bonds between the aryl groups and the nanotubes proposed in our studies are significantly more stable than the ester-bonded groups described very recently by other authors. [32] Moreover, the SWCNTs_naph/laccase biocathode demonstrates an almost constant electrode potential with changing loads of the biofuel cell, and does not change with time even when relatively high current flows through the circuit (Fig. 2B). At present, the limiting factor is the depletion of oxygen at the electrode surface. The mediatorless, more powerful and at the same time more stable biofuel cell presented in our paper is a promising step towards practical enzymatic fuel cells

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Figure captions

Fig. 1 Cyclic voltammograms of biocathodes (A) 1 and (B) 2 in the deaerated and saturated with dioxygen 0.15 M McIlvaine buffer, pH 5.3 solutions and (C) anode, in the same buffer with 5 mM NAD^+ (black line) and after addition of 80 mM glucose (red line). Scan rate: $1mV \cdot s^{-1}$.

Fig. 2 (A) Characteristics of the biofuel cell with bioelectrode 2 as the cathode in oxygen saturated McIlvaine buffer solution, pH 6.0 containing 5 mM NAD^+ and 80 mM glucose. Potential of the cathode simultaneously measured vs. SCE (red line). (**B)** Changes of current with time under 1 MΩ loading for the biofuel cell with biocathode 2 (blue line), and biocathode 1 (black line), in McIlvaine buffer, pH = 6.0, with 5 mM NAD^+ and 80 mM glucose. Cathode potential vs. time against SCE (red line).

Fig.1

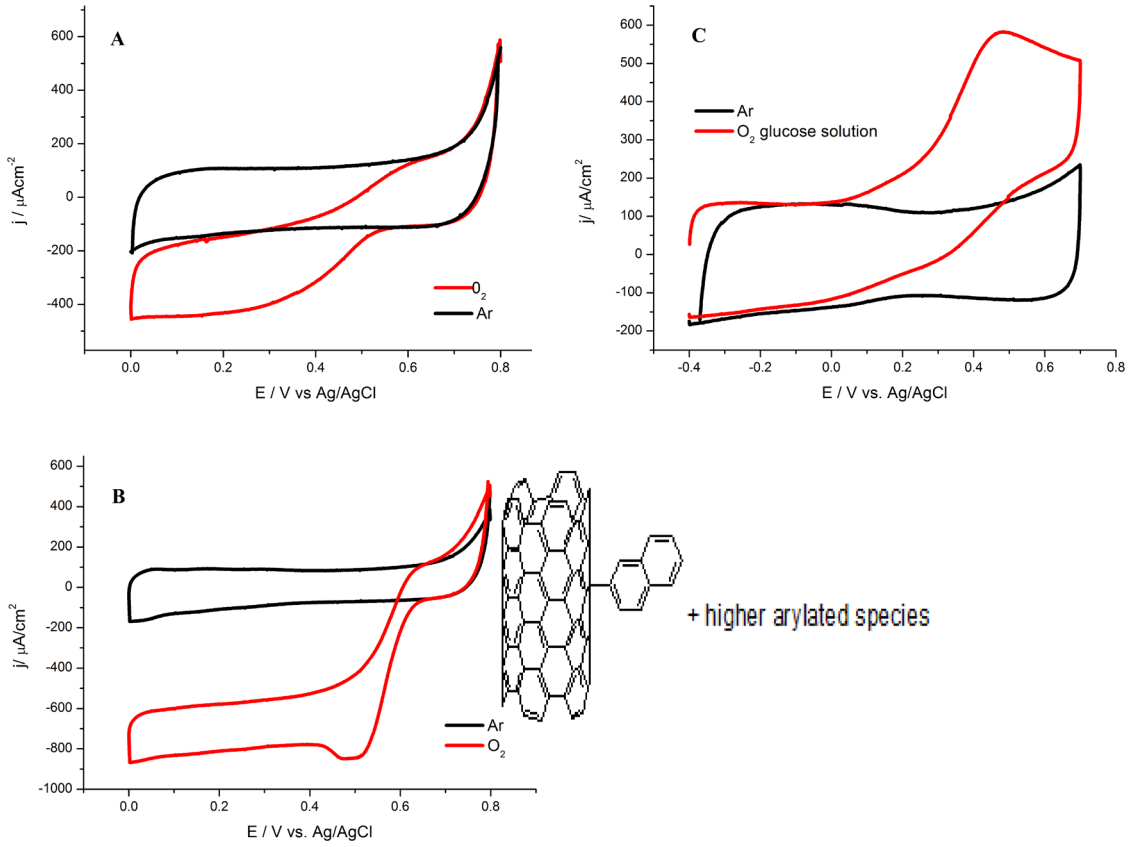
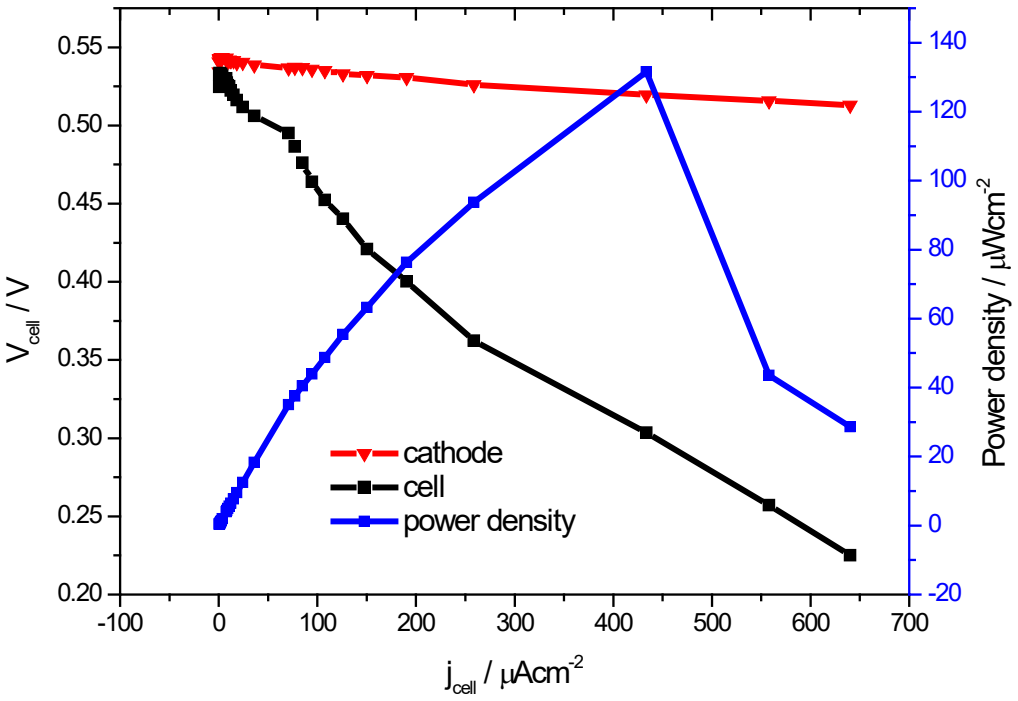


Fig. 2

A



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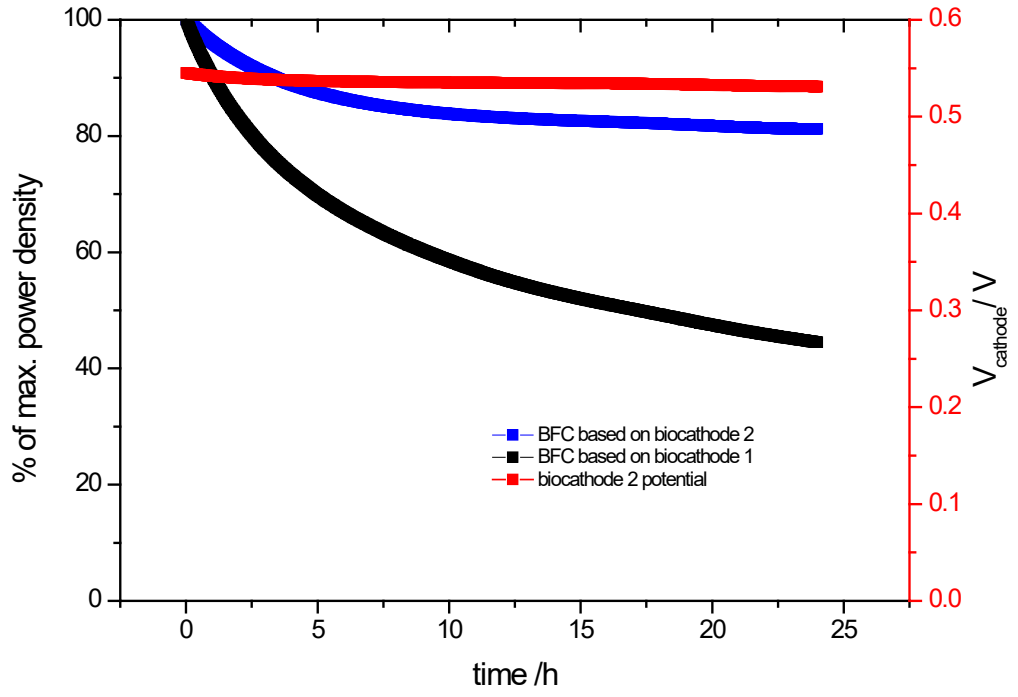

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