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Enhanced performance of electrospun carbon fibers modified with carbon nanotubes: promising electrodes for enzymatic biofuel cells

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Abstract

New nanostructured electrodes, promising for the production of clean and renewable energy in biofuel cells, were developed with success. For this purpose, carbon nanofibers were produced by the electrospinning of polyacrylonitrile solution followed by convenient thermal treatments (stabilization followed by carbonization at 1000, 1200 and 1400°C), and carbon nanotubes were adsorbed on the surfaces of the fibers by a dipping method. The morphology of the developed electrodes was characterized by several techniques (SEM, Raman spectroscopy, electrical conductivity measurement). The electrochemical properties were evaluated through cyclic voltammetry, where the influence of the carbonization temperature of the fibers and the beneficial contribution of the carbon nanotubes were observed through the reversibility and size of the redox peaks of $K_3Fe(CN)_6$ versus Ag/AgCl. Subsequently, redox enzymes were immobilized on the electrodes and the electroreduction of oxygen to water was realized as a test of their efficiency as biocathodes. Due to the fibrous and porous structure of these new electrodes, and to the fact that carbon nanotubes may have the ability to promote electron transfer reactions of redox biomolecules, the new electrodes developed were capable of producing higher current densities than an electrode composed only of electrospun carbon fibers.

1. Introduction

The concept of biofuel cells has been studied since the 1960s [1], but it was only in the 1990s that a bigger interest appeared in using them as an alternative route to produce low power densities. However, performances have not yet reached the key targets to bridge the gap between laboratory works and industrial applications [2]. Two critical issues in

enzyme-based biofuel cells are short lifetime and poor power density, both of which are related to enzyme stability, electron transfer rate, and enzyme loading on the electrode [3]. Thus, the material of which the electrode is composed is of extreme importance in a biofuel cell, since it can influence both the electron transfer rate and the enzyme loading rate in the cell. According to Logan *et al* [4], a good electrode material must present, among others: high electrical conductivity,

strong biocompatibility, large surface area and appropriate mechanical strength.

In this context the study of electrodes consisted of electrospun carbon nanofibers (CNFs) seems attractive. The fiber mats produced by this technique have properties like high porosity with interconnected pores, pore sizes in the same magnitude of the fibers diameters, high specific surface, high surface-to-volume ratio, and the ability to control these properties according to the chemical composition of the precursor solution [5]. Furthermore, the high specific surface of these nanofibers favors the immobilization of a bigger amount of enzymes, a key point for enzymatic biofuel cells [3].

In this work, in order to enhance the performance of such electrodes, and meet the demands of high performance biofuel cells, a modification of the electrospun electrodes is proposed through the adsorption of carbon nanotubes (CNTs) in the surface of the fibers. Carbon nanotubes consist of cylindrical graphite sheets with nanometer diameter, and have attracted intensive attention for their impressive electrical conductivity, high chemical stability and mechanical strength [6, 7]. The unique electronic properties suggest that CNTs have the ability to promote electron transfer reactions of redox biomolecules [8, 9]. Besides, the incorporation of CNTs on fibers has shown to be an interesting way to boost the properties of the original material, such as the electrical conductivity [10–13], thermal properties [12, 13] and mechanical properties [14].

The carbon nanofibers were obtained by electrospinning of polyacrylonitrile (PAN) solution, followed by convenient stabilization and carbonization thermal treatments. A dipping method has been used in order to adsorb CNTs in the surface of the fibers. After preliminary works on nanostructured electrodes [15], investigations on CNFs@CNTs ability to work as a bioelectrode on biofuel cells were made, and a comparison between the pure CNFs electrode and the CNFs@CNTs electrodes was attempted. Redox enzymes have been immobilized in the surface of the electrodes and the electroreduction of oxygen to water was realized as a test of their efficiency.

2. Experimental details

2.1. Materials

Polyacrylonitrile (PAN, $M_w = 150\,000$), *N,N*-dimethylformamide (DMF), laccase from *Trametes Versicolor* (20 U mg⁻¹ solid), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS), Nafion[®] 117 solution 5 wt%, potassium hexacyanoferrate(II) trihydrate (K₄Fe(CN)₆·3H₂O), sodium phosphate dibasic dihydrate (Na₂HPO₄·2H₂O) and sodium phosphate monobasic monohydrate (NaH₂PO₄·H₂O) were purchased from Sigma-Aldrich and used without further purification. The phosphate buffer was prepared with Na₂HPO₄·2H₂O and NaH₂PO₄·H₂O (pH 5 or pH 7, 0.1 M). Carbon Vulcan was a gift from CABOT Corporation and used as received. Double-walled carbon nanotubes (DWNTs) were synthesized by a catalytic chemical vapor deposition (CCVD)



Figure 1. Images of the PAN fibers mat (white) and CNFs mat (black).

route reported earlier [16]. Multi-walled carbon nanotubes (MWNTs) were purchased from Nanocyl (Belgium).

2.2. Preparation of the carbon nanofibers

A PAN solution in DMF (10 wt%) was stirred at 60 °C for 4 h. A web of PAN fibers was obtained by an electrospinning process under high voltage (25 kV) (power supply Model T1CP 300 304n, Germany). The feed rate and the tip–collector distance were 2.0 ml h⁻¹ and 15 cm, respectively. The electrospun nanofibers were stabilized in an air environment at 250 °C for 2 h (heating rate was 2 °C min⁻¹) using an ashes furnace (Thermolyne 4800). Then the stabilized nanofibers were carbonized at 1000, 1200 or 1400 °C (1 h dwell) in high-purity nitrogen atmosphere (heating rate 2 °C min⁻¹) using a tubular furnace (Vecstar VTF-4, England). The resulted carbon nanofibers were cut into strips of size 30 mm × 5 mm approximately. Color changes went along with these heating treatments: white PAN fibers turned to brown (stabilized fibers) then finally black (CNFs) (figure 1).

2.3. Double and multi-walled carbon nanotubes

Some characteristics of the CNTs used in this work are listed in table 1. The average number of walls (N) and average external diameter (d_{ext}) of the CNTs were determined from the measurement of about 100 CNTs on high-resolution transmission electron microscopy images. The theoretical density of the CNTs (ρ) was calculated using the CNT density chart [17]. It was attempted to evaluate CNT length (L) on transmission electron microscopy images, although it is very difficult to do for the DWNTs because they tend to form bundles. Nevertheless, they were significantly longer than the MWNTs. The high-frequency range of the Raman spectra (Jobin-Yvon LabRAM HR 800 spectrometer, laser excitation at 632.82 nm) showed the D band (approximately 1320 cm⁻¹) and the G band (approximately 1580 cm⁻¹). The ratio between the intensity of the D band and the G band (I_D/I_G) was equal to 0.17 and 1.90 for the DWNTs and MWNTs, respectively. An increasing I_D/I_G value corresponds to a higher proportion of sp³-like carbon, which is generally

Table 1. Average number of walls (N), average external diameter (d_{ext}) and approximate length (L) of the CNTs, theoretical density (ρ), specific surface area (S_{CNT}) and carbon content (C_n) in the CNT samples; the balance was mostly water and residual metal catalyst.

Specimen	N	d_{ext} (nm)	L (μm)	P (g cm^{-3})	I_D/I_G	C_n (wt%)	S_{CNT} ($\text{m}^2 \text{g}^{-1}$)
DWNTs	2	2.0	>5	1.8	0.17	88.4	923
MWNTs	8	10.2	<1.5	1.8	1.90	91.8	242

attributed to the presence of more structural defects in the CNTs. The carbon content in the samples (C_n) was determined by flash combustion. The specific surface area of the samples (S_{CNT}) was measured by BET method using N_2 adsorption at liquid- N_2 temperature. The obtained values are in good agreement with calculations derived from geometrical data [18].

2.4. Adsorption of carbon nanotubes on the surface of carbon nanofibers

The adsorption of carbon nanotubes was realized by dipping the electrospun carbon nanofiber mats in a dispersion of carbon nanotubes, either double or multi-walled. The CNTs were dispersed in distilled water (0.5 mg ml^{-1}) in ultrasonic bath. The dips were realized during 30 s and the electrodes were then dried in ambient conditions. The electrodes were denominated according to their carbonization temperature (1000, 1200 or 1400 °C) and the kind of CNTs adsorbed (double, DWNT, or multi-walled, MWNT). For example, the sample 1200 + MWNTs refers to the CNFs treated at 1200 °C and immersed for 30 s in MWNTs dispersion. Three samples did not receive any surface modification with CNTs, and were simply denominated as 1000, 1200 and 1400. Note that no surfactant was added in the CNTs dispersions. The presence of a surfactant was not desired among the nanofibers to prevent further chemical interferences in the samples. Kim *et al* employed a comparable technique for the adsorption of carbon nanotubes on nylon 6 nanofibers, although they used Triton X-100 and sodium dodecyl sulfate as surfactants in water [11]. Gao *et al* realized the dips simultaneously with ultrasonication, and tested the effect of adding or not a surfactant in the CNT dispersion [12]. They observed that although the surfactant guarantees the CNT uniform dispersion, it actually prevented the CNTs from adsorbing onto the nanofibers, indicating that some interaction between CNTs and surfactants may take place, which adds to the fact that we did not use a surfactant in this work. The quantification of the amount of MWNT adsorbed was performed by weighting the electrodes before and after adsorption. We have measured a mean value of the amount of carbon nanotube adsorbed per unit of geometric surface of the electrode, which is $81 \mu\text{g}_{\text{CNT}}/\text{cm}^2_{\text{electrode}}$. Since the electrodes are not dense but composed of electrospun fibers, it is also interesting to quantify the amount of MWCNTs per gram of electrospun fibers. We have measured a mean value of $35 \mu\text{g}_{\text{CNT}}/\text{mg}_{\text{FIBRE}}$.

2.5. Preparation of the cathode

The bioelectrodes to be employed in the electroreduction of oxygen were prepared by adsorption of enzymes and

mediators on the surface of the electrodes by drop casting technique. This procedure consisted in depositing on the extremity of the electrodes (geometrical surface of 0.25 cm^2) a droplet of $20 \mu\text{l}$ of a mixture containing laccase (7.5 mg ml^{-1}), ABTS (2.4 mg ml^{-1}), carbon vulcan (7.5 mg ml^{-1}) and Nafion (5.0 vol%) in phosphate buffer (pH 5). The samples were then dried at 5 °C overnight. The enzyme loading was estimated to $600 \mu\text{g cm}^{-2}$.

2.6. Characterization techniques

Scanning electron microscopy (SEM) micrographs were taken on a HITACHI S4500 analyzer for the non-modified electrodes, and field-emission-gun scanning electron microscopy (FESEM, JEOL JSM 700F) was used to observe the electrodes modified with CNTs. Electrochemical measurements were performed on a potentiostat Autolab (Eco chemie, Netherlands) at 25 °C in phosphate buffer, with a conventional three-electrode system composed of a stainless steel auxiliary electrode, an Ag/AgCl reference electrode, and a square of $0.5 \times 0.5 \text{ cm}^2$ of CNFs mat as working electrode. The electrodes thickness of approximately $600 \mu\text{m}$ was kept constant from the electrospinning processing time, and was measured with an optical microscope. Cyclic voltammograms (CV) were measured in potassium hexacyanoferrate trihydrate (10 mM) at pH 7 with a scan rate of 10 mV s^{-1} . Linear scan voltammetry measurements were determined in dioxygen-saturated phosphate buffer at pH 5 after stabilization of the cathode open circuit potential at scan rate of 10 mV s^{-1} . The current density was determined from the geometric surface area of the electrodes.

3. Results and discussion

Carbon nanofibers (CNFs) were prepared by electrospinning of a PAN solution in DMF (10 wt%) as described in the experimental part. A mat of thin and continuous electrospun fibers has been reproducibly produced. For the conversion of polymer fibers into carbon, a stabilization step was followed by a carbonization step. Carbon nanotubes were adsorbed on the surface of the CNFs by a dipping technique.

3.1. Characterization of electrospun carbon fibers modified with carbon nanotubes

The morphology of the electrospun fibers was observed by SEM (figure 2). The fibers present regular and smooth fibrous morphology. During the chemical conversion of PAN to carbon, structural changes were induced and the average diameters of the fibers decreased from 450 nm (PAN fibers

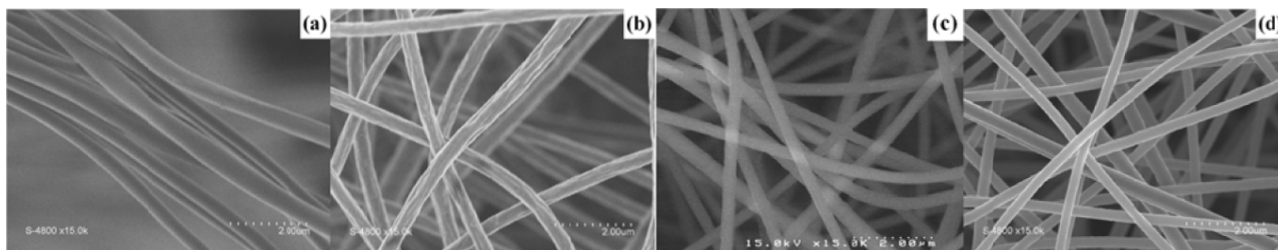


Figure 2. SEM images of electrospun fibers at different carbonization temperatures. (A) Polymer fibers without any heat treatment. (B)–(D) Fibers carbonized at 1000, 1200 and 1400 °C respectively. Scale bar is 2 μm .

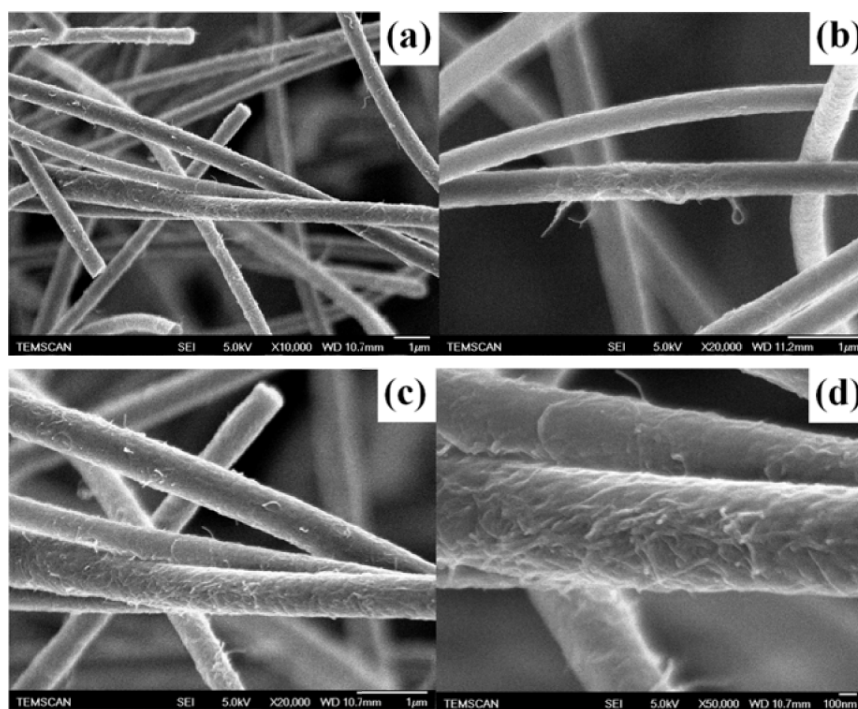


Figure 3. FESEM images of the sample 1000 + MWNTs showing the presence of multi-walled carbon nanotubes on the surface of electrospun carbon fibers.

not heat-treated, figure 2(A)) to 420 nm ($T = 1000\text{ }^{\circ}\text{C}$, figure 2(B)), 330 nm ($T = 1200\text{ }^{\circ}\text{C}$, figure 2(C)) and 300 nm ($T = 1400\text{ }^{\circ}\text{C}$, figure 2(D)), due to the resulting weight loss [19]. The mean diameters were measured on about 50 fibers for each sample from SEM images.

After dipping in the nanotubes suspension then drying, the different samples were observed by field-emission-gun scanning electron microscopy (FESEM). No significant structural difference was observed with the different starting fibers mat (1000, 1200 and 1400 °C). Concerning first the multi-walled nanotubes, typical images of 1000 + MWNTs are shown in figure 3. Numerous MWNTs are observed on the surface of the electrospun fibers (figures 3(A) and (B)). Higher magnification images (figures 3(C) and (D)) show that the CNTs, isolated and in small bundles, are fairly adsorbed on the surface of the fibers. CNTs bridging two different fibers are very rarely observed, which may reflect their short length (approximately 1.5 μm) with respect to the free space between fibers.

The suspension of DWNTs in water, without any surfactant, was unstable yielding a lack of reproducibility and homogeneity in these series of experiments. As an illustration, figure 4 shows a typical SEM image of sample 1400 + DWNTs. This image illustrates the presence of CNT agglomerates, which reflect the instability of the DWNTs suspension. One can also notice in figure 4 the presence of bridging DWNTs, which illustrate their higher length compared to MWNTs. However, since the formation of agglomerates was not desirable, the study was continued only with MWNTs, because they could create a uniform coating of CNTs over the fibers, as observed in figure 3.

The variations in the structural changes of the electrospun carbon nanofibers as a function of the carbonization temperature and of the presence of adsorbed CNTs were studied by Raman spectroscopy. Figure 5 presents the spectra of the sample 1200 with and without MWNTs. Raman spectra manifest D peak centered around 1329 cm^{-1} , and G peak centered around 1577 cm^{-1} , characteristic of disordered carbon and graphite, respectively, both of the bands are

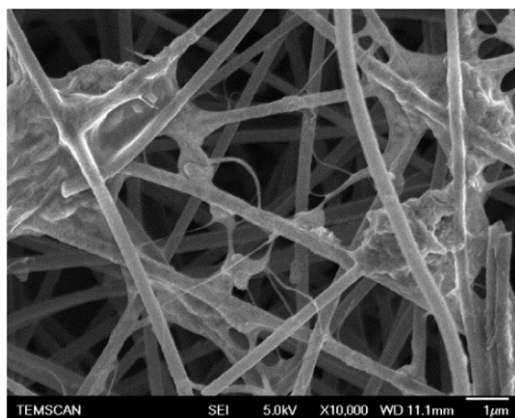


Figure 4. FESEM image of sample 1400 + DWNTs, illustrating the presence of CNTs bridges between the carbon fibers.

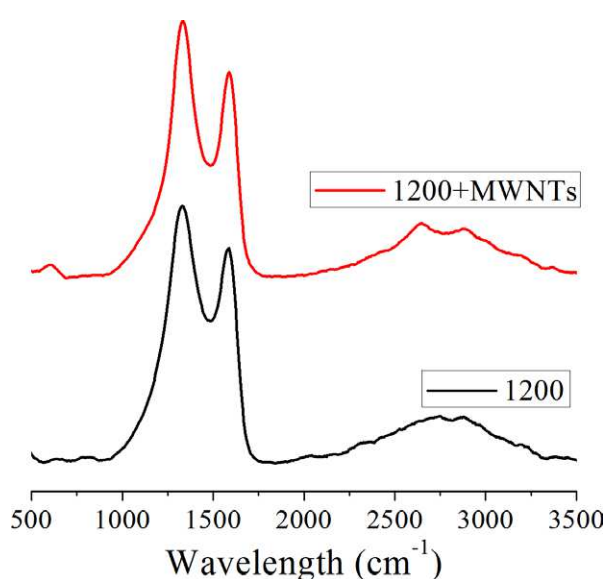


Figure 5. Raman spectra of the electrode 1200 with and without adsorbed MWNTs.

attributed to sp^2 bonded species [20]. It is well known that the R -value, the relative intensity ratio of the D band to the G band (I_D/I_G), depends on both the degree of graphitization and the alignment of graphitic planes. The lower the R -value, the higher the amount of sp^2 (graphite) groups is in the sample. The R -values of the electrodes are presented in figure 6. A decrease in R is observed along with increasing carbonization temperature (from 1000 to 1400 °C), representing the transformation into a more graphitized carbon. Such behavior has been observed by several authors who studied carbon materials such as CNFs [21–24].

For samples with adsorbed MWNTs, R -values are smaller than the corresponding samples without MWNTs (but treated at the same temperature), and also decrease for higher temperatures. This behavior attests that the presence of MWNTs increases the relative rate of sp^2 -carbon into the sample which is expected to lead to an enhanced electrical conductivity.

Finally, all the samples presented G' peak centered on 2740 cm^{-1} , additional to D and G ones. G' is approximately

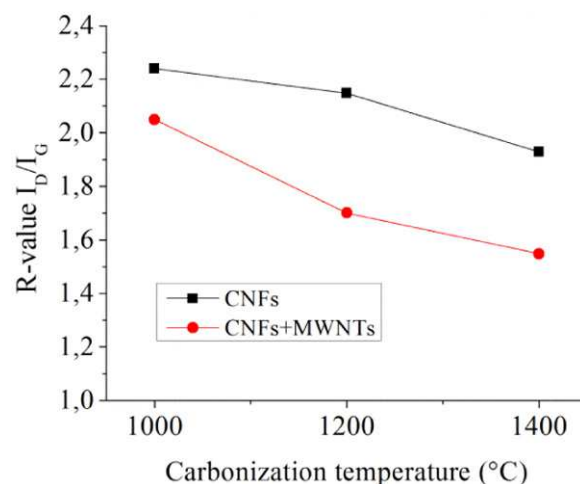


Figure 6. Ratio between D and G peaks (R) values obtained from Raman spectra according to the carbonization temperature of carbon fibers with no modification (■), carbon fibers adsorbed with MWNTs (●).

2 times the frequency of D peak (i.e. the overtone of D peak), although the features of D and G' are the opposite [21]. It appears only for carbons treated higher than 1000 °C, and gets narrower and grows in amplitude as the heat treatment temperature and graphitization level increases [21].

3.2. Electrical property

The electrical conductivity of the electrodes was measured by the four-point probe Van der Pauw method [25]. The values obtained are presented in figure 7 and they lay between approximately 1 and 13 S cm^{-1} . Similar values were observed by several authors who studied carbon fibers [23, 24, 26, 27]. Kim *et al* developed PAN-based carbon nanofibers with conductivity of approximately 2 S cm^{-1} when treated at 1000 °C, and approximately 15 S cm^{-1} when treated at 1500 °C [23, 24]. Guo *et al* obtained a conductivity of approximately 5 S cm^{-1} for hybrid material composed of CNFs containing MWNTs, prepared through the electrospinning of CNTs suspended in PAN solution followed by thermal treatments [27]. However, according to our studies, the four-point probe technique was not able to prove with certainty the beneficial effects of the presence of carbon nanotubes on the electrical conductivity of the materials. This is probably because the characteristics of the material are not appropriated to be measured by points, due to its non-massive nature (with voids between the fibers). When the four points touch the fibers in order to make the conductivity measurements, the electric current tends to flow in certain carbon fibers or carbon nanotubes that are in contact in that moment, so that it is not representative of the real behavior of the whole material. In order to show the relation between the different electrodes developed in this work, the electronic conductivity was evaluated by cyclic voltammetry technique.

3.3. Electrochemical properties

CNFs and CNFs@CNTs mats were characterized by cyclic voltammetry in phosphate buffer solution (pH 7) containing

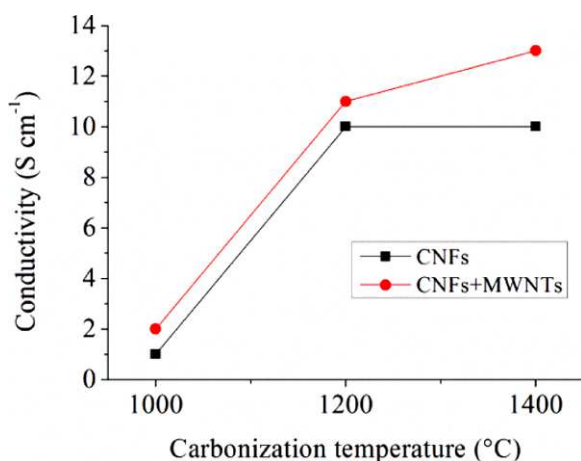


Figure 7. Electrical conductivity values measured by Van der Pauw four-point probe method of carbon fibers with no modification (■) and carbon fibers adsorbed with MWNTs (●).

10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ from 0 to 0.5 V versus Ag/AgCl. When used as an electrode, the resulting electrospun mat did not need adding any polymer binder and the structure affords an easy handling. The influence of the carbonization temperature was studied upon the separation of the peak potentials (ΔE_p) and peak current height (I_{p_a}) for $\text{K}_3\text{Fe}(\text{CN})_6$ (figure 8(a)). The voltammograms are characterized by a pair of well-defined redox peaks for $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ at $E_{1/2} = 0.18$ mV versus Ag/AgCl. When the carbonization temperature increases from 1000 to 1400 °C, ΔE_p and I_{p_a} decreased. The trend of ΔE values ($\Delta E_{1400} < \Delta E_{1200} < \Delta E_{1000}$) indicates that the 1400 electrode presents the best reversibility in terms of ΔE due to its more organized and graphitized structure compared to 1000 and 1200 electrodes, as already observed by Raman. This result indicates an easier electronic conductivity that agrees with the morphology of the corresponding carbon fibers. In the case of the I_{p_a} trend, the peak current decreases with the heating treatment, which is attributed to the weight loss of the material during the carbonization step. The higher the treating temperature was,

the higher the weight loss of the material is. Thus, since a commitment between smaller ΔE and larger I_{p_a} is necessary, the 1200 electrode was selected as the one that meets the better this condition.

The benefit of adsorbing MWNTs on carbon fibers was illustrated by the evolution of both ΔE_p and I_{p_a} . For all the samples, the reversibility of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ was notably improved and effective electroactive surface area of the electrodes was increased in the presence of MWNTs. As shown in the case of the 1200 electrode (figure 8(b)), ΔE decreases from 250 to 200 mV after immobilization of the MWNTs in the nanofibers mat and the anodic peak current is magnified 2-fold, suggesting that the presence of CNTs significantly improves the electronic conductivity and increases the effective electroactive area of the mats, which is due to the 3D structure of this CNFs@CNTs nanocomposite material. This effect was, however, not observed for the electrodes modified with DWNTs, likely as a result of the agglomerates formed on the fibers (see supplementary information for more data on electrochemical properties of electrodes modified with DWNTs available at stacks.iop.org/Nano/24/245402/mmedia).

3.4. Bioelectrocatalysis of O_2 on electrospun carbon fibers modified with carbon nanotubes

Enzymatic reduction of O_2 is an important topic in biofuel cells development [28]. For this reason, development of cathodes modified with redox enzymes is a challenge since it can allow to perform oxygen reduction at low overpotential [29]. The porous structure and the large surface area of CNFs mats makes them ideal for enzyme immobilization leading to high content of electrically contacted enzyme molecules per unit of geometric surface area of the electrode and thus higher catalytic current density [30]. Besides, CNTs have been extensively used for the construction of bioelectrodes, due to their ability to promote electron transfer reactions between immobilized enzymes and electrode [8]. In this work, the advantages of electrospun carbon fibers and CNTs were

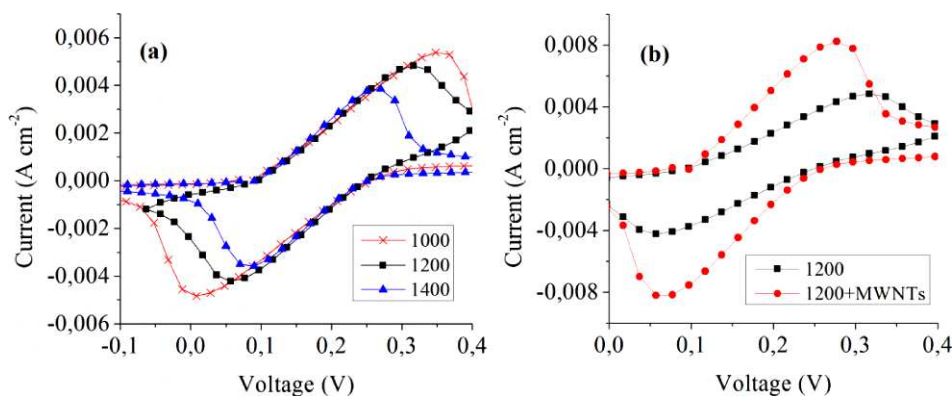


Figure 8. (a) Cyclic voltammetry of 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at CNF electrodes without MWNTs adsorbed, carbonized at 1000 (×), 1200 (■) and 1400 °C (▲). (b) Cyclic voltammetry of 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at CNF electrodes carbonized at 1200 °C with adsorbed MWNTs (●) and without MWNTs (■). Electrolyte: phosphate buffer pH 7, scan rate 10 mV s⁻¹.

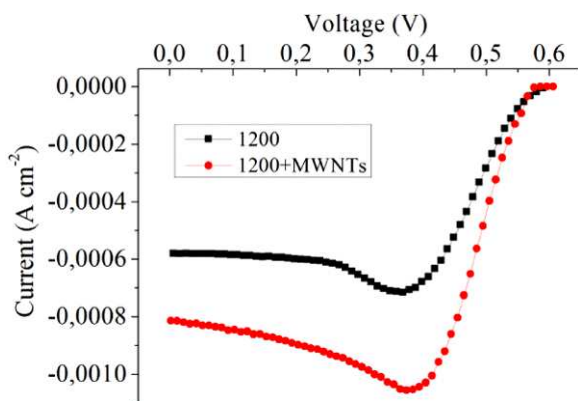


Figure 9. Polarization curves of a laccase/ABTS modified electrode in O_2 -saturated phosphate solution (pH 5, 0.1 M). Comparison between electrodes carbonized at $1200\text{ }^\circ\text{C}$ with MWNTs adsorbed (●) and without MWNTs (■) on CNFs. Scan rate 3.3 mV s^{-1} .

combined to develop suitable conductive support for enzyme immobilization. The enzyme laccase was chosen as a common enzyme that catalyzes the four-electron reduction of O_2 completely to H_2O in the presence of the electron transfer mediator ABTS [31]. By oxidizing the mediator $ABTS_{red}$, laccase acquires the necessary electrons to reduce dioxygen into water, and $ABTS_{ox}$ is reduced on the electrode surface. The CNFs@CNTs electrodes were covered on the surface by a mixture of laccase, ABTS and carbon vulcan particles entrapped in Nafion polymer. Figure 9 presents linear scan voltammetry measurements (LSV) to evaluate the electrochemical performance of the biocathodes towards O_2 reduction, in buffer solution (pH 5) saturated in oxygen. The polarization curves show high current density values, in the range $600\text{--}900\text{ }\mu\text{A cm}^{-2}$, associated to the enzymatic O_2 reduction. The oxygen reduction current begins at 0.6 V versus Ag/AgCl, without overpotential, and current densities feature a semi-plateau that indicates the control of the catalytic reaction by diffusion of the oxygen to the electrode surface.

In this work, current densities are significantly improved for electrospun CNFs modified with CNTs ($900\text{ }\mu\text{A cm}^{-2}$), which make such new electrodes really attractive as cathodes for biofuel cells. Specially, our system presents competitive efficiency with other reported values focusing on the immobilization of the couple laccase/ABTS by encapsulation in silica matrix on porous carbon paper supports [32] ($450\text{ }\mu\text{A cm}^{-2}$ at pH 6 with estimated laccase loading of $190\text{ }\mu\text{g cm}^{-2}$), by entrapment within layered double hydroxides [33] ($70\text{ }\mu\text{A cm}^{-2}$ at pH 6 with estimated laccase loading of $600\text{ }\mu\text{g cm}^{-2}$) or by entrapment in polypyrrole on porous carbon tubes [34] ($300\text{ }\mu\text{A cm}^{-2}$ at pH 4.8, with estimated laccase loading of $190\text{ }\mu\text{g cm}^{-2}$).

4. Conclusions

Nanostructured hierarchical carbon electrodes have been successfully developed in the present work and show promising performances for applications in biofuel cells. The electrodes were composed of a mat of electrospun carbon fibers that did not need addition of any polymer binder, and

which structure allows an easy handling. Multi-walled carbon nanotubes were able to be fairly adsorbed on the surface of the CNFs through the dipping of fiber mats in a CNTs dispersion. Double-walled CNTs were also attempted to be adsorbed on the fibers, but with less success due to their instability in solution. Indeed, it was observed through FESEM images that DWNTs formed agglomerates instead of adsorbing uniformly on the surface of the fibers. Because of that, our study continued only with MWNTs. Cyclic voltammetry technique allowed the evaluation of important features that were not possible to be analyzed by other techniques, due to the fibrous structure of the electrodes. One of these features is the electrical conductivity, which increased in electrodes that contained MWNTs. The best preparation conditions involved PAN nanofibers carbonized at $1200\text{ }^\circ\text{C}$, representing the best compromise between good electrical transfer ability and high surface. Thanks to the electrical properties of carbon nanotubes combined with the structure of the electrospun carbon nanofibers, an improvement in the performance of the electrodes was demonstrated when laccase was immobilized in the presence of ABTS as a redox mediator. The current density improved from $600\text{ }\mu\text{A cm}^{-2}$ (CNFs electrode) to approximately $900\text{ }\mu\text{A cm}^{-2}$ (CNFs@CNTs electrode) when the electrodes were employed in the electroreduction of oxygen. This results show that carbon nanotubes adsorbed carbon nanofibers are a promising material in the development of new electrodes for enzymatic biofuel cells.

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