



Bacterial electroactivity and viability depends on the carbon nanotube-coated sponge anode used in a microbial fuel cell

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ABSTRACT

The anode material is vital to improve the power generation of a microbial fuel cell (MFC). In this study, a carbon nanotube (CNT)-coated sponge with macro-porous structure, high surface area, and high conductivity was constructed as an anode to encapsulate *Escherichia coli* K12 (*E. coli* K12) cells. To achieve high power generation of the MFC, the optimal concentration of the CNT coating the sponge was found to be 30 mg mL⁻¹. At this concentration, a maximum power density of 787 W m⁻³ and a chemical oxygen demand (COD) removal of 80.9% were obtained with a long stable electricity generation process in batch mode. This indicates that the biofilm on the CNT (30 mg mL⁻¹)-coated sponge possessed excellent electroactivity and stability. Scanning electron microscope (SEM) images confirmed that the CNT-coated sponge provided a suitable microenvironment for *E. coli* K12 cells to maintain their attachment and colonization. Additionally, a CNT-dependent viability phenomenon of the *E. coli* K12 cells was discovered after electricity generation. This CNT-dependent viability of the *E. coli* K12 cells was stable and sustainable after storage at -20 °C in a milk tube for one year.

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1. Introduction

A microbial fuel cell (MFC) is a device that uses microbial cells as catalysts to convert organic or inorganic wastes into electricity [1,2]. Numerous bacterial species have shown the ability to oxidize organic compounds to produce electrons [3,4]. In early 1983, *Escherichia coli* (*E. coli*) was first selected as an electrogenic bacterium to generate electricity in an MFC [5], because *E. coli* cells can produce endogenous mediators and form a biofilm in an MFC [6–10]. Thus, *E. coli* is usually used as a model electrogenic bacterium in MFC research, because of its easy availability, rapid growth and reproduction, and excellent electrochemical activity [6]. The key to maintaining the electrochemical activity and power output ability of *E. coli* cells is the use of a suitable anode material that serves as a support for the growth, enrichment, and attachment of the microbial cells. Therefore, it is important to select an ideal electrode material that can not only facilitate the attachment of an electrogenic bacterium and the formation of a biofilm but also promote efficient electron transfer from microbes to electrodes.

The physicochemical properties and structure of anode materials play an important role in electricity generation [11,12]; it directly affects bacterial attachment, electron transfer, and substrate oxidation. To date, conventional carbon materials such as carbon cloth, graphite felt and

carbon paper have been widely used in MFCs because of their good conductivity and biocompatibility [13–15]. Unfortunately, the application of these materials has been limited owing to their low specific surface area and poor electrocatalytic activity [11]. Compared with conventional carbon materials, the carbon nanotube (CNT) is advantageous for MFCs, because of its large specific surface area, high mechanical strength, and ductility [16–18]. Furthermore, it has been reported that a CNT can promote electron transfer as an electrode material, and thus, increase the electricity generation of MFCs [19,20]. Nevertheless, a bare CNT cannot to serve as a stand-alone anode material, it must be attached to a carrier to form an electrode with a high specific surface area and conductivity. A good CNT carrier should possess the following properties: be beneficial for CNT loading; be suitable for microbial colonization and attachment; possess good biocompatibility; and support efficient electron and substrate transfer. Therefore, it is key to select a suitable CNT carrier for CNT-based electrode construction.

In recent years, three-dimensional (3D) macro-porous materials were widely employed as anode materials, owing to their high specific surface area, good biocompatibility, and stable chemical characteristics [21–23]. When microbial cells grow on a planar anode, such as a carbon cloth or carbon paper, they are exposed on the surface of the anode, which leads to poor electrocatalytic activity [24]. In contrast, a 3D macro-porous structure can provide a large specific surface area, reduce the diffusion resistance effect, and enhance the electron transfer process by maximizing the contact of microbial cells with the anode [23,25]. Furthermore, a 3D macro-porous sponge is a commonly available material with low cost and high strength, and is a suitable CNT carrier that

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shows promise as an electrode material for MFCs. Thus, a CNT-coated sponge, a common and inexpensive material, not only offers a high specific surface area but also provides high conductivity and efficient substrate and electron transfer.

In this study, a CNT-coated sponge anode with an open 3D macro-porous structure was constructed, and *E. coli* K12 cells were used as an anodic electrogenic bacterium. The performances of MFCs with different CNT-coated sponge anodes were compared. The nanotoxicity of CNT toward *E. coli* K12 cells before and after electricity generation was also explored.

2. Methods and materials

2.1. Electrode materials and MFC construction

Different concentrations of CNT suspensions (1, 10, 20, 30 and 40 mg mL⁻¹) were prepared by dispersing multi-walled CNT (Chengdu Organic Chemicals CO., Ltd., China) in 10 mg mL⁻¹ sodium dodecylbenzene sulfonate (SDBS) [21]. The prepared CNT suspensions were then sonicated in a bath to disperse the CNT for 1 h. A CNT-coated sponge was fabricated by dipping a polyurethane sponge (Shenzhen Junfa Packaging Products Co., Ltd., China) into the CNT suspension for 12 h, and then drying at 90 °C. The physicochemical properties of the CNT-coated sponges are listed in detail in Table 1.

A classic H-shaped MFC was constructed by connecting two 130 mL glass flasks as we previously described [25]. *E. coli* K12 was used as an anodic electrogenic bacterium, and a CNT-coated sponge served as an anode and a carbon paper (Daofeng Co. Ltd., China) was employed as a cathode. The anodic media and cathodic electrolyte of the MFC were prepared as we previously described [25]. All MFCs were operated at a fixed current of 0.3 mA.

2.2. Analytical methods

The electrochemical activity of the anolyte was measured using cyclic voltammetry (CV). A glassy carbon electrode (GCE), a Pt sheet, and a saturated calomel electrode (SCE) were used as working electrode, counter electrode, and reference electrode, respectively. The electrolyte solution contained 5 mL phosphate buffer solution (PBS, 50 mM, pH 7.0) and 10 mL anolyte sample of an electricity generation process at stable power output period. Additionally, the potential of the MFC and the chemical oxygen demand (COD) value of anolyte were measured as we previously described [25].

2.3. Characterization of the CNT-coated sponge

The morphologies of CNT-coated sponges before and after microbial colonization were characterized using a Nova NanoSEM 450 field emission scanning electron microscope (SEM, JSM6700F). For SEM sample preparation, a small piece of CNT-coated sponge anode was removed and immediately fixed in an anaerobic glutaraldehyde solution (2.5%, v/v). Then, the sample was washed three times with PBS (100 mM, pH 7.3), and immobilized in an osmium tetroxide solution (1%, v/v) for 1.5 h. After washing with ultrapure water, the sample was dehydrated by immersing in a gradient ethanol series (30%, 50%, 70%,

80%, 90%, and 95%) for 15 min at each concentration. After dehydration in 100% ethanol twice, the sample was subjected to critical-point drying by using an EM CPD300 (Leica, Germany). The desiccated sample was coated with evaporated gold and viewed by using a Nova NanoSEM 450 SEM.

2.4. CNT toxicity measurement

To measure CNT toxicity, *E. coli* K12 cells were cultivated in a Luria-Bertani (LB) medium and harvested in the exponential phase (the optical density was approximately 0.68 to 0.70 at 620 nm) of growth by centrifugation at 4000 ×g for 10 min. The pellet was washed twice with sterile NaCl solution (0.9%, w/v) to eliminate complicating effects associated with macromolecules and other constituents in the culture medium. The pellet was then resuspended in sterile NaCl solution (0.9%, w/v) to achieve an initial concentration of 0.1 g mL⁻¹ before exposure to CNT. Different amounts of CNT were added in NaCl solution (0.9%, w/v) to form 1, 10, 20, 30, 40 mg mL⁻¹ CNT suspensions. Bacterial suspension (5 mL) and CNT suspension (5 mL) were added to a 50 mL flask and fully mixed using a vortex mixer. The cell suspension was then incubated at 30 °C for 2 h in a dark shaker. A flask containing 5 mL bacterial suspension and 5 mL NaCl solution (0.9%, w/v) was used as a control. The toxicity of the CNT toward *E. coli* K12 cells was evaluated by serial dilution on LB agar plates, and the number of colony forming units (CFU) was compared with the control after 24 h incubation at 30 °C [26]. All treatments were prepared in duplicate and each experiment was repeated at least three times to ensure data repeatability.

3. Results and discussion

3.1. The physicochemical properties of CNT-coated sponges

The anode material functions not only to immobilize microbial cells but also directly involved in the electron transfer between microbial cells and the anode [18]. Therefore, the physicochemical properties of the anode material are crucial to improve electricity generation. In this study, the physicochemical properties of CNT-coated sponges, prepared with different CNT concentrations, were first investigated. As shown in Table 1, the thicknesses of the sponge samples were all uniform (2.0 ± 0.1 mm). The average weight density and conductivity of the CNT-coated sponges increased gradually as the CNT concentration rose from 1 to 40 mg mL⁻¹. This result demonstrates that the CNTs were successfully loaded onto the sponge, because of the favorable conditions created by the open 3D macro-porous structure. In addition, there was no linear correlation between the average weight density of the CNT-coated sponge and the conductivity. A sharp increase in conductivity was observed at the concentration of 40 mg mL⁻¹ CNT, indicating more CNT loading onto the sponge.

3.2. MFCs performance with a CNT-coated sponge as the anode

After construction of the CNT-coated sponges, the performance of the MFCs with different concentrations of CNT-coating on the sponge anodes was investigated in batch mode. Fig. 1A shows the typical electricity generation cycles of MFCs with 1–40 mg mL⁻¹ CNT-coated sponges as anodes. When a 1 mg mL⁻¹ CNT-coated sponge served as the anode, the MFC showed the shortest stationary phase, lasting approximately 336 h, with a low voltage output of 0.2 V. With a 10 mg mL⁻¹ CNT-coated sponge as the anode, the stationary phase of the MFC lasted approximately 362 h. From 0 to 250 h, the voltage of the MFC reached 0.59 V in the first 10 h and then, decreased gradually from 0.59 V to 0.15 V, which was a better performance than the 1 mg mL⁻¹ CNT-coated sponge. For the 20 mg mL⁻¹ CNT-coated sponge anode, the MFC performance improved greatly with a long stationary phase lasting approximately 827 h. From 0 to 210 h, the voltage of the MFC increased gradually to 0.55 V, and maintained a voltage range of

Table 1
The physicochemical properties of CNT-coated sponge.

Sponge with CNT (mg mL ⁻¹)	Thickness (mm)	Average weight density (kg m ⁻³)	Conductivity (S m ⁻¹)
0	2.0 ± 0.1	18.0	0.000
1	2.0 ± 0.1	29.5	5.043 × 10 ⁻⁸
10	2.0 ± 0.1	36.3	2.312 × 10 ⁻²
20	2.0 ± 0.1	41.5	1.416 × 10 ⁻¹
30	2.0 ± 0.1	50.7	1.677 × 10 ⁻¹
40	2.0 ± 0.1	86.1	2.229

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