Biosensors and Bioelectronics 101 (2018) 60–65

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel three-dimensional carbonized $PANI_{1600}$@CNTs network for enhanced enzymatic biofuel cell

Zepeng Kang[a], Kailong Jiao[a], Jin Cheng[a], Ruiyun Peng[b], Shuqiang Jiao[a], Zongqian Hu[b,*]

[a] State Key Laboratory of Advanced Metallurgy, University of Science and Technology Beijing, Beijing 100083, PR China
[b] Beijing Institute of Radiation Medicine, Beijing 100850, PR China

ARTICLE INFO

Keywords:
3D carbon composite
Polyaniline
In situ polymerization
Biofuel cell

ABSTRACT

A novel three-dimensional (3D) carbon composite of $PANI_{1600}$@CNTs with rhizobium-like structure is prepared by in-situ polymerization of aniline monomers around and along the functionalized carbon nanotubes (CNTs) and then carbonized at 1600 °C for enzymatic biofuel cells (EBFCs). The SEM and TEM images clearly show that the carbonized PANI grew seamlessly on the surface of CNTs and presented the rhizobium-like structure. The carbonized PANI acts like conductive "glue" and connects the adjacent tubes together, which can assemble the CNTs into a 3D network. The $PANI_{1600}$@CNTs composite modified glassy carbon electrodes based on glucose oxidase (GOx) and laccase (Lac) exhibit high electrochemical performance. A glucose//O_2 EBFC constitutes of the fabricated anode and cathode performs a maximum power density of 1.12 mW cm^{-2} at 0.45 V. Furthermore, three of the fabricated EBFCs in series are able to lightening up a yellow light-emitting diode (LED) whose turn-on voltage is about at 1.8 V. This work may be helpful for exploiting novel substrates by carbonizing the composites of conducting polymer with nano materials at high-temperature for immobilization of enzymes in the EBFCs or biosensor fields.

1. Introduction

Enzymatic biofuel cells (EBFCs) is a type of fuel cell that utilizes enzymes as the electrocatalysts to catalyze the oxidation of fuel and/or the reduction of oxygen or peroxide to convert chemical energy into electricity (Rasmussen et al., 2016). However, EBFCs are limited by the relatively slow rate of electron transfer between enzyme and electrode, which is a major barrier for improving the power output (Chen et al., 2015b). Because the active centers of the redox enzymes are usually buried inside the protein matrices, establishing a reliable and efficient electrical contact between the biocatalyst and the electrode surface is the urgently required (Blaik et al., 2016; Prasad et al., 2014). A common approach of overcoming the electron transfer issue is by using a redox mediator molecule to facilitate electron transfer, namely the mediated electron transfer (MET) process (Blaik et al., 2016). However, the additional reagents, such as redox mediators, can be harmful to the biological activity of enzyme and reduce the open-circuit potential (OCP). The mediator-less electron transfer between biocatalysts and electrode surface is thus more favored (Navaee and Salimi, 2015).

GOx is one of the common oxidases in the application of bioanode towards glucose oxidation (Atanassov et al., 2007; Minteer et al., 2007). However, direct electrochemistry of GOx is still a big issue, while some researchers claimed the direct electron transfer (DET) process is possible and other groups insisted it is not. Due to the size of the protein (roughly 160 kDa) and the location of the FAD (deeply buried in the center of the protein), it is hard to believe DET can be occurred (Liang et al., 2015). Still, some researchers detected a quasi-reversible peak near −0.5 to −0.4 V (depending on pH and reference electrodes) at the GOx-modified electrodes and they claimed to obtain DET (Gao et al., 2014; Kowalewska and Jakubow, 2017; Liu et al., 2007).

Lac is a typical multicopper oxidase and has been intensively studied as biocathode electrocatalysts for oxygen reduction in biofuel cells by exploiting direct and mediated electron-transfer reactions (Barrière et al., 2006; Karnicka et al., 2008; Prasad et al., 2014; Zhang et al., 2014). However, achieving the DET system of Lac is still very hard due to the complicated catalytic mechanism and the strict enzyme molecular immobilized orientation (Matijošytė et al., 2008; Tominaga et al., 2015). Therefore, most studies are focused on mediated electron-transfer reaction for obtaining high effective electron transfer (Barrière et al., 2004; Zhang et al., 2014). Developing a new type electrode materials for immobilization of Lac and its mediators is the key step to construct the biocathode (Prasad et al., 2014).

In recent years, considerable efforts have been made to develop new materials as well as stable and efficient immobilization techniques for

* Corresponding author.
E-mail address: huzongqian@hotmail.com (Z. Hu).

http://dx.doi.org/10.1016/j.bios.2017.10.008
Received 1 September 2017; Received in revised form 30 September 2017; Accepted 2 October 2017
Available online 07 October 2017
0956-5663/

obtaining the DET of GOx and immobilizing the Lac and its mediators. For example, various conductive nanoparticles (Fang et al., 2016; Gutierrez-Sanchez et al., 2012; Huang et al., 2012) organic polymers (Crepaldi et al., 2014; Peng et al., 2013; Uk Lee et al., 2013) or carbon nanomaterials (Babadi et al., 2016; Gao et al., 2014) have been developed and used as an ideal conducting channel to promote efficient DET. Among the various conductive nanomaterials, carbon, with special morphology, high electrical conductivity, stable mechanical and thermal properties, plays an important role in the EBFCs fields (Chen et al., 2015b).

Carbonizing the nanostructured polymers, such as polyaniline (PANI) and polypyrrole (PPy), is a typical way to obtain various types of microporous carbon nanomaterials (Gavrilov et al., 2011; Janošević et al., 2012; Stejskal et al., 2010). In this work, we selected PANI as the carbon precursor, a novel 3D composite of PANI@CNTs with rhizobium-like structure were prepared by in-situ polymerization of aniline monomers along and around the surface of CNTs. The composite was carbonized at 1600 °C in accordance with our previous work (Kang et al., 2017). The carbonized composite of $PANI_{1600}$@CNTs was then used as the substrate for immobilization of GOx and Lac and their electrochemical properties were sufficiently studied.

2. Materials and methods

2.1. Synthesis of PANI and PANI@CNTs composite and carbonization

The PANI was synthesized by chemical oxidation polymerization of aniline monomers (Janošević et al., 2011). The detailed preparation processes of the PANI@CNTs composite were referenced to the previous reports (Ma et al., 2008; Zhou et al., 2010). Briefly, 200 mg of functional CNTs and 1.14 g of APS were suspended or dissolved in 80 mL of H_2SO_4 (1 M) solution, respectively, then put 373 mg of the aniline into the CNTs turbid liquid and sonicated for 30 min. After sonication, the mixture was stirred for 2 h by a magnetic stirring apparatus. Lastly, taking the APS solution into the mixture slowly under the condition of stirring. After that, the ultimate mixture was placed quietly for 24 h at the room temperature. The schematic diagram of the preparation procedures of PANI@CNTs composite was shown in Scheme 1. Carbonization was carried out through gradual heating (at 2 °C min^{-1}) to the target temperature 1600 °C at a nitrogen atmosphere and the product was denoted $PANI_{1600}$@CNTs.

2.2. Preparation of working electrode

For preparing the bioanode, a glassy carbon electrode (GCE) was sequentially polished using a slurry of 0.5 μm and 0.03 μm alumina power and successively washed by ultrasonication in deionized water, 1 M HNO_3, deionized water and ethanol for 3 min, respectively. Then the electrode was activated electrochemically in 0.1 M sulfuric acid by cyclic voltammetry (CV) until the CV curve to invariant (Inamuddin et al., 2014). Thereafter, the electrode was washed and dried in argon, then 6 μL of carbonized $PANI_{1600}$@CNT (5 mg mL^{-1}, prepared by deionized water) suspension was dropped on the surface of the cleaned GCE by using a microinjector, and then the electrode was dried in the air. The obtained electrode was denoted as $PANI_{1600}$@CNT/GCE. After that, 5 μL of GOx solution (4 mg mL^{-1}, prepared by PBS, pH 4) was spread onto the surface of $PANI_{1600}$@CNT/GCE electrode and then the electrode was left in a refrigerator (4 °C) for 6 h. The obtained electrode was denoted as GOx/$PANI_{1600}$@CNT/GCE. Finally, 4 μL of Nafion solution (1 *v/v*%, prepared by ethyl alcohol, 99.99%) was spread onto the surface of GOx/$PANI_{1600}$@CNT/GCE. Then left it in a refrigerator (4 °C) for 2 h. The final electrode was denoted as Nafion/GOx/$PANI_{1600}$@CNT/GCE. For comparison, Nafion/GC, Nafion/GOx/GC, Nafion/$PANI_{1600}$/GC, Nafion/GOx/CNTs/GC and Nafion/$PANI_{1600}$@CNT/GC electrodes were prepared through the same procedures as above.

The biocathode was fabricated as follows. Briefly, 6 μL of 5 mg mL^{-1} $PANI_{1600}$@CNT suspension was cast on the surface of an GCE, air-dried, followed by successive casting 4 μL of 10 mg mL^{-1} Lac solution (prepared by PBS, pH 4.0), and 3 μL of Nafion solution, and each casting was done after the previous cast had been air-dried. The as-prepared enzyme electrode was denoted as Nafion/Lac/$PANI_{1600}$@CNT/GCE. All the prepared enzyme electrodes were stored in PBS at 4 °C when not in use.

2.3. Electrochemical testing and BFC performance

All electrochemical experiments were carried out in an electrochemical workstation (CHI 660 C, CHI Instrument, Shanghai, China). The conventional three-electrode comprised a working electrode of modified glassy carbon (3 mm diameter), counter electrode of Pt (surface area: 1 cm^2), and reference electrode of Ag/AgCl (3 M KCl). The electrochemical measurements of bioanode were carried out in 0.1 M sodium phosphate buffer solution (PBS, pH 7.2), which was purged with high-purity argon for at least 30 min prior to experiments. An argon environment was kept over the solution in the cell. The electrochemical measurements of biocathode were carried out in 0.1 M Britton-Robison (B-R, pH 5.0) with or without 0.5 mM ABTS for oxidation of the oxygen. All experiments were performed at ambient temperature.

The glucose//O_2 EBFC was fabricated in two-compartments using acrylic glass and the anodic and cathodic compartments were separated by the Nafion 211 membrane. The Nafion/GOx/$PANI_{1600}$@CNT/GCE and the Nafion/Lac/$PANI_{1600}$@CNT/GCE were acted as the bioanode and the biocathode, respectively. The electrolyte of anodic compartment was the Ar-saturated 0.1 M PBS (pH 5.0) with 0.1 M glucose. The cathodic compartment was filled by the 0.2 M B-R buffer (pH 5.0) with 0.5 mM ABTS and continuously bubbled with the oxygen. The measurements of power density were referenced to previous report (Liu et al., 2012).

3. Results and discussion

3.1. Microscopic and spectroscopic characterization

Fig. 1 shows the SEM and TEM images of the (A, D) CNTs, (B, E) PANI@CNTs and (C, F) $PANI_{1600}$@CNTs composites. As can be seen in the Fig. 1**A** and **D**, the CNTs presented the typical nanotube structure and smooth surface. In Fig. 1**B,** it can be clearly seen that the surface of the graphite-structure CNTs was covered by a compact original PANI layer with a diameter of 60–80 nm. Fig. 1**E** shows the corresponding images of TEM, the PANI@CNTs composite exhibited a well-defined boundary and presented a core-shell structure. Obviously, the conducting polymer of PANI played a role of “glue” and connected the adjacent tubes together, which could make the CNTs into a three-dimensional network. Fig. 1**C** shows that the presence of nanotubes with a smaller diameter compared with that in the PANI@CNTs composite, which might be caused by the gases emission, such as CO, CO_2, CH_4, etc., during the calcination process. The amplified image (inset) exhibits a three-dimensional network of coadjacent nanotubes. Fig. 1**F** shows that the carbonized PANI distributed uniformly on the surface of CNTs and presented the rhizobium-like shape. The rhizobium-like carbon grew seamlessly on the surface of the CNTs and connected the adjacent tubes together, which could effectively reduce the resistance at the intertube junctions in the $PANI_{1600}$@CNTs network. All these results indicated that the $PANI_{1600}$@CNTs composite could be an excellent materials for immobilization of enzymes in the application of biofuel cells or biosensors.

Fig. S1 shows the FT-IR spectra of (curve a) original CNTs, (curve b) functional CNTs, (curve c) aniline-CNTs and (curve d) PANI@CNTs. The stretching bands at 1620 cm^{-1} and 1380 cm^{-1} are the vibration of C = C and C–H (curve a). The increased stretching band for C = O at