

ARYL AND N-ARYLAMIDE CARBON NANOTUBES FOR ELECTRICAL COUPLING OF LACCASE TO ELECTRODES IN BIOFUEL CELLS AND BIOBATTERIES

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Abstract

Single walled carbon nanotubes (SWCNTs) were equipped with aryl residues by chemical reactions. These insoluble materials were used to substitute classical soluble mediators, which help to transfer electrical charge between the conducting electrode and the redox active center of enzyme molecules. The effect of different aryl residues on the efficiency of the catalytic reduction of dioxygen in the presence of laccase was systematically studied using voltammetry and measuring the power output of a biofuel cell.

Keywords: biocathode, biobattery, chemically modified carbon nanotubes, electron transfer, laccase.

I. INTRODUCTION

Biofuel cells are of great importance due to predictable practical applications. A special feature of biofuel cells is clean electric energy production by conversion of chemical energy. The conversion could be carried out at ambient temperature, in neutral aqueous system and with high output that may achieve 100%. Important advantage of biological fuel cells (BFCs) over conventional fuel cells is low cost of their components. BFC and biobatteries use organisms, tissues or enzymes as catalysts and natural organic compounds as the fuels [see review articles: 1, 2, 3, 4, 5]. Specific type are implantable biofuel cells [6]. They are able to

produce electrical energy by redox processes of glucose and oxygen always present in physiological fluids. Such implantable devices may power devices implanted in practice such as monitors of human health, microvalves, drugs dispensers, and sensors [7, 8, 9,10]. Enzymatic biofuel cells are favorable also due to the specificity and selectivity of reactions running at the electrodes, thus they also could be constructed without membrane, separating the catholite and anolite. In case of enzymatic biofuel cells the substrates and products are environmentally friendly and the whole system could be made of biodegradable elements. Biofuel cells could be easily miniaturized. In order to achieve high durability and the possibly highest efficiency a variety of constructions of biofuel cells and biocathodes in particular for this purpose were elaborated [2, 4].

The biocatalysts used in case of biocathode are bilirubin oxidase or different multicopper oxidases, e.g. laccases, catalyzing four electron ($4e^-$) dioxygen reduction directly to water. The enzyme of choice frequently applied in biocathode compartment is laccase [e.g. 11] Active site of laccases contain four copper atoms organized as T1, T2 and T3 centers. The reaction sequence of oxygen reduction is presented well by [12, 13, 14]; whereas the mechanism and reaction kinetics were studied [15]. The scheme of reactions that proceed on the enzymatic cathode is shown in Figure 1.

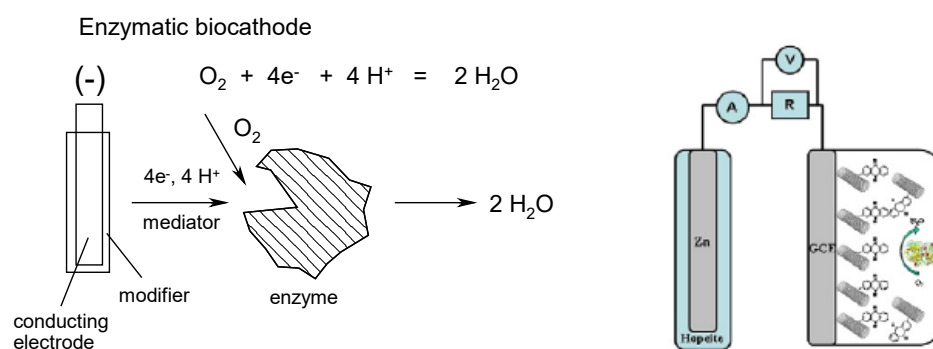


Fig. 1. Left: schematic of dioxygen reduction on biocathode upon catalytic action of an enzyme. Right: scheme of the respective biobattery [16].

The principle of bioanode operation is analogous to that of biocathode showing similar advantages and disadvantages. Both electrode processes appear to be simple, however, for



practical purpose the cell system and its elements should be properly optimized [4, 17]. The issues that still required to be solved are connected with the limited stability of purposely modified electrodes, transport limitations of the substrates and products of the redox reactions, not efficient direct electron transfer (DET) due to large distance between the electrode surface and the enzyme active center, and limited number of enzyme molecules, that can be electrically coupled to the electrode [16]. Parts of the electrochemical cells needing deeper research and improvements are: conducting electrode material, the mode of electron transfer, and high activity and selectivity of the applied enzyme.

As the electrode substrate first of all carbonaceous materials, e.g. different types of graphite or carbon aerogel [18] are applied. Glassy carbon (GC) structured with these carbonaceous materials are most frequently used as electrodes that replace smooth, expensive and not always desired metals, e.g. platinum. Structuring the conducting material also enormously increases physical surface area of electrode causing rise of the formal current density. In particular, important are hydrophobic carbon nanotubes (CNTs) possessing unique structural, mechanical and electric properties and showing splendid properties for enzymatic catalysis. CNTs can be used for the construction of extended conducting networks, and at the same time, for increasing the effective surface area of the electrode [4, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30]. In addition, CNTs can be easily modified non-covalently by adsorption, or by covalent bonding at the edges or defect sites, and on side-walls [31]. Functionalization of CNTs is also performed to increase biocompatibility and biomolecular recognition, to facilitate dispersion in macromolecular matrix, for highly specific electronic biosensors, and so on [32, 33]. Chemical modification of the nanotube side-wall or its terminus is generally needed to control dispersion of CNTs, a crucial parameter for the construction of devices [e.g. 34].

For the bioelectrocatalytic reduction of dioxygen mainly bilirubin oxidase or multicopper laccases are used. However, the distance between the enzyme active center and



the conducting electrode is too large for free flow of electric charge. Therefore, well water soluble redox compounds, named mediators, are typically used to facilitate/allow transfer of electrons between the conducting electrode and the T1 redox center of laccase [2, 4]. To the most frequently used mediators belong heavy metal complexes or soluble organic compounds. An example is ABTS [ammonium 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)], a compound with redox potentials almost equal to redox potentials of laccase [23, 35, 36, 37, 38, 39, 40]. Mediated electron transfer (MET) achieved by using such mediator in biocathode results in high catalytic current density. However, leaching of the mediator(s) from the electrochemical cell causes progressive drop of cell power. Moreover, leaching from implanted cells could be hazardous to the implant bearer. Due to the listed above negative aspects adsorptive (physical) immobilization of mediators on carbonaceous materials was proposed [31], however slow leaching owing to desorption cannot be avoided.

The above reasons lead us to the application of solid insoluble and conducting materials to which e.g. ABTS was chemically attached. In our recent papers, ABTS was chemically bonded in different arrangements to carbon nanotubes; the resulting materials structuring glassy carbon electrode show excellent electron transfer properties [10, 41, 42, 43, 44]. Covalent binding of ABTS to the walls or terminus of nanotubes is advantageous since it completely eliminates leaching of mediator to the solution (or from the biofuel cell) that significantly improves the catalytic efficiency and leads to a stable biocathode system. However, ABTS by itself slowly damages laccase molecules. Hence other groups derivatizing CNTs are desired to play the role of electron carrier.

Banks et al. [45] modified basal plane of pyrolytic graphite electrodes *via* abrasive immobilization of nanotubes with covalently attached anthraquinone residues. The aromatic residue operated as an effective mediator for the electrocatalytic reduction of dioxygen, which was observed at -0.29 V *vs.* SCE. It is almost identical with the potential of anthraquinone



redox processes, proving the mediated electron transfer (MET) mechanism. More stimulating was observation made by Blanford et al. who chemically coupled anthracene to the surface of pyrolytic graphite electrode by electroreduction of diazonium salt. The aryl groups of modified carbonaceous material were shown to enter the hydrophobic pocket at T1 center of copper oxidoreductase facilitating direct electron transfer (DET) between the electrode and active center of laccase [46]. In this case the anthryl residue is not electrochemically redox active in the potentials range applied to study biocathode properties.

Direct electron transfer (DET) of electrons between the active site of the enzyme and the electrode surface is more attractive than mediated electron transfer (MET) because of its simplicity and rather high power-output. On unmodified electrode surface, laccase molecules adsorb only weakly, thus such system is only slightly catalytically active. Attachment of substrate-like lipophilic anthracene based units to the surface of pyrolytic graphite greatly enhances the adsorption of the enzymes [46]. The authors suggest, that each anthracene moiety provides a rigid, hydrophobic, π -electron conductor with the appropriate length, width and projection angle to insert tightly into the hydrophobic pocket of laccase. They described the enzyme-anthracene unit interaction as ‘plugin-socket’ attachment.

The carbonaceous Blanford’s material anthrylated by free radical procedure has a fairly complicated structure. Doppelt et al. [47] perceived, that free radicals, generated by thermolysis or electrochemical reduction of diazonium salts in order to functionalize carbonaceous materials, react to a great extent with aryl groups already attached to the carbon surface and form higher aromatic and azo aromatic units. Successive reactions of arylated pyrolytic graphite with radicals was also suggested by Blanford et al. [46] and proven in case of arylated carbon nanotubes by Sadowska et al. [48]. The Doppelt’s approach led to the formation of layers of branched aryl structures thicker than monomolecular. This explains relatively high catalytic currents of oxygen reduction and additional voltammetric peaks appearance [47].



Sosna et al. electrochemically prepared monolayers of anthraquinone and anthracene residues covalently bonded *via* spacers to GC electrodes. The authors used these structures as platforms adsorbing *Trametes hirsuta* laccase providing direct electric contact with the active site of enzyme [14]. The enzyme adsorption was evidenced by different techniques (XPS, EIS, voltammetry). The catalytic dioxygen reduction started at 0.6 V vs. SCE electrode; however, the catalytic current density was less than 1 $\mu\text{A}/\text{cm}^2$. It indicates that the derivatization degree of rather smooth GC electrodes is low, which means that only a small part of laccase is adsorbed and electrically connected with the electrode.

Considering the above, it was expected that conducting electrode nanostructured with the properly chemically modified nanotubes (CNT) would be beneficial for electron transfer properties of bioelectrodes and the whole biofuel cell. In all experiments oxidoreductase *Cerrena unicolor* was applied. It catalyses dioxygen reduction directly to water excluding harmful for living organisms intermediate oxygen derivatives on lower oxidation states.

Having in mind implantability of biofuel cells, carbon nanotubes as the substrate for the immobilization of electron carriers were chosen due to low density, high electric conductivity and large surface area, i.e. properties of high importance for bioelectrodes. Carbon nanotubes are also insoluble in water, highly resistant toward oxidation and reduction, and are not harmful for living cells [49]. Nanostructuring the glassy carbon conducting electrode with (single wall) carbon nanotubes (SWCNT) and with modified SWCNTs in particular would transform the 2D surface into the 3D fully electrically connected assembly. It allows significantly more laccase molecules to be plugged in by the electrode surface and, as a result, leading to significant increase of the catalytic dioxygen reduction current. On the other hand, the side wall functionalization of CNTs changes the local sp^2 into sp^3 hybridization of neighboring carbon atoms. Consequently, the CNT–substituent bond is perpendicular to the side wall. This should



effectively tie the lipophilic pocket that shields the enzyme active center and increase its affinity towards SWCNTs.

The aim of this study was to recognize how different aromatic residues and their different attachment to CNTs can affect transfer of electrons between enzyme active center and working electrode. The stimulating results [2, 14, 46] discussed above with modified carbonaceous materials prompted us to examine electrocatalytic properties of single walled carbon nanotubes (SWCNTs) equipped with anthryl and antraquinyl residues [11, 16, 43, 48, 50–53]. Due to promising results the set of aryl residues was extended by new type of derivatives. Figure 2 shows the reaction schemes leading to SWCNTs modified with aryl substituents.

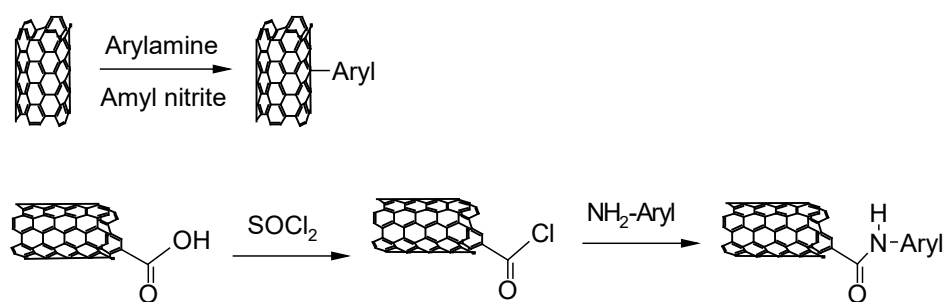


Fig. 2. Scheme of synthesis of aryl-SWCNT and N-arylamides.

The studied derivatives cover: 1° - materials with aromatic residues joined to side walls of SWCNTs by direct carbon-to-carbon bond **1 – 5**, always accompanied by higher aromatic residues formed as a result of successive reaction with an excess of radicals, exemplified as **1A–1C**, **2A–2C** and **5A–5C** (Fig. 3) [11, 16, 43, 46–48, 50–53], and 2° - indirectly by N-arylamide bond of terminal carboxylic group (or -COOH groups created at defect sites). In the last case, the products **6–10** (Fig. 4) [53] could not be contaminated by extended, branched aromatic systems. Use of free radicals or condensation reactions allows more or less preferential functionalization of walls or terminus of carbon nanotubes [31, 46–48, 53].

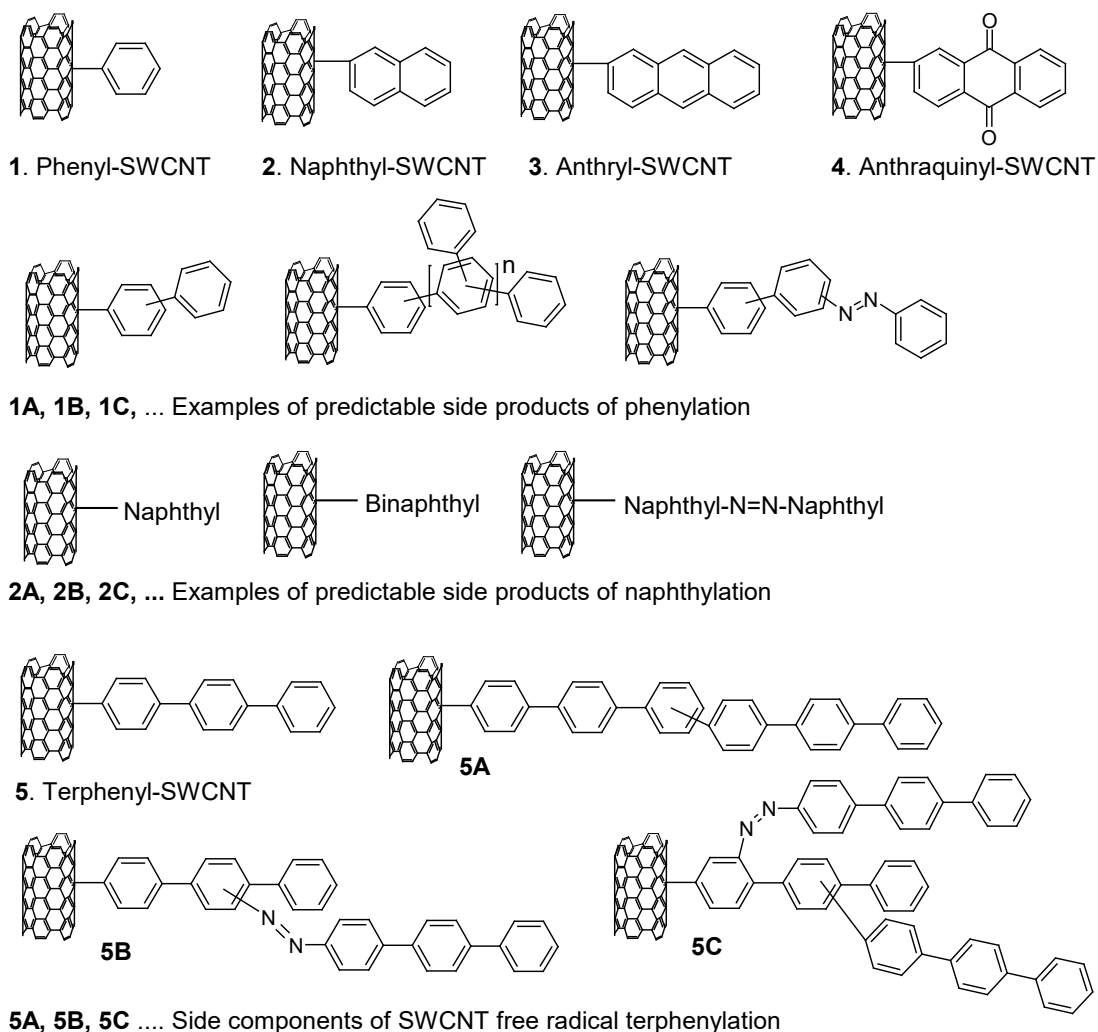


Fig. 3. Free radical arylated SWCNTs with direct C-C bond and examples of likely formed side products of arylation and azo-coupling of arylated SWCNTs

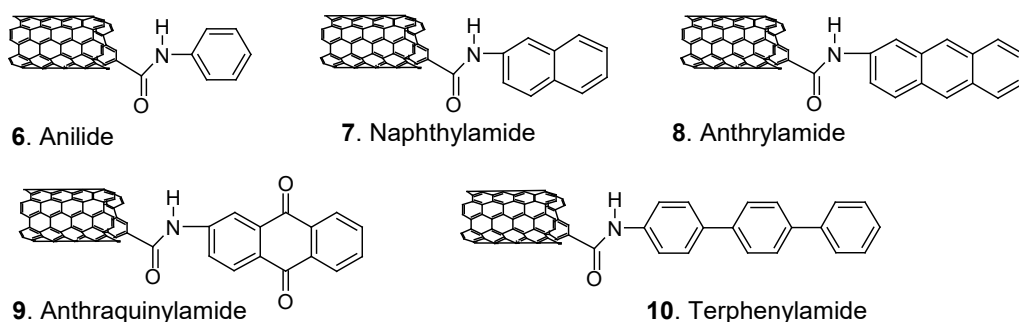


Fig. 4. Terminal amides of aromatic amines

The products were characterized by thermogravimetry and by Raman spectroscopy. The above materials were applied on glassy carbon electrode and the modified electrodes characterized by electrochemical methods. The results are summarized in Table 1.

Table 1. Values of current densities of oxygen reduction for GC electrodes covered first with carbon nanotubes modified with aryl residues and then with a mixture of laccase and Nafion, and measured at potential equal 0.2 V at a sweep rate of 1 mV/s. Supporting electrolyte: McIlvaine buffer (pH 5.2)/0.2 M NaNO₃.

GC ELECTRODE COVERED with side wall modified SWCNTs (Fig 3)	j_{beg} [$\mu\text{A}/\text{cm}^2$]	j_{cat} [$\mu\text{A}/\text{cm}^2$]	$j_{\text{cat}} - j_{\text{beg}}$ [$\mu\text{A}/\text{cm}^2$]	References
Mixture of phenylation products 1 and 1A – 1C (Fig. 3)	-51.5±2.4	-150.3±23.9	-98.8±21.1	[53]
mixture of naphthylation products 2 and 2A – 2C	-51.0±8.0	-241.2±7.0	-190.2±1.0	[53]
Mixture of anthryl derivative 3 + side products of anthrylation	-22.6 ±5.7	-238.5±32.5	-215.8±33.0	[16]
Mixture of anthraquinyl derivative 4 + side products	-27.3±5.0	-214.5±14.5	-187.2±12.0	[16]
Mixture of terphenyl derivatives 5 – 5C	-12.5±2.6	88.1±17.5	-75.6±14.9	this paper, [54]
GC ELECTRODE MODIFIED with arylamides (Fig 4)				
Starting SWCNTs-COOH	-64.1±3.2	-131.9±4.6	-67.8±1.4	this paper
Anilide 6 / laccase + Nafion	-0.23±0.65	-31.7±1.1	-11.5±1.2	this paper [54]
Naphthylamide 7	-47.5±9.9	-232.7±11.0	-185.2±1.1	this paper [54]
Anthrylamide 8	-121.2±15.9	-215.0±25.9	-93.8±10	[16]
Anthraquinylamide 9	-29.3±2.4	-181.0±14.0	-151.7±16.4	[16]
Terphenylamide 10	-35.6±17.9	-185.3±62.0	-149.7±44.1	this paper [54]

II. METHODOLOGY

The inorganic reagents from POCh (Gliwice, Poland), and the organic reagents from Aldrich, were used without further purification. Pristine single-walled carbon nanotubes (>90%) purchased from CheapTubes.com (Brattleboro, USA; [for SEM image see 48] before using for chemical modification were suspended in 4 N hydrochloric acid and sonicated for 4 h at

50°C to remove metal ions. The solid was collected by filtration and exhaustively washed with water to neutrality then with methanol and dried in vacuum [55, 56]. The commercial oxidized SWCNTs (CheapTubes) were used as received. Water was distilled and passed through Milli-Q purification system.

For detailed description of Laccase *Cerrena unicolor* C-139 preparation see [16]. Crude laccase from the fermentor scale cultivation was purified by ion exchange chromatography on DEAE-Sepharose (fast flow) [57] and lyophilized. The concentration of isolated and frozen (-18°C) enzyme was $C_{\text{lacc}} = 178 \text{ } \mu\text{g}/\text{cm}^3$ and activity 186,000 nkat/dm³. After lyophilizing, the laccase activity [58] dissolved in 1 ml of water was 3,000,000 nkat/dm³ and $C_{\text{lacc}} = 1.5 \text{ mg}/\text{cm}^3$.

Thermogravimetric analyses (TGA/DTA) were conducted using Universal V4.3A TA Instrument in argon atmosphere at a heating rate of 10°/min. The derivatization degrees were calculated according to recommendations [59–61] assuming temperature of 600°C as sufficiently high for complete detachment of substituents. The calculated results are collected in Table 2 and 3.

Raman spectra were collected using the Witec confocal Raman microscope system (Ulm, Germany) equipped with a fiber coupled Melles Griot (Carlsbad, CA) argon ion laser operating at 514.5 nm focused through a 60×objective. Collected light was dispersed through a triple monochromator (600 g/mm, 500 nm blaze angle) and detected with a thermoelectrically cooled (-60°C) charge-coupled device. A small amount of carbon nanotubes in form of powder was placed on a microscope slide and covered with a coverslip. Laser power at the sample was approximately 5 mW. The Raman data are collected in Table 2 and 3.

Arylation of SWCNTs on walls [c.f. 53]: To a mixture of 50 mg of carbon nanotubes (~4.2 mmol of C), 5.2 mmol of appropriate amine (aniline, 2-aminoanthracene, 2-



aminoanthraquinone or aminoterphenylene) chlorobenzene (or *o*-dichloro-benzene) (5 ml) and acetonitrile (5 ml) sonicated for 15 min, amyl or isoamyl nitrite (5.2 mmol) was added. The mixture was sonicated at 65°C for 6h and then diluted with DMF, sonicated for few minutes and centrifuged. This procedure was repeated several times, and then the mixture was washed with toluene, methanol, ethyl acetate, diluted acetic acid, acetone etc. until the washings become clear, colorless and not fluorescing. Washing with hot pyridine cleans the products very effectively. Residual pyridine was removed by washing with toluene, diluted hydrochloric acid, and exhaustively with methanol. Finally the product was dried in vacuum. In case of naphthylation the respective amount of 2-aminonaphthalene hydrochloride was used followed by addition of 1 ml of dry pyridine. Structures of aryl residues attached to the walls of SWCNTs including side products are collected in Fig. 3.

Synthesis of terminal amides of SWCNTs: To 50 mg of carboxylated nanotubes (SWCNT-COOH) suspended in thionyl chloride (2 ml) 0.01 ml dry pyridine was added. The mixture was sonicated at 65°C for 5 h. Then 2 ml of dry toluene was added and the volatiles were removed under reduced pressure. To the residue 1 mmol of corresponding aromatic amine was added followed by 2 ml of dry pyridine. The mixture was sonicated at 50-60°C for 4 h. Methanol (4 ml) was added to the cooled mixture, sonicated for a while and centrifuged. Washing with methanol was repeated several times. Finally the product was washed with methylene chloride and dried. Structures of the obtained materials are shown in Fig. 4.

Preparation of biocathode and electrochemistry: A GCE biocathode with 0.07 cm² working surface was covered with suspension of modified carbon nanotubes and then with a mixture of laccase dissolved in McIlvaine buffer (pH 5.2) and Nafion solution [44]. One electrode requires ~40 µg modified SWCNTs and ~8 µg of laccase.

GCE/SWCNT-aryl/laccase + Nafion electrode preparation: Onto GCE surface 10 µl suspension of side wall arylated SWCNTs (1–5) in ethanol (4 mg/ml) was dropped. After



drying, 10 μl of enzyme/Nafion casting solution was pipetted onto the electrode and allowed to dry at room temp.

Laccase solution was prepared by dissolving 1 mg of enzyme in 0.64 ml McIlvaine buffer containing 0.2 M NaNO_3 (pH 5.2). 1% Nafion was prepared by dissolution of 5% Nafion in ethanol. Mixture of laccase and Nafion was prepared by mixing 50 μl of Nafion and 50 μl laccase solution. The biofuel cell parameters were examined in dioxygen saturated McIlvaine buffer solution (pH 5.2)/0.2 M NaNO_3 and in 0.2 M phosphate buffer (pH 7.1).

Electrochemical experiments were done in three electrode arrangement with Ag/AgCl (KCl sat.) reference electrode, platinum foil as the counter electrode and glassy carbon electrode (GCE, BAS) as the working electrode with surface area of 0.071 cm^2 . Cyclic voltammetry experiments were carried out using ECO ChemieAutolab potentiostat. All electrochemical measurements were done at $22\pm 2^\circ\text{C}$. All current densities were calculated using geometrical area of the electrode.

The biofuel cell parameters were examined in dioxygen saturated McIlvaine buffer solution (pH 5.2)/0.2 M NaNO_3 and in 0.2 M phosphate buffer (pH 7.1) [16]. Fig. 1 (right) shows the configuration of the biobattery. Open circuit voltage (OCV) was measured in all experiments. The cell voltage (V_{cell}), the anode voltage (V_{a}) and the cathode voltage (V_{c}) were measured under varying loadings in the range from 1 $\text{k}\Omega$ to 10 $\text{M}\Omega$. To minimize the power loss caused by fuel depletion during the test, measurements under each load were restricted to 5 s. The anodes for the Zn–O₂ hybrid fuel cells (called further biobatteries) were zinc wires (0.25 mm diameter, Goodfellow) coated with a Nafion film by dipping in 0.5% Nafion solution in ethanol and dried for 5 min at ambient conditions. A hopeite layer ($\text{Zn}_3(\text{PO}_4)_2\cdot 4\text{H}_2\text{O}$) was formed on metal surface during discharging in buffer solution [62, 63]. The hopeite film blocks dioxygen transport to the Zn surface, thus enabling the necessary transport of Zn^{2+} while preventing Zn corrosion. The open cell voltage for such hybrid



batteries is high due to negative value of Zn/Zn^{2+} redox potential ($E_0 = -0.76$ V vs. NHE [64]). The potential of such zinc electrode is constant. The lengths of the Zn–O₂ electrodes were adjusted to obtain the same surface area as that of the biocathode [16, 65].

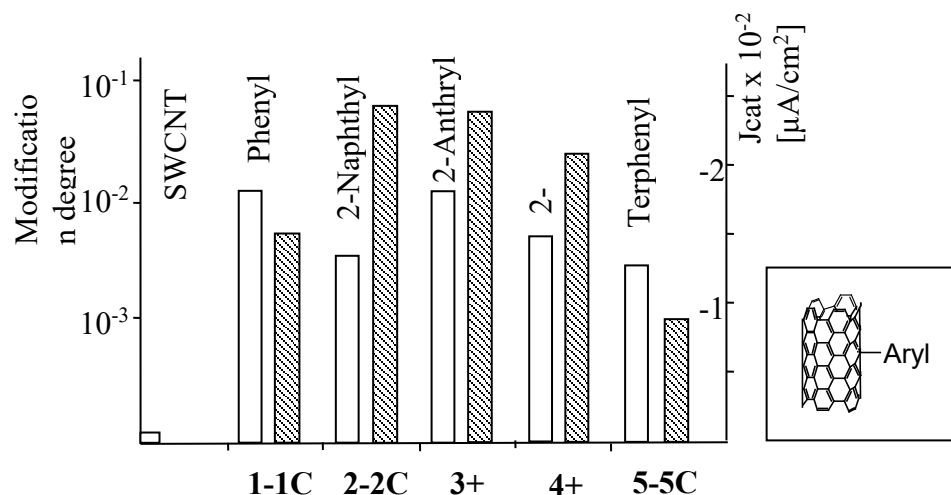


Fig. 5. Comparison of SWCNTs arylation degree (TGA data - empty bars) with the catalytic current density obtained using the respective arylated SWCNTs for electrode modification (dashed bars): phenyl (1–1C), terphenyl (5–5C), naphthyl (2–2C), anthryl (3 + side products), anthraquinyl (4 + side products). See Table 2 below.

Table 2. TGA and Raman characteristics of arylated SWCNTs and electrocatalytic properties of GC modified electrodes

SWCNT Sample, See Fig. 3	Mol of moiety/ mol carbon up to 600°C, TGA	Jcat/SWCNTs modification degree ^a	Raman data			Electrochemistry j_{cat} [$\mu A/cm^2$] and References
			D/G	G'/G	G'/D	
Pristine SWCNTs	0%		0.12	0.32	2.78	
1 – 1C	1.08×10^{-2}	~14	0.195	0.300	1.543	-150.3±23.9 [53]
2 – 2C	5.1×10^{-3}	~47	0.119	0.329	2.761	-241.2±7.0 [53]
3 + anthrylation side products	$2,8 \cdot 10^{-3}$	~85	0.72	0.72	1.01	-238.5± 33.0 [16]
4 + anthraquinyla- tion side products	6.5×10^{-3}	~33	0.26	0.45	3.61	-214.5±14.5 [16]
5 – 5C	4.4×10^{-3}	~20	0.178	0.377	2.118	-88.1±17.5 this paper, [54]

^a arbitrary units

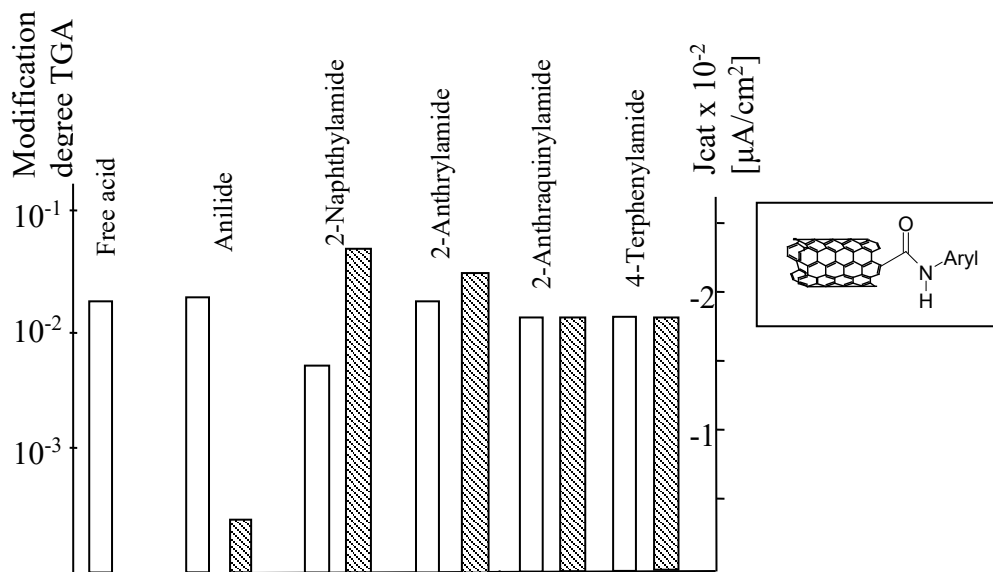


Fig. 6. Comparison of SWCNTs N-arylamide modification degree (TGA data - empty bars) with the catalytic current density obtained using the respective N-arylamide SWCNTs for electrode modification (dashed bars): phenyl **6**, naphthyl **7**, anthryl **8**, terphenyl **9** and anthraquinyl **10** amides of SWCNTs. See Table 3 below.

Table 3. TGA and Raman characteristics of N-arylamide SWCNTs and electrocatalytic properties of GC modified electrodes

SWCNTs sample, See Fig. 4	TGA Mol moiety/ mol of carbon up to 600°C	J _{cat} /SWCNTs modification degree ^a	Raman data			Electrochemistry j _{cat} [μA/cm ²] and References
			D/G	G'/G	G'/D	
SWCNTs-COOH	3.1x10 ⁻²	~4	0.091	0.291	3.195	-131.9±4.6 this paper
Anilide 6 ;	2x10 ⁻²	~2	0.143	0.302	2.109	-31.7±1.1 this paper
Naphthylamide 7	8.7x10 ⁻³	~27				-232.7±11.0 this paper, [54]
Anthrylamide 8	1.6x10 ⁻²	~13	1.18	0.58	0.49	-215.0±25.9 [16, 54]
Anthraquinylamide 9	1.06·10 ⁻²	~17	0.17	0.42	2.52	-181.0±14.0 this paper, [16]
Terphenylamide 10	1.1x10 ⁻²	~17	0.064	0.240	3.775	-185.3±62.0 this paper, [54]

^a arbitrary units

III. RESULTS

Syntheses

Arylation of hydrochloric acid pretreated SWCNTs carried out by free radical reaction was assumed to proceed on side walls [31, cf. 48]. The first step was generation of diazonium compounds from aromatic amines by treating with amyl or isoamyl nitrite. The diazonium intermediates were thermally decomposed in the presence of carbon nanotubes under permanent sonication at 60–65°C to form arylated nanotubes (Fig. 3, materials **1 – 5**). The formulas may not reflect the real structures since the radicals generated are able to react not only with carbon nanotubes but also with aryl groups that in the first step were bonded to the SWCNT side wall [48]. Similar consecutive reactions of arylated graphite and glassy carbon were observed earlier [46, 47, 14]. However, free radical reaction affects not only carbon nanotubes, but also diazonium precursor applied in large excess. The side reaction causes formation of great amount of high molecular polycyclic aromatic compounds seriously contaminating the desired products. Washout of the impurities is troublesome; mostly there are only slightly soluble in many solvents and due to lipophilic properties they are strongly adsorbed on SWCNTs. Therefore the reaction products were exhaustively washed with a range of hot solvents, each time after vigorous sonication. The washing was repeated dozens of times until the clear and colorless extracts do not show fluorescence. The nanotubes were assumed to be fully washed when extracts were not fluorescing. Because aromatic compounds are strongly adsorbed on nanotubes, advantageous is extraction with hot pyridine followed by washing with toluene, methanol and exhaustive drying under reduced pressure. Results of experiments are summarized in Fig 5 and in Table 2.

Amides **6 – 10** (Fig. 4) were obtained activating first the SWCNT-COOH with thionyl chloride [66] followed by reaction with aromatic amines in dry pyridine under sonication at 60°C for 5-6 hours. In this case removal of excessive solvent and other reagents was rather



easy to perform. The products are more homogenous as they do not contain branched aromatic residues. The results are shown in Figure 6 and in Table 3.

In both synthetic procedures centrifugation or filtration was applied to separate modified nanotubes.

Samples of all modified SWCNTs were characterized by TGA analysis and by Raman spectroscopy. Data for some materials were presented earlier. Both techniques confirmed chemical functionalization; more details can be found in [43, 44, 48, 50].

Calculation of the TGA data show that the number of aryl residues in products **1–5** (Fig. 3 and Table 2) per SWCNT's carbon atom are 1.2×10^{-2} , 4.4×10^{-3} , 5.1×10^{-3} , 2.8×10^{-3} and 6.5×10^{-3} , respectively. It means roughly one substituent per 100 to 800 carbon atoms. The diversified modification degrees may be attributed to different reactivities of free radicals generated from various diazonium salts. It should be also noted, that the arylation reaction proceeds in heterogeneous system that does not provide adequate conditions for reactions standardization. As mentioned above, formation of branched structures of aryl derivatives in the free radical reaction is very probable; thus the number of aryl moieties directly bonded to SWCNTs surface carbons is definitely lower.

Unfortunately, the TGA data are not very accurate. In case of aromatic substituents (with extended structure in particular) thermal removal proceeds fully presumably at temperatures exceeding recommended 600°C [59–61]. Hence, the determined modification degree considering decomposition till 600°C seems to be too low. The modification degrees calculated from TGA data up to 800°C are higher and are in the range of $0.8\text{--}2.4 \times 10^{-2}$ mol of aryl residues per mol of carbon. Interestingly, a deeply colored side product isolated from the arylation reaction under TGA conditions evaporates to a great extent above 600°C . Above this temperature also some fractions of carbon nanotubes are probably evaporated.



The modification degrees in case of N-arylamides of SWCNTs **6–10** compared to direct arylation are approximately higher two times or by one order of magnitude; the values of TGA data collected to 600°C are 3.1×10^{-2} for the starting carboxylic acid and then tends to decrease to 2×10^{-2} , 0.87×10^{-2} , 1.6×10^{-2} , 1.06×10^{-2} and 1.1×10^{-2} for anilide, 2-naphthylamide, 2-anthrylamide, 2-anthraquinylamide and 4-terphenylamide, respectively (Fig. 4 and Table 3). With except of 2-naphthylamide, this order corresponds to the increasing size and thus to the decreasing reactivity of the acylated arylamines. Contrary to the free radical arylation products, the N-aryl amides do not undergo branching. In case of materials **6–10** the temperature of 600°C is high enough for complete detachment of substituents; all the released products are volatile.

The TGA data and characteristics of Raman spectra are collected in Table 2 and 3. An example of TGA/DTG is shown in Figure 7.

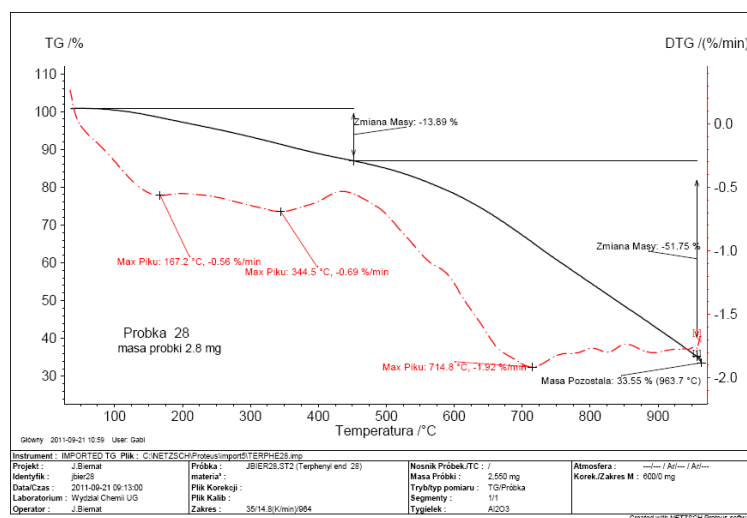


Fig. 7. TGA/DTG of a mixture of SWCNT terphenylated by free radical reaction.

The products of SWCNT derivatization were also studied by Raman spectroscopy. The so-called G-mode is a characteristic signal of the graphitic layers and refers to the tangential vibration of the carbon atoms. The second characteristic band is a typical sign for defective graphitic structures (D-mode). Chemical modification of SWCNTs side walls somehow destroys carbon network, so successful modification is revealed as the D band intensity

increase. Measuring the D/G ratio of band intensities the degree of functionalization could be qualitatively assessed and used to compare different samples. Among arylated nanotubes the highest functionalization seems to be in the case of SWCNT derivatized with anthryl residue **3**, found also from TGA results. On the other hand for the remaining arylated species the D/G intensity ratios are substantially lower (Table 2), although it is not in exact accordance with TGA analyses. These results also correlate only to some extent with electrocatalytic properties (see below). Due to purity requirements the materials **3** and **4** were extremely washed with a huge amount of different solvents; partial separation of different types and broken tubes in particular may affect the resulting Raman data.

It should be underlined, that Raman spectra for side wall arylated SWCNTs reveal presence of modes at approximately 1430 cm^{-1} , which can be attributed to -N=N- and aryl ring modes. This is a proof for successive reaction of free radicals and diazonium salts with aromatic residues bonded to CNTs [48, 47].

The D/G intensities in Raman spectra of arylamides **6–10** (Table 3) are only little differentiated and indicating lack of the side walls participation in amidation reactions. The derivatization degree for the amides are more or less consistent with the derivatization degree of the starting SWCNTs-COOH.

Results of electrochemical measurements of electrocatalytic currents of biocathodes in biobatteries are summarized in Table 2 and 3. Table 4 presents the parameters of selected biobatteries based on different arylated SWCNTs.

Table 4. Characteristics of biobatteries based on GC biocathodes covered with functionalized SWCNTs and laccase in Nafion film. Anode: Zn/ hopeite-phase. McIlvaine buffer solution + 0.2 M NaNO_3 (pH 5.2). The loading is $20\text{ k}\Omega$ in each case.

Biocathode modified with arylated SWCNTs:	P_{\max} [$\mu\text{W}/\text{cm}^2$]	j [$\mu\text{A}/\text{cm}^2$]	R [$\text{k}\Omega$]	E [OCV] [V]
1 – 1C	1105.0	884.0	20	1.54
2 – 2C	968.1	827.4	20	1.54

3 + higher arylation products	1001.5	841.6	20	1.53
4 + higher arylation products	750.3	728.4	20	1.53
5 – 5C	779.7	742.6	20	1.59

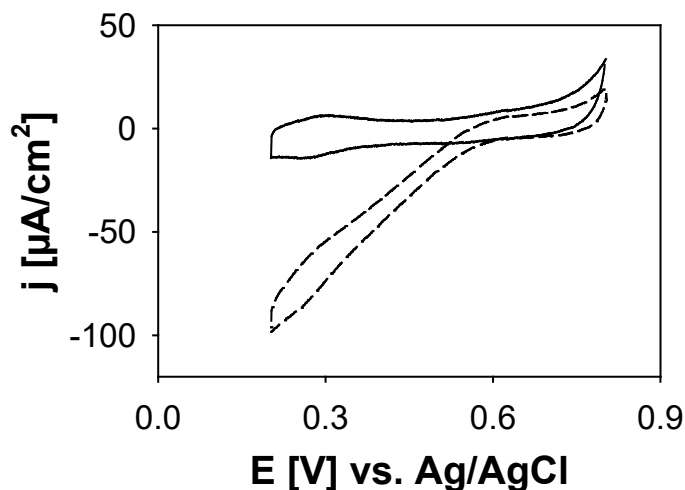


Fig. 8. Cyclic voltammograms recorded in (—) deoxygenated and (---) saturated with dioxygen solution using electrodes covered with terphenylated SWCNTs (**5–5C**) /laccase in Nafion layer in McIlvaine buffer solution (pH 5.2)/0.2 M NaNO₃, scan rate 1 mV/s.

IV. DISCUSSION

Electron conducting properties of aromatic systems is well known phenomenon. It explains facile charge exchange between side wall arylated carbon nanotubes deposited on basal cathode and the active center of the applied enzyme. Although the concentration of aryl residues on SWCNTs is low, the system conducts electrons quite well. The catalytic currents observed for preparations **1–5** change with the derivatization degree. The best currents are observed for naphthyl- (**2**) and anthryl-SWCNTs (**3**). Some differences may be ascribed to different branching of aryl substituents (see Fig. 3) causing variations in steric hindrance that affects the enzyme adsorption [46]. The adsorption depends on the best fit of the shape of aromatic substituent(s) and setting of the lipophilic surrounding of the active center. Considering the catalytic currents for different arylated SWCNTs it could be stated that the sizes of aryl residues are not the main factor; e.g. the terphenylated material **5** shows the

lowest influence on the catalytic current. Catalytic properties of material **5** are the most similar to the phenylated carbon nanotubes **1**. This could mean structural similarity of formed mixtures **1–1C** and **5–5C**. It may also mean that the products **5–5C** due to consecutive free radical reaction have too big volume to be well adopted by lipophilic pocket of the enzyme.

The structure and size of aryl residues also affect charge transfer [67]. The behavior of anthraquinyl residue is difficult to explain. The anthraquinone redox potentials and its similarity to the behavior of anthraquinylated carbonaceous material [45] was considered to be an evidence for mediated electron transfer mechanism. However, the catalytic currents caused by naphthyl and anthryl residues are very similar and suggest rather direct electron transfer. It may also mean that electron withdrawing carbonyl groups do not substantially affect electron transfer; it could also mean that high polarity of carbonyl groups do not substantially affect the enzyme adsorption.

The above observations lead to conclusion that charge transport could be facilitated by other aromatic residues, e.g. terminal N-aryl carboxamides of carbon nanotubes. Application of this type of derivatives was reported by us in conference presentations, in publication [43] and in patent application [54]

The TGA data show that in case of N-arylamides the derivatization degree is roughly higher by one order of magnitude compared to side arylated products (Figure 6 and Table 3). However, the catalytic properties of biocathodes modified with arylamides **6–10** are generally poorer. On the other hand the synthesis and purification of N-arylamides is really facile. In addition, the structure of amides is much better defined as successive reactions could not proceed.

Interesting is the ratio (in arbitrary units) of electrocatalytic properties of aryl or arylamides to the degree of SWCNTs functionalization (Table 2 and 3). The above statements



are better exposed and show the highest catalytic improvements of side naphthyl, anthryl, anthraquinyl residues and N-naphthylamide of SWCNTs.

Concluding, it should be emphasized that the above presented relations are showing only some trends in this kind of chemistry. The main reason for only qualitative descriptions is relatively low accuracy of experimental results that are insufficient for more accurate explanations of observed phenomena.

Recently, S. Minter applied 2-anthryl ester of terminal carboxylated multiwall carbon nanotubes for construction of biocathode [68]. This type of derivative belongs to “active” esters known from their susceptibility to react fast with nucleophiles. Finally it appeared paper [69] in which the authors immobilized laccase on CNTs modified with anthraquinone residue. These papers emphasize importance and value of our previous reports.

V. CONCLUSIONS

Systematic studies of catalytic activity of biocathode components, its constitution and texture explain differences in electrode behavior. It was found that the most effective charge conducting substituents attached to SWCNTs are phenyl, naphthyl, anthryl, terphenyl and anthraquinyl residues. The structure of spacers linking the aryl residues to nanotubes by various bonds less evidently affect the electrode behavior.

By many respects the described biocathodes fulfill parameters anticipated for bioelectrodes applicable in implantable cells. The measurements show dependence of the biocathode behavior on components of cathodic compartment and, in particular, on material applied to assist charge exchange. The CNTs with aromatic residues play well the role of insoluble molecular wires and the power and current densities are very high. The last parameters presumably depend on the best fit of the aromatic substituent dimensions attached to SWCNTs and the size of the hydrophobic hole of enzyme active site.

Combination of biocathode with zinc electrode of constant potential forming biobattery is useful for characterization of modified electrodes. Powers of the biobatteries upon 20 k Ω loading equal >1.5 V is high and is well adapted to the requirements of the most common electric/electronic devices.

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