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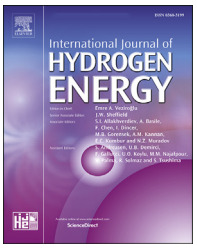

Long-term effect of carbon nanotubes on electrochemical properties and microbial community of electrochemically active biofilms in microbial fuel cells

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ABSTRACT

Carbon nanotubes (CNTs) have been widely exploited to improve anodic performance, but information is needed on their long-term stability for improvement. Herein, we prepared a novel CNTs-modified graphite felt (CNTs-GF) by a simple and scalable process and evaluated its long-term performance using anaerobic sludge as inoculum. the MFC with CNTs-GF yielded a sustained enhancement of power output, increasing from 1.93 ± 0.09 W m^{-2} after 1 month to 2.10 ± 0.05 W m^{-2} after 3 months and reaching 2.00 ± 0.10 W m^{-2} after 13 month, indicating the enhancement in electricity generation by the CNTs was not declined over one year. However, the bare GF showed a declining tendency of performance during 13 months. The long-term enhancement can be explained by the facts that the CNTs-GF was beneficial to electrochemically active biofilms (EABs) growth and interacted better with EABs and increased the extracellular electron transfer. Community analysis showed an increase in *Geobacter* in response to CNTs modification. These results demonstrated that CNTs modification could sustain a superior long-term enhancement in MFC performance.

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Introduction

Microbial fuel cells (MFCs) are capable of harvesting electrical energy directly from decomposition of organic matter and thus hold a great promise to become an energy-efficient technology for sustainable wastewater treatment [1,2]. These MFCs devices are generally composed by anodic and cathodic chambers separated by a proton or cation exchange membrane. In the anodic chamber, the organic matter oxidation is carried out by microorganisms, while electrons passed to the anode from their metabolism [3,4]. This is a complex process that has been most extensively studied in several ways, suggesting different electron pathways

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including direct transfer [5], mediated transfer [6,7], or long-range charge transport along filamentous pili [8,9]. The low rate of extracellular electron transfer (EET) from the electrochemically active biofilms (EABs) to the solid electrode, however, have been found to be the major limiting factor in the process of electricity production [10]. Specifically, the electrochemical properties and surface structure of anode material directly affect bacteria attachment, electron transfer efficiency, and substrate oxidation [11,12]. Currently, the power density of MFCs is not yet considerable and too low for their applications. Considerable efforts, such as modification of anode materials, have thus been made to improve the EET efficiency between EABs and anodic electrode [13,14].

Nanoscale materials favoring electroactive biofilm formation have been widely exploited to improve anodic performance due to their unique properties (i.e., higher active surface area, excellent conductivity, tailored nanostructure to adsorb bacteria) [14–24]. For instance, nanoscale composite, such as PANI/mesoporous TiO_2 [20], grapheme-sponges [16], three-dimensional (3D) PANI/grapheme [17], biomineralized iron sulfide nanoparticles [25], polyaniline/grapheme [24], and nano polypyrrole/polythiophene [21], was employed as anode materials to facilitate electron transfer and provide multiplexed and highly conductive pathways, resulting in increased current generation. Particularly, Carbon nanotubes (CNTs) have been usually used to modify anodes in MFCs due to their unique electrical and structural properties. Sharma et al. [26] found that the maximum power density achieved using CNT-Pt modified graphite as anodes could be six times higher than that of bare graphite. Also, the multi-wall CNTs-modified anodic electrode fabricated by layer-by-layer assembly technique [27] or self-drop-coating technique [28] improved the MFCs performance. A CNT-coated textile [15] or sponge [29,30] offered an open 3D space for internal colonization of EABs with efficient electrolyte transport and increased electrical conductivity, which led to the improvement of EET efficiency. Recently, Erbay et al. [31] developed anodes with multi-wall CNTs directly grown in the radial direction from the wires of stainless steel meshes, providing extremely large 3D surfaces and showing an improved power production. In addition, use of natural or conducting polymers with CNTs as anodes, such as chitosan/CNTs [32], polypyrrole–CNTs [33,34], and PANI–CNTs [35] mixtures, have also been demonstrated to enhance the current production as a result of increased conductivity of the bacterial network. Mishra et al. [36] found that the carbon cloth modified MWCNT-MnO2/PPy nano-composite electrodes had a high potential and good biocompatibility that showed a great realized mediator less MFCs for bio-electricity production from sewage waste water. Specially, functionalized MWCNTs have been investigated in MFCs, in which the MWCNTs and their functionality promoted biofilm formation, biodegradation and electron transfer on anodes [37].

Despite recent progress in successful application of CNTs, discussion about long-term operated anodes was barely focused. As well known, the growth of EABS in anode could be affected by electrochemical properties and surface structure and rates of diffusion of substrates [11,38]. The activity and microbial diversity of the biofilms could vary with time. This possibility and related processes with CNTs-modified anodes have not been systematically studied. It is not clear whether and how nanomaterials-modification can enhance anodic performance in MFC over long-term operation, which is more convincing to determine the comprehensive property of anode modification for application. Combining electrochemical and biological analyses will offer a comprehensive and in-depth understanding of the long-term effects of CNTs and present some consultations for further improvement in bioanode performance.

In this study, anodic electrode was modified by CNTs and used in the MFCs, which were operated for 13 months to investigate its long-term performance. Electrochemical properties (current generation, cyclic voltammetry and electrochemical impedance spectroscopy analyses) were conducted to evaluate the EET between EABs and the anode. Finally, Biological properties (morphology and microbial community) were investigated to determine whether the modification of CNTs on anodes caused any change.

Materials and methods

CNTs-electrode preparation

CNTs (diameter of 40–60 nm, Shenzhen Nanotech Port Co., Ltd.) were ultrasonically dispersed into a mixture of H_2SO_4–HNO_3 (volume ratio: 3:1) for 5 h then filtered through a polytetrafluoroethylene filter membrane (pore diameter 0.4 μm) and washed with de-ionized water until the mixture with pH 6–7 was obtained, as previously described [39]. Aqueous CNTs ink was fabricated by adding the carboxylated CNTs into de-ionized water with sodium dodecylbenzene sulfonate (SDBS) surfactant (0.18% for CNTs and 1% for SDBS by weight). Then, the suspension liquid was ultrasonicated for another 1 h to obtain a homogeneous solution. A piece of Graphite felt (GF, 2.5 × 3.0 × 0.5 cm, Beijing Sanye Co., Ltd.), with a projected surface area of 7 cm^2, was dropped into the CNTs ink for about 30 s, removed and oven-dried at 80 °C for an hour to obtain a CNTs-modified electrode. The dipping–drying process was conducted in triplicate to produce suitable CNTs-modified GF, which was used for morphological analysis and used as a bioanode in MFC.

Microbial inoculum and reactor operation

A dual chamber MFC was adopted. Both of the anodic and cathodic chamber were cylindrical with 2.4 cm long and 4.9 cm in diameter. The bare GF electrodes (2.5 × 3.0 × 0.5 cm) and CNTs-modified GF were twined with titanium wire and used as the anode electrodes. The bare GF was also used as cathode electrodes with the same shape and size. A cation exchange membrane (Zhejiang Qianqiu Group Co., Ltd.) was placed between the two chambers and the bare GF electrode was used as the cathode. The bioanode was inoculated with anaerobic sludge (obtained from Lijiao Sewage treatment plant, Guangzhou, China) and a solution containing sodium acetate (1000 mg L^{-1}), a phosphate buffer solution (PBS, 100 mM), minerals (12.5 mL L^{-1}), and vitamin solution (12.5 mL L^{-1}) as described in our previous study [39]. The cathodic chamber was fed with 50 mM $K_3[Fe(CN)_6]$ and

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