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Carbon Nanotubes Chemically Derivatized with Redox Systems as Mediators for Biofuel Cell Applications

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The aim of this study was designing of nanostructured bioelectrodes and assembling them into a biofuel cell with no separating membrane. Carbon nanotubes (CNTs) chemically connected with residues of typical mediators, i.e. ferrocene (Fc) and 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) deposited on glassy carbon electrodes (GCE) were found useful as mediators for the enzyme catalyzed electrode processes. The electrodes were in turn covered with glucose oxidase from *Aspergillus niger* AM-11 and laccase from *Cerrena unicolor* C-139, respectively, incorporated in a liquid-crystalline matrix. The nanostructured electrode coating with the cubic phase film containing enzymes acted as the catalytic surface for the enzymatic reactions that is oxidation of glucose at anode and reduction of oxygen at cathode. For the system with mediators anchored to CNTs the catalysis was almost ten times more efficient than on bare GCE electrodes: catalytic current of glucose oxidation was 1 $mAcm^{-2}$ and oxygen reduction current exceeded 0.6 $mAcm^{-2}$. The open circuit voltage of the biofuel cell was 0.43 V. Application of the carbon nanotubes increased maximum power output of the constructed biofuel cell to 100 μWcm^{-2} without stirring the solution. It is ca. 100 times more efficient than using the same bioelectrodes without nanotubes on the electrode surface.

K e y w o r d s: biofuel cell, laccase, glucose oxidase, cubic phase, carbon nanotubes

1. Introduction

Function of the enzymatic biofuel cell is to convert chemical energy into electrical current using redox enzymes as biocatalysts. The electric output of such system is

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expected to supply devices which require relatively low power, for example sensors, micro-devices and pacemakers. An essential feature of the enzymatic biofuel cell is that power can be generated with the use of energy reach components i.e. glucose and dioxygen, present in the human body. For some applications operability of the biofuel cells at temperatures of 20–40°C and at pH close to neutral is crucial. These properties make the biofuel cells attractive for unique applications where drastic conditions are not appropriate [1–4]. Limitations in the operation of common biofuel cells are connected with fuel or oxidant mass transport to the active centers of enzymes and diffusion of mediators between enzyme and electrode surface, which slows down the reaction. To minimize these effects rotation of electrodes, stirring or flow-cells are employed. Stirring would be not practical; therefore the biofuel cell working under motion-less conditions is considered in the present paper.

In the most of the biofuel cells primary alcohols or carbohydrates are used as substrates and respective dehydrogenases or oxidases as the anode biocatalysts. As glucose oxidation catalyst, efficient and stable glucose oxidase is most frequently used. Energy reach glucose is widely distributed in organisms; its redox potential at pH 7 is negative enough for construction of bioanodes. The active center of the enzyme is localized ca. 13 Å below the molecule surface. Electrons exchanged in enzymatic redox reaction may be transferred directly to the electrode surface, however, with small rate. For efficient biofuel cells appropriate mediators are needed to make the process more effective. In this work, carbon nanotubes derivatized with residues of typical mediators are used to increase catalytic efficiencies of the electrodes. Enzymes catalyzing $4e^-$ reduction of dioxygen directly to water are widely used to construct bioelectrodes [1–5]. Recently, engineered multicopper oxidase CueO from *Escherichia coli* was applied for catalytic oxygen reduction in air saturated buffer [6].

Research in the biofuel cell field is nowadays focused on the electrode structure improvement, which should result in decrease of dioxygen reduction overpotential, improvement of durability and achievement of higher current densities. Up to date, the best biofuel cells are mainly based on osmium complexes as mediators or on metal electrodes, e.g. platinum. There are also few direct electron transfer-type (DET-type) biofuel cells presented in literature [7–11]. Fructose/dioxygen biofuel cell of DET-type was based on D-fructose dehydrogenase and laccase as biocatalysts. The maximum power density reached 850 μAcm^{-2} at the cell voltage of 410 mV, however, under stirring conditions [9]. Another DET-type biofuel cell based on cellobiose dehydrogenase and bilirubin oxidase had 3 and 4 μAcm^{-2} at the cell voltage of 0.37 and 0.19 V in phosphate buffer and human serum, respectively [11]. To increase the amount of enzyme molecules contacting directly with the electrode its surface can be nanostructured with carbon nanotubes that additionally provide contact between the enzymes and the electrode surface. It was shown that carbon nanotubes possess splendid properties for the enzymatic catalysis, either for oxygen reduction or glucose oxidation [12–19]. Due to π–π interactions between aromatic compounds and graphene like carbon nanotubes surface non covalent immobilization of mediators

can be realized [18, 20]. On the other hand structural defects of CNTs can be utilized for covalent attachment of mediators to the electrode surface [21]. Recently, we have demonstrated that liquid-crystalline cubic phase is a convenient matrix for enzyme immobilization since it holds the enzyme in active form near the electrode surface facilitating fast transport of substrates and products through the film and provides a biocompatible environment. Cubic phase was applied for laccase, glucose oxidase and pyranose oxidase immobilization [22–26]. The lipidic cubic phase can be characterized as a curved, non-intersecting bilayer with two separated water channels. Monoolein (MO) is an example of a compound forming such lipidic cubic phase. It is highly viscous, transparent and at hydration over 20% is stable in contact with water. When the layer of monoolein cubic phase is used for hosting the enzymes, no additional membranes separating catholyte from anolyte are needed. Recently, we reported biocatalytic dioxygen reduction catalyzed by laccase with the use of 2,2′-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS) as mediator, incorporated in the liquid-crystalline cubic phase [22]. ABTS exhibits good mediating properties in the cubic phase and reversible electrochemistry close to the formal potential of laccase.

In the present work the nanostructured electrodes based on the functionalized carbon nanotubes were employed for the application in the biofuel cells. Mediator-functionalized carbon nanotubes were proposed for improving the parameters of the biofuel cell based on electrodes modified with glucose oxidase and laccase embedded in the cubic phase. Residues of ABTS covalently attached to single-walled carbon nanotubes (SWCNTs) act as efficient mediators in the systems. Leaching of the mediator from the film is eliminated. Two types of chemically modified nanotubes are employed: the previously described SWCNT-ABTS-end and SWCNT-ABTS-side [27] in the cathodic part of the biofuel cell. In the anodic part tetrathiafulvalene (TTF) or single- or multi-walled carbon nanotubes modified with ferrocene (Fc) residues act as mediators of the glucose oxidation process. Cubic phase, in 3-component mixture of monoolein-TTF-H_2O, could relatively strongly hold TTF, due to its high hydrophobicity.

2. Experimental

2.1. Materials and Equipment

Glucose oxidase (GOD) was obtained using a mutant of filamentous fungi *Aspergillus niger* AM-11 from the culture collection of the Department of Industrial Microbiology (M. Curie-Skłodowska University, Lublin, Poland). The method of isolation was described in [28]. The activity of GOD was determined [29].

Laccase from *Cerrena unicolor* C-139 was obtained from the culture collection of Regensburg University and deposited in the fungal collection of Department of Biochemistry (Maria Curie Skłodowska University, Poland) under strain number

139. Laccase from the fermenter scale cultivation was isolated as reported [30], lyophilized and the enzyme activity was determined [31]. The activity of laccase solution was 1,150,110 nkatdm^{-3} and C_{lacc} = 1.18 mgcm^{-3}.

Monoolein (1-oleoyl-rac-glycerol) (MO) and D-(+)-glucose were purchased from Sigma and were used as received. McIlvaine buffer was prepared by mixing 0.1 M citric acid and 0.2 M disodium phosphate (both from POCh (Polish Chemicals Co.)). All solutions were prepared using Milli Q water (18.2 MΩ cm^{-1}), Millipore, (Bedford, MA, USA). Stock solutions of D-(+)-glucose were prepared at least 24 h before the experiment to reach equilibrium between α and β anomers.

Cyclic voltammetry experiments were performed using an ECO Chemie Autolab potentiostat with GPES software in a three-electrode arrangement with Ag/AgCl reference electrode and a platinum foil as the counter electrode. The working electrode was a glassy carbon electrode (GCE) modified with pristine or derivatized carbon nanotubes and covered with monoolein cubic phase film. The carbon nanotubes were purchased from CheapTubes.com. Characterization of the pristine CNTs used was done using Raman spectroscopy, XPS, electrochemistry and was presented in our previous paper [32].

The biofuel cell parameters were examined in dioxygen saturated 0.1 M McIlvaine buffer solution pH 6.8 containing 15 mM glucose. Figure 1 shows the configuration of the biofuel cell. Open circuit voltage (OCV) was measured in all experiments. The cell current (Icell) and the cell voltage (Vcell) were measured under varying loading in the range from 1 kΩ to 10 MΩ; the cell current and voltage were measured after stabilization of the system. Raman spectra were collected using a Witec confocal Raman microscope system (Ulm, Germany) equipped with a fiber-coupled

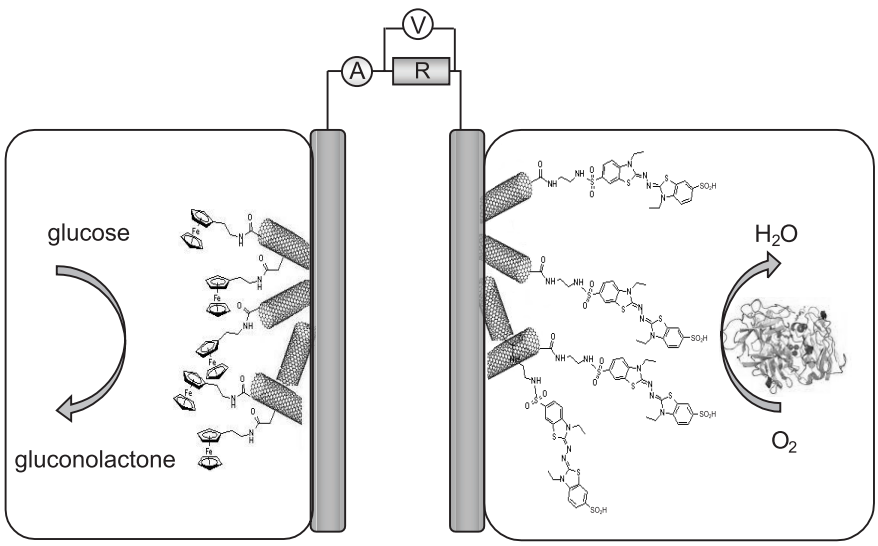

Fig. 1. Schematic representation of biofuel cell circuit

Melles Griot (Carlsbad, CA) argon ion laser operating at 514.5 nm. The light was dispersed through a triple monochromator (600 g/mm, 500 nm blaze) and detected with a thermoelectrically cooled (−60°C) charge-coupled device [32].

The X-ray fluorescence measurements were carried out using a spectrometer with an X-ray tube (IS601.5, Italstructures) producing a beam collimated to 4 mm in diameter by means of a Pb collimator, and an X-ray detection system (AXAS, Ketek) equipped with a thermoelectrically cooled silicon drift detector of 10 mm^2 area (VITUS).

Thermogravimetric analyses (TGA) were done using an Universal V4.3A TA Instrument. The measurements were carried out in argon atmosphere at a heating rate of 10°/min.

2.2. Electrode Preparation Procedure

To prepare the liquid-crystalline cubic phase, the monoolein was melted and mixed with appropriate amount (chosen from phase diagram for the monoolein–water system) of the enzyme solution until a transparent and highly viscous cubic phase was formed. The cubic phase containing TTF consisted of three components: MO, H_2O, TTF in 60:37:3 wt. % ratio, respectively. Due to high TTF hydrophobicity, its leaching to the buffer solution was not observed [28]. Prior to modification of the glassy carbon electrodes (GCE) with CNTs, their suspension in ethanol (1 $mgml^{-1}$) was sonicated for 30 min, and then 6 μl of this mixture was dropped onto the electrode. The electrodes were left to dry and then covered with a cubic phase film incorporating glucose oxidase (and TTF in some cases) for the anode, and laccase from *C. unicolor* for the cathode.

2.3. Preparation and Characteristics of Modified Carbon Nanotubes

SWCNT-Fc derivative (Fig. 2) was prepared by a combination of methods known from the literature [33–35]. The combination was implemented in order to introduce as many functional groups as possible. SWCNTs or MWCNTs were oxidized using HNO_3/H_2SO_4 mixture to produce carboxylic groups on the ends/defect sites of the nanotubes. This material was treated with iodoacetonitrile and benzoyl peroxide at 75°C. To convert the carbonitrile into carboxylic acid, the material was refluxed in 10% KOH solution in ethanol. The washed product was converted into acid chloride with thionyl chloride and the isolated product was treated with an excess of 2-ferrocyletylhylamine. The structures of SWCNT-Fc and MWCNT-Fc (both prepared analogously) are presented in Fig. 2.

Procedures for modification of the carbon nanotubes with residues of ABTS: SWCNT-ABTS-end and SWCNT-ABTS-side have been described recently [27, 36]. To obtain SWCNT-ABTS-end the pristine carbon nanotubes were oxidized as shown in Fig. 2 and the ends/defect sites derivatized material was in turn boiled

with an excess of thionyl chloride (c.f. Fig. 2) for 6 h to afford chlorocarbonyl derivative; its reaction at 70°C with an excess of ethylenediamine produces respective aminoethylamide (Fig. 3). The last material upon reaction with ABTS dibromide yielded SWCNT-ABTS-end.

To obtain carbon nanotubes modified on the side walls pristine SWCNTs (bearing negligible number of carboxylic groups on CNTs ends) were functionalized under free radical conditions. To a suspension of SWCNTs in dry benzene an excess of iodoacetonitrile and benzoyl peroxide were added, and the mixture was heated for 24 h at 75°C. The exhaustively washed preparation was suspended in KOH solution

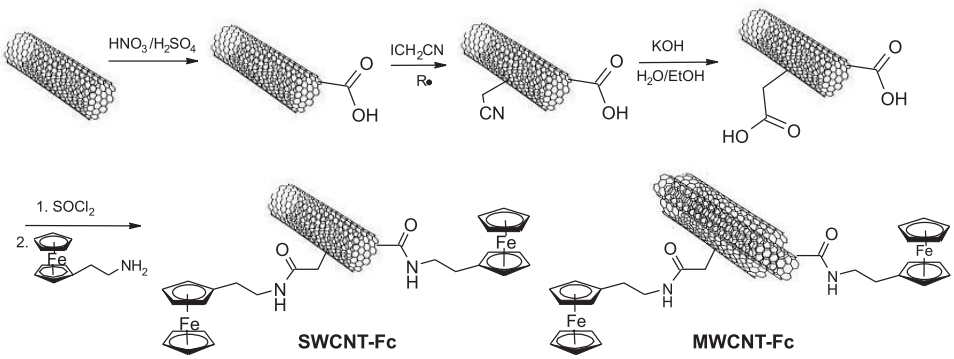

Fig. 2. Synthesis of SWCNT-Fc and MWCNT-Fc [cf. 28, 36]

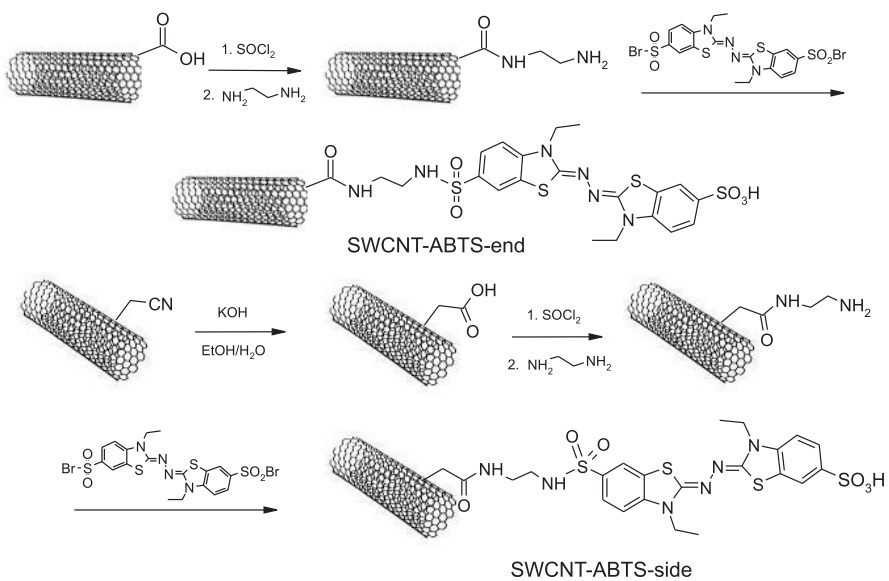

Fig. 3. Synthesis of SWCNT-ABTS-end, and SWCNT-ABTS-side [27, 36]

in aqueous ethanol, refluxed for 6 hours, washed, dried and treated with an excess of thionyl chloride (analogously as in Fig. 2). The material with side chlorocarbonyl groups was treated first with an excess of ethylenediamine and finally with ABTS dibromide to produce SWCNT-ABTs-side [27, 36].

The reacting mixtures on all steps were sonicated to increase the reaction rate. The time required for particular reaction steps was in the range of 6 to 24 hours. The structures of CNTs modified on ends and on side walls are shown in Fig. 3.

2.4. Characterization of Pristine and Modified Carbon Nanotubes

Initially, pristine SWCNTs were characterized using SEM imaging. The diameters were below 1.5 nm; the sample contained ~5% of amorphous carbon. The absence of metallic reaction catalysts (e.g. Fe or Co) used in nanotube synthesis was confirmed by X-ray fluorescence (XRF). XRF spectra of pristine and ABTS modified SWCNTs fully overlap with the background indicating that concentrations of the catalysts are below the detection limit of the instrument. For SWCNT-Fc and MWCNT-Fc the recorded XRF data confirm the presence of iron after anchoring ferrocene residues onto nanotubes.

Both pristine and derivatized SWCNTs were also characterized by Raman spectroscopy. The relative intensity ratios of the one-phonon double resonance D band to the first-order tangential G band, (I_D/I_G), were utilized to monitor purity and functionalization degree of the nanotubes. Further evidence suggested that the relationship of I_D/I_G was more accurate if the intensities were measured relative to the intensity of the second-order G′ band (I_G'). The relationship of I_D/I_G measured in the relation to G′ band intensity is presented in Table 1.

I_D/I_G ratio increased from 0.12, for pristine nanotubes, to 0.36 after derivatization with ferrocene unit. Changes were observed also in the case of $I_{G'}/I_D$ and $I_{G'}/I_G$ ratios. $I_{G'}/I_D$ ratio decreased from 2.8 for the unmodified SWCNTs to 0.68 after functionalization with ferrocene. In this case the $I_{G'}/I_G$ ratio was smaller for derivatized nanotubes and was 0.24 (0.32 for pristine SWCNTs). Analogous changes are found for the ABTS functionalized SWCNTs. The data collected confirm covalent functionalization for all samples.

Table 1. Relative intensities ratios of Raman bands for pristine and modified SWCNTs

Sample	I_D/I_G	$I_{G'}/I_D$	$I_{G'}/I_G$
Pristine SWCNT	0.12	2.78	0.32
SWCNT-CH_2CN	0.40	0.79	0.31
SWCNT-CH_2-COOH	0.60	0.39	0.23
SWCNT-ABTS-side	0.34	0.96	0.32
SWCNT-ABTS-end	0.34	0.92	0.31
SWCNT-Fc	0.36	0.68	0.24

TGA measurements were performed for pristine SWCNTs and for the ABTS-modified material. No mass loss was observed for pristine SWCNTs up to 600°C. The decomposition of the functionalized materials proceed in few steps (Table 2). For SWCNT-ABTS-end below 200°C residual solvent is removed (~2% of total mass). Between 220 and 420°C the ABTS moiety is detached resulting in 5% mass loss. In case of SWCNT-ABTS-side the mass loss referring to residual solvent removal is not observed. The mass loss corresponding to the attached moiety decomposition is about 10% and it proceeds between 220 and 420°C.

Table 2. TGA analysis of pristine and ABTS-modified SWCNT

Sample	Mass loss below 200°C (%)	Mass loss in the range of 200–420°C	Estimated percentage of SWCNT (%)	Mol of moiety/mol of carbon	Assumed m.w. of detached moiety
Pristine SWCNT	0	0	100	–	–
SWCNT-ABTS-side	0	10	90	2.2×10^{-3}	597
SWCNT-ABTS-end	2	5	92	1.1×10^{-3}	583

3. Results

3.1. Glucose Oxidation on GC Electrodes Nanostructured with SWCNT-Fc and MWCNT-Fc and Covered with Cubic Phase Film Containing Glucose Oxidase

Due to insolubility and high electric conductivity carbon nanotubes are widely used to improve electron transfer between the electrode surface and enzyme redox sites. More, CNTs increase the total physical surface area of the electrode.

For the same purpose, the carbon nanotubes SWCNTs-Fc and MWCNTs-Fc with covalently attached ferrocene moieties were applied in this work onto GCE and covered with a film of the liquid-crystalline cubic phase embedding glucose oxidase catalyzing glucose oxidation. Redox reactions were monitored using cyclic voltammetry (Fig. 4). The formal potential in the case of SWCNT-Fc was equal to +0.240 mV *vs*. Ag/AgCl, whereas in the case of MWCNT-Fc redox potential decreased to +0.170 mV. The area under ferrocene peak, which is proportional to the charge exchanged with the electrode is larger for SWCNTs than for MWCNTs, indicating that more ferrocene groups are available in the former case.

Applicability of these electrodes for the oxidation of glucose was checked (Fig. 4A). Cyclic voltammograms of the GC electrode chemically "decorated" with MWCNTs-Fc and covered with the cubic phase containing glucose oxidase (GOD) were recorded in the absence and presence of glucose in argon-saturated buffer. After addition of glucose to the solution the anodic current increases and the cathodic current decreases. It reflects ferrocene-mediated bioelectrocatalysis of glucose oxidation. The catalytic process starts at 100 mV. When SWCNT-Fc instead of MWCNT-Fc

was used as the mediator, the catalytic process shifts to ca. 0 mV, it is 100 mV more negative than using MWCNTs (Fig. 4B). This is beneficial from the viewpoint of the bioanode application in a biofuel cell. Simultaneously, the maximum value of current density increased from 300 to 1,000 μAcm^{-2}.

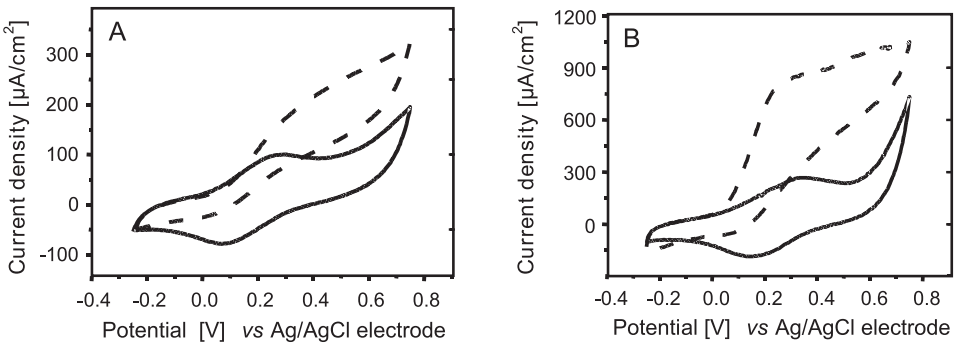

Fig. 4. Biocatalytic oxidation of glucose on GCE modified with A – MWCNT-Fc, and B – SWCNT-Fc. In both cases the GCE were covered with cubic phase incorporating glucose oxidase. Voltammograms recorded in 0.1 M McIlvaine buffer, pH 6.8. Glucose concentration 0 mM (solid line) and 15 mM (dashed line). Scan rate –1 mV/s

3.2. Dioxygen Reduction on Electrodes Modified with SWCNT-ABTS

On a bare GC electrode, dioxygen reduction proceeds with large overpotential ($E_{1/2} = -0.6$ V). In order to use laccase for catalytic reduction of dioxygen, usually soluble or bound to the electrode surface mediators are required. The most common organic mediator used in this case is ABTS, mainly because of its redox potential, which is close to that of laccase. To facilitate electron transfer between laccase and electrode, and to increase physical surface of glassy carbon the ABTS-derivatized single-walled carbon nanotubes SWCNT-ABTS-end, and SWCNT-ABTS-side (Fig. 3) were applied. Oxygen reduction catalyzed with laccase was studied using cyclovoltammetery in the 0.1 M McIlvaine buffer pH = 5.2 (Fig. 5). Pair of peaks observed for GCE/SWCNT-ABTS-end, and GCE/SWCNT-ABTS-side, corresponds to the electrode process of ABTS moiety. Redox potentials in both cases are close to 430 mV. This is almost identical with the potential of free ABTS on bare GCE, 472 mV *vs*. Ag/AgCl at pH 4.0 [37], and 505 mV *vs*. Ag/AgCl at pH 7.0 [37]. Cyclic voltammograms recorded on the GC electrode modified with SWCNT-ABTS-end or SWCNT-ABTS-side, and covered with the monoolein cubic phase layer containing laccase from *Cerrena unicolor* in the 0.1 M McIlvaine buffer, pH 5.2 show efficient catalysis of dioxygen reduction for both types of the CNTs covered electrodes. Maximum current density of dioxygen reduction reached 600 μAcm^{-2} for the GC electrode modified with SWCNT-ABTS-side (Fig. 5B) and 400 μAcm^{-2} when

SWCNT-ABTS-end (Fig. 5A) was used. For comparison, when SWCNTs with adsorbed ABTS were used the maximum current density was only 90 μAcm^{-2}, and it was even less, i.e. 24 μAcm^{-2} when ABTS was dissolved in the buffer solution.

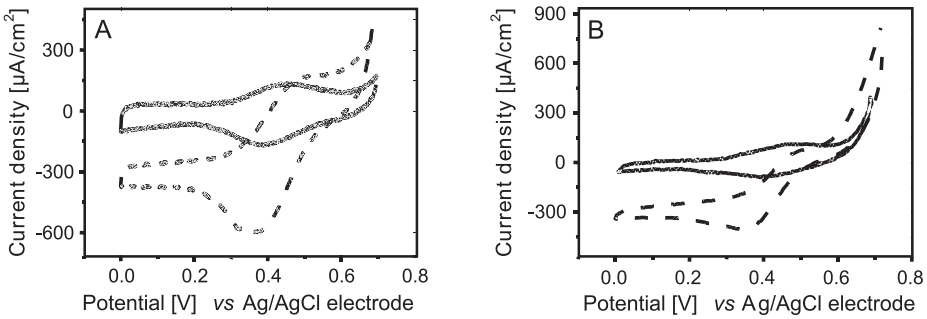

Fig. 5. Biocatalytic oxygen reduction at A – GC/SWCNTs-ABTS-end, and B – GC/SWCNTs-ABTS--side modified with cubic phase incorporating laccase. Voltammograms recorded in deoxygenated (solid line) or oxygenated (dashed line) 0.1 M McIlvaine buffer, pH 5.2. Scan rate – 1 mV/s

As shown for SWCNT-ABTS-end in Table 3, catalytic current density per one molecule of ABTS is larger indicating high efficiency of ABTS chemically anchored to the ends of carbon nanotubes. On the other hand, the total number of ABTS groups present on the electrode in case of walls derivatization (SWCNT-ABTS-side) is much larger, hence total catalytic current density for the SWCNT-ABTS-side is larger making them favorable for the application in the biofuel cell (Table 3).

Table 3. Characteristics of laccase catalyzed oxygen reduction current on glassy carbon electrodes (GCE) covered with SWCNTs derivatized with ABTS

	Q (C)	I_{cat}/A^* (μA/cm^2)	Γ (mol/cm^2)	I_{cat}/Γ (A/mol)
SWCNT-ABTS-end	6.2×10^{-4}	400	9.2×10^{-8}	4,347
SWCNT-ABTS-side	1.6×10^{-3}	600	2.3×10^{-7}	2,608

* Electrode surface area A = 0.071 cm^2

As noted above, the use of SWCNT-ABTS-end- or SWCNT-ABTS-side-based electrodes allowed decreasing the overpotential of dioxygen reduction to 0.5 V *vs*. Ag/AgCl. This is convenient for their application as biocathodes. Application of the electrodes modified with the ABTS derivatives of SWCNTs led also to high catalytic currents and mediator leaching to the solution phase was avoided. Leaching of mediator was monitored by placing the electrodes in argon-saturated solutions and measuring heights of the ABTS peaks. Lack of changes proved that no leaching took place.

3.3. Biofuel Cell Performance

The constructed electrodes were used in a glucose/O_2 biofuel cell. The cover of the cell used in our experiments was made of glass, solution volume was 20 ml. The variable loads, in the range from 1 kΩ to 10 MΩ, were applied between the anode and the cathode to determine the cell current (I_{cell}) and the cell voltage (V_{cell}). Figure 3 displays the voltammograms recorded for two systems used for the biofuel cell construction differing only on the anode side. The anode was the GC electrode covered with:

- pristine carbon nanotubes and modified with the cubic phase containing *A. niger* glucose oxidase and TTF as the mediator (Fig. 6A), or
- SWCNTs with covalently bound ferrocene (SWCNT-Fc) mediator covered with the liquid-crystalline film containing glucose oxidase (Fig. 6B).

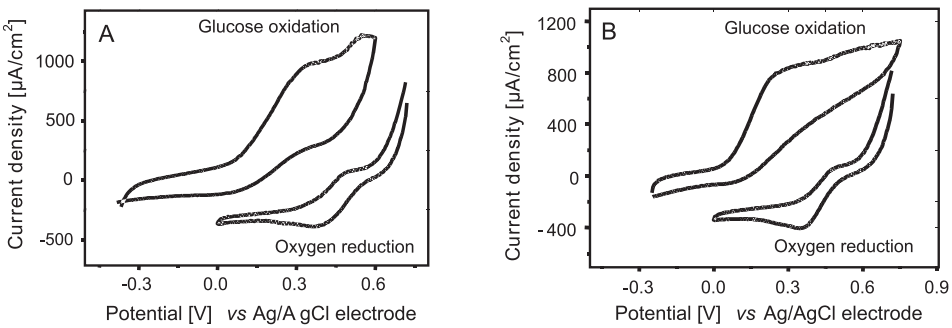

Fig. 6. A – Cathode – oxygen reduction catalyzed by laccase and ABTS covalently bound to SWCNT; anode – glucose oxidation on electrode modified with pristine SWCNT, and glucose oxidase and adsorbed TTF embedded in cubic phase. B – Cathode – oxygen reduction catalyzed by laccase and ABTS covalently bound to SWCNT; anode – glucose oxidation on electrode modified with SWCNT-Fc and glucose oxidase embedded in cubic phase. v = 1 mV/s

The catalytic current density for glucose oxidation was ca. 1 $mAcm^{-2}$ for the GC cathode nanostructured with SWCNT-ABTS-end, and covered with the lipid cubic phase film containing laccase. The onsets of catalytic anodic and cathodic currents are separated by ca. 500 mV. The open circuit potential measured was ca. 450 ± 40 mV and 430 ± 50 mV for systems based on TTF incorporated in the lipidic matrix, and that with SWCNTs-Fc, respectively. Figure 7 displays the dependence of power density on current density for the tested systems. In case of the TTF-based system, power density reached 60 ± 20 $\mu W/cm^2$ while in case of SWCNT-Fc power density increased to 100 ± 30 μWcm^{-2}. Continuous supply of oxygen stabilizes the biofuel cell.

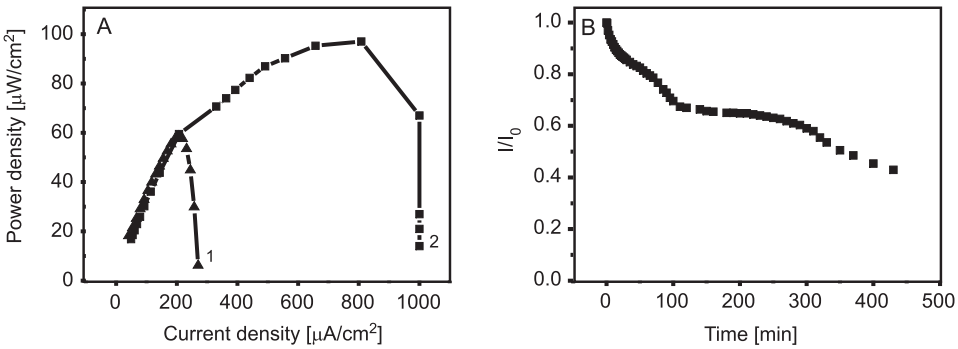

Fig. 7. A – Dependence of power density on current density for systems: (1) anode – TTF+GOD; cathode – ABTS + laccase; (2) anode SWCNT-Fc + GOD; cathode SWCNT-ABTS-end+laccase. B – time stability test: anode – SWCNT-Fc + GOD; cathode – SWCNT-ABTS-end + laccase. Electrolyte: 0.1 M McIlvaine buffer, pH 6.8

In case of the biofuel cell without nanotubes, measured maximum power density was only ca. 7 μW/cm^2. This is similar to the reported in most papers for quiescent solutions [8, 11]. Powers exceeding 100 μWcm^{-2} obtained when both cathode and anode are nanostructured with SWCNTs derivatized with appropriate mediators are becoming interesting practically. In the biofuel cell presented in this paper, a 40% decrease of initial power was observed after the first 100 min of continuous work under 90 kΩ load (Fig. 7B); at longer time scale, power decreases more slowly. This disadvantage needs to be excluded.

4. Conclusions

The nanostructered bioelectrodes were designed and tested as electrodes in the biofuel cell. The presented biofuel cell construction uses neither metal electrodes nor osmium-redox polymers commonly employed as mediating units. The mediators in the form of ferrocene or ABTS residues covalently bonded to the carbon nanotubes applied on glassy carbon led to novel effective nanomaterial for the electrode nanostructuring. In such approach there is no leaching of the mediator to the solution, a difficulty often reported for soluble or adsorbed mediators. Laccase and glucose oxidase used as the biocatalysts were placed in the lipid liquid-crystalline film covering the electrode nanostructured with the single-wall carbon nanotubes. The catalytic current of glucose oxidation was 1 mAcm^{-2} and oxygen reduction current exceeded 0.6 mAcm^{-2}. Powers exceeding 100 μWcm^{-2} obtained when both cathode and anode were nanostructured with the SWCNTs derivatized with appropriate mediators are becoming interesting practically. More work is needed to improve the long-term stability of the biofuel cells.

Acknowledgments

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