ACS APPLIED MATERIALS & INTERFACES

Conductive Carbon Nanotube Hydrogel as a Bioanode for Enhanced Microbial Electrocatalysis

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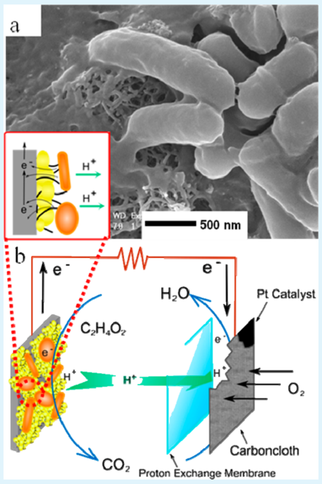
Supporting Information

ABSTRACT: Enhancing microbial electrocatalysis through new material design is essential to the efficient and stable operation of bio-electrochemical system (BES). In this work, a novel conductive carbon nanotube (CNT) hydrogel was fabricated by electrodepositing both CNT and chitosan onto a carbon paper electrode and used as a BES anode electrode. The microscopic, spectroscopic, and electrochemical analytical results show that the CNT hydrogel exhibited an excellent electrochemical activity. In the BES tests, the current generation and the maximum power density of the MFC with the CNT hydrogel increased by 23% and 65%, respectively, compared with the control. This demonstrates that the utilization of such a hydrogel offers an effective approach to enhance the current generation of BES. The great conductivity of CNT and the high content of oxygen-containing functional groups (C—OH, C═O, etc.) on their surface were found to be responsible for the improvements. Our work provides a facile way to prepare appropriate BES electrodes and offers a straightforward and effective route to enhance the BES performance.



KEYWORDS: *carbon nanotube (CNT), electrocatalysis, electron transfer, hydrogel, bio-electrochemical systems (BES)*

INTRODUCTION

Microbial electrocatalysis at electrodes is the decisive aspect of all microbe-electrode interaction-based systems. A microbial bioelectrochemical system (BES), such as microbial fuel cell (MFC), applies the ability of electrochemically active bacteria donating electrons to an anode.[1] BES has received extensive attention for extracting energy from organic wastes and converting them into commodity chemicals.[1−5] However, owning to its low power density and poor long-term stability, its application is still limited.[6] To make this technology more practical, many efforts have been made to improve its efficiency through exploring the limiting factors, such as microbial metabolism,[7] microbial electron transfer,[4] proton exchange membrane,[8] internal and external resistance of the electrolytes,[9] reactor configuration,[10] efficiency of cathode oxygen diffusion and supply.[11] Among these limiting factors, the electron transfer efficiency is recognized to be essential in increasing current and thus improving the MFC performance.[12,13]

Development of anode materials with a high conductivity and electrochemical activity is an appropriate solution to sort out the aforementioned problem. Several methods have been developed to accelerate electron transfer and improve MFC performance through applying different chemical catalysts, nanomaterial-biofilm matrix, and bioactive redox compounds.[14−17] Besides high conductivity, chemical stability, biocompatibility, resistance to decomposition, and catalytic activity, optimal anodes preferably require porous structure to allow internal colonization and strong interaction, including affinitive mechanical contact and higher electrical conductivity, between anode surface and microbial biofilms to facilitate extracellular electron transfer.[18]

As a representative of a new class of nanomaterials, carbon nanotubes (CNTs) are composed of graphitic carbons with one or several concentric tubules and were recently used for fabricating MFC anode.[19−21] CNT/polyaniline hybrid material was coated onto metal foams with polytetrafluoroethylene as a binder,[20] which increased MFC current density. However, the MFC current output was limited because polyaniline is redox-active only in acidic solution (e.g., pH < 3). Polyaniline is usually deprotonated and nonconducting at neutral pH,[22] and polytetrafluoroethylene is not conductive. In more recent studies, CNTs were coated on a glassy carbon electrode with a diameter of 3 mm,[19] carbon paper,[21] and sponge[23] with electrostatic interactions. These could result in enhanced MFC

Received: January 28, 2014
Accepted: May 12, 2014
Published: May 12, 2014

performance. However, the thin CNTs film was not stable for a long-term operation, especially under anaerobic corrosive conditions. For other effective anode fabrication, chemical vapor deposition method has been used, but it is complex and also expensive.[24,25] Polymer-based conducting hydrogels are expected to serve to electrically connect redox centers of enzymes to electrodes, which have been extensively used in biofuel cells and biosensors.[26] However, a CNT-doped hydrogel for enhanced microbial electrocatalysis has not been reported so far.

In this study, we report the fabrication of a CNT hydrogel and demonstrate its effectiveness and stability when it was used in MFC. Such a self-assembled CNT hydrogel was prepared through a simultaneous electrodeposition of both CNT and chitosan onto a carbon paper electrode. To the best of our knowledge, this is the first attempt to develop a CNT hydrogel to enhance the current generation of MFC.

■ MATERIALS AND METHODS

Chemicals. CNTs with a diameter of 20−40 nm and a length of 5−15 μm were purchased from Nanoport Co., China, and pretreated according to the previous report.[27] Other chemicals are of analytical grade and were used without further purification.

Preparation of the CNT Hydrogel. Chitosan solution (1%, wt %) was prepared by ultrasonically dissolving chitosan flakes in 1% acetic acid and 0.01 M KCl solution at 40 °C. pH was adjusted to 5.0 using 1.0 M NaOH. A certain amount of CNTs was added into the chitosan solution with aid of ultrasonic agitation for 1 h. After the CNTs were treated with nitric acid and sulfuric acid by sonication, they became completely dispersed in the chitosan solution to form a black solution. Electrochemical deposition was conducted through dipping the carbon paper (3×3 cm^2, 190 μm thickness, Toray Co., Japan) into the CNT-chitosan solution at −3 V for 5 min. Unless mentioned otherwise, the contents of chitosan and soluble CNT were 1.0% and 0.5 mg/mL, respectively. The carbon paper was then removed from the solution and rinsed with water to obtain a CNT hydrogel-modified electrode.

Characterization of the CNT Hydrogel. After the CNT hydrogel-modified carbon paper was dehydrated in air, a piece (0.5×0.5 cm^2) of electrode was cut down for field-emission scanning electron microscope imaging (SEM, Sirion200, FEI Ltd., the Netherlands). X-ray photoemission spectroscopy (XPS) analysis was performed using an ESCALAB 250 instrument (VG Instrument Ltd., U.K.) with a monochromatic Al Kα source (1253.6 eV). Cyclic voltammetric analysis was performed using a CHI660C electrochemical workstation (Chenhua Instrument Co., China) with a conventional three-electrode cell. The CNT hydrogel-modified carbon paper served as the working electrode, whereas Ag/AgCl (KCl sat.) and a Pt wire were used as the reference and counter electrodes, respectively. CV was conducted between −0.8 and 0.8 V in 50 mM Na_2HPO_4 and NaH_2PO_4 solution at pH 7.0 with pre-determined scan rates. The electrolyte solutions were purged with N_2 for 15 min to reduce the dissolved oxygen in solution before electrochemical measurement. Electrochemical impedance spectroscopy (EIS) measurements were conducted over a frequency range from 0.1 Hz to 100 kHz at −0.2 V with a perturbation signal of 5 mV in a 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture with 0.1 M KCl as the supporting electrolyte.

MFC Set-up and Inoculums. When the electrodes were held with a potentiostat (CHI 1030A, Chenhua Instrument Co., China) at −0.1 V (an Ag/AgCl KCl sat.), a dual-chambered fuel cell was constructed. The volume of each chamber with the electrode was approximately 80 mL of medium with a 20 mL headspace. The two chambers were separated with a proton exchange membrane (PEM, GEFC-10N, GEFC Co., China). The CNT hydrogel-modified electrode and the raw carbon paper were respectively poised as the working electrode at a constant potential. The raw carbon paper was used for the control experiment. A Ag/AgCl reference electrode was introduced into the anode-working electrode chamber by embedding it in a butyl rubber stopper. Raw carbon paper (3×3 cm^2) was used as the counter electrode, as the counter electrode chamber was filled with potassium ferricyanide of 50 mM $K_3Fe(CN)_6$ in 50 mM phosphorus buffer solution (PBS). Current was measured directly by the potentiostat every 45 s.

When electrodes were operated as fuel cells, 100 mL single-chamber MFCs (80 mL of medium with a 20 mL headspace) were used to evaluate their current generation. The carbon cloth with Pt 0.05 mg/cm^2 (GDL, GEFC Co., China) was used as the cathode. The coated side of the cathode was placed facing PEM, with the uncoated side directly exposed to air. The cell voltage (V) across a 1000 Ω resistor was automatically collected every 10 min using a data acquisition system (USB2801, ATD Co., China). Cell voltages at different external resistances were measured to prepare the polarization curves and also used to calculate the power (P) according to $P = IV$. At each resistance, MFCs were operated for at least 1 h to ensure a stable current output. Power density was calculated using the current normalized by the anode projected area.

Five milliliters of highly diverse anaerobic sludge from a full-scale wastewater treatment anaerobic reactor was inoculated to enrich electrochemically active bacteria. All of the MFCs were operated following according to a previous report.[28] The anodic electrolyte solution was replaced when the voltage decreased to less than 30 mV. To enrich electrochemically active microorganisms, the suspended sludge in the anode chamber was allowed to settle down for at least 5 min after 1 week operation, then the supernatant was removed and nutrient medium was amended to recover the volume. To evaluate the electrode stability, the two electrodes were autoclaved at the end of the test. Then, the MFCs were restarted using the two autoclaved electrodes as anode following the procedures described above. To quantify the biomass attached on electrodes, total proteins of biomass on the electrodes were assayed following a previous report.[29] Briefly, electrodes were removed from the bioreactor and the washed biomass was incubated in 1 mL of 0.2 N NaOH for 30 min at 90 °C to solubilize the attached proteins. The supernatant was analyzed using the bicinchoninic acid assay (Sangon Biotech Co., China) according to the manufacturer's instructions.

■ RESULTS AND DISCUSSION

Fabrication and Characterization of the Self-Sssembled CNT Hydrogel. The procedure for constructing the CNT hydrogel on the rough carbon paper electrode is illustrated in Figure 1a, which is based on a method for assembling chitosan hydrogel onto patterned surface.[30,31] When a potential of −3 V was applied, H^+ in the CNT colloidal solution could be reduced to hydrogen and electrodeposition occurred, as a local high pH on the cathode surface induced a sol-gel transition of the pH-responsive chitosan (chitosan is soluble at pH below its pK_a of 6.0−6.5).[32] The porous CNT hydrogels were formed on the carbon paper surface as the template of hydrogen bubbles (Figure 1b).

The image in Figure 1c shows that the freshly prepared CNT hydrogels were deposited on the carbon paper. The hydrogels, with a thickness of ∼3 mm could be stored in distilled water for 1 week without noticeable change in structure. When the hydrogels were stored in air, they were observed to dehydrate in 1 day. The carbon fiber surface of the raw carbon paper could be clearly identified with slick structure, as shown by the SEM image in Figure 1d. Figure 1e reveals that the CNT hydrogel did not aggregate, suggesting that the CNT hydrogels were formed through a one-step electrodeposition process.

XPS elemental analysis was performed to probe the surface functional groups on the modified carbon paper. The increased O1s photoemission peak was shown on the carbon paper covered by the CNT hydrogels (Supporting Information (SI)

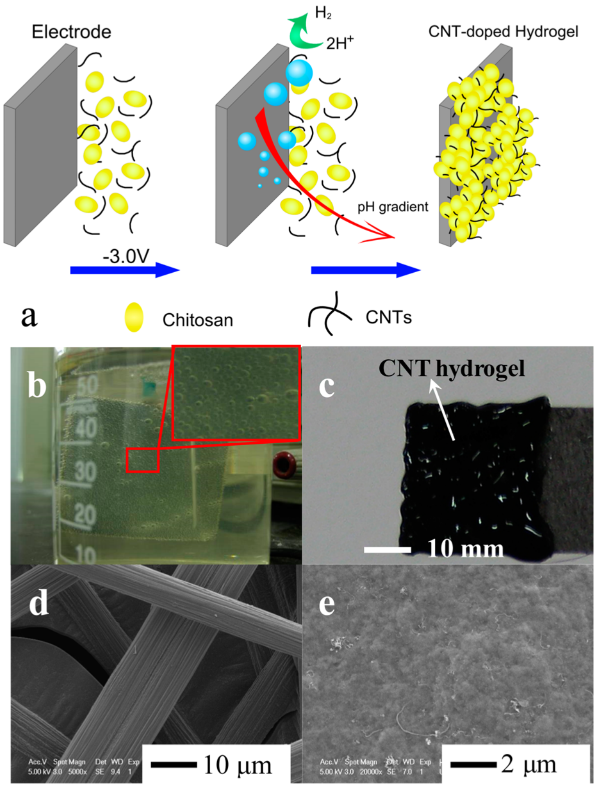


Figure 1. Schematic of the CNT hydrogeld electrode preparation (a), images of the hydrogen bubbles produced as template in the deposition process (b), CNT hydrogel on carbon paper (c), SEM images of carbon paper (d), and CNT hydrogel-modified carbon paper (e).

Figure S1). The analysis of C 1s spectra shows that an asymmetric peak from sp^2 hybridized carbons centered at 284.5 eV was generated from the carbon paper, which was usually used for all other fittings of the sp^2 hybridized graphite-like carbons (Figure 2a and b). Using this asymmetric peak as a reference, the C 1s peaks of the CNT hydrogels were fitted to three Gaussian−Lorentzian shape peaks, which were attributed to C—N or hydroxyls C—OH (286.2 ± 0.1 eV), and carbonyls C=O (quinone structures) (287.8 ± 0.2 eV) (Figure 2b).[33]

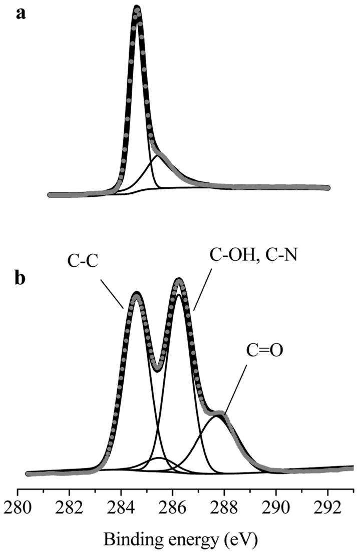


Figure 2. XPS C 1s spectra of the carbon paper (a), CNT hydrogel-modified carbon paper (b).

Electron Transfer Kinetics of the CNT Hydrogel Electrode. The electron transfer kinetics of a redox probe, $Fe(CN)_6^{3-}/^{4-}$ at the two different electrodes were explored with EIS. The measured EIS results show the well-defined single semicircles over the high frequency range, followed by the short straight lines in the low-frequency region for the two samples (Figure 3a). The diameter of the semicircle corresponds to the interfacial charge-transfer resistance (R_{ct}), which usually represents the resistance of electrochemical reactions on the electrode.[20] A smaller R_{ct} indicates a faster electron-transfer rate. Figure 3a shows that R_{ct} was remarkably reduced as the CNT hydrogel was coated on the carbon paper, implying that the CNT hydrogel accelerated the electron transfer. This might be ascribed to the enhanced electron transfer rate by the formation of the CNT hydrogel. This result suggests that the hydrogel was an ideal material to enhance the microbial electron transfer.

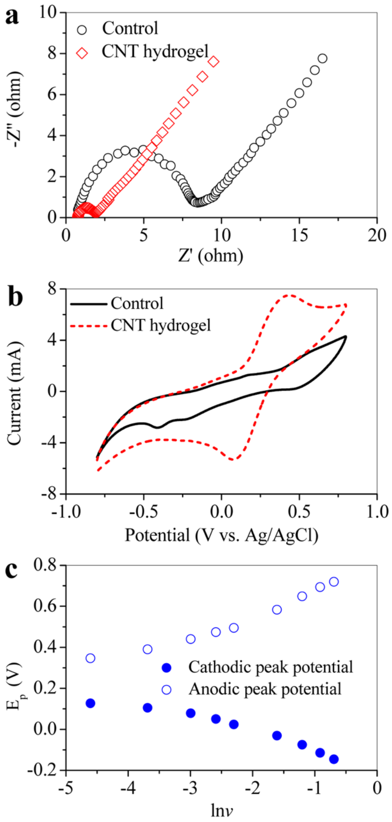


Figure 3. Nyquist plots of 10 mM $[Fe(CN)_6]^{3-}/^{4-}$ in 0.1 M KCl from 100 kHz to 0.1 Hz at an ac amplitude of 5 mV under open-circuit potential conditions (a), CVs of 50 mM PBS in 0.1 M KCl (pH 7.0) at a scan rate of 50 mV/s (b), semilogarithmic dependence of the cathodic peak potential, anodic peak potential, and the scan rate for CNT hydrogel-modified carbon paper in PBS solution (pH 7.0) (c).

CV analysis was conducted in 50 mM PBS (pH 7.0) to further explore the electron transfer kinetics in the CNT hydrogel-modified carbon paper. The raw carbon paper had no redox response in PBS in a potential ranging from −0.8 to 0.8 V (Figure 4b), because there were no redox functional groups on the raw carbon paper, as demonstrated by the XPS analysis (Figure2a). However, a pair of surface peaks was observed for the CNT hydrogel-modified electrode (Figure 3b), indicating

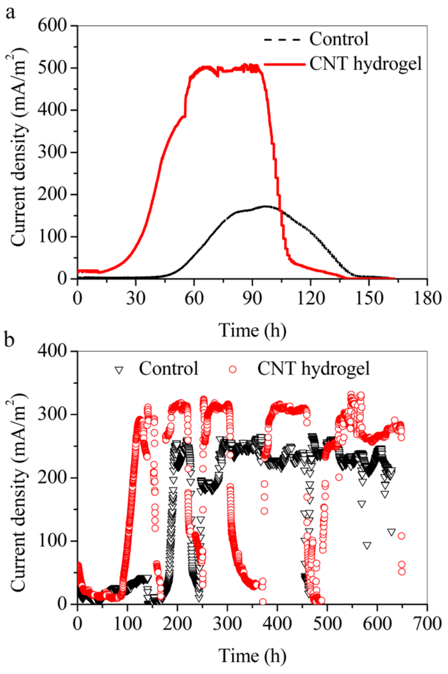


Figure 4. Electricity generation with the different electrodes poised at −100 mV vs an Ag/AgCl reference electrode (a); performance of the MFCs with different anode materials (b).

nearly reversible or quasi-reversible electron-transfer kinetics. In addition, the potential scan rate influenced the cyclic voltammetric behavior of the CNT hydrogels (Figure 3c). Laviron model is usually used to estimate the electron transfer rate constant of the surface reaction, k_s:[34]

$$E_{pc} = E^{o'} + \frac{RT}{\alpha nF} \ln \frac{RTk_s}{\alpha nF} - \frac{RT}{\alpha nF} \ln v \tag{1}$$

$$E_{pa} = E^{o'} + \frac{RT}{(1-\alpha)nF} \ln \frac{(1-\alpha)nF}{RTk_s} + \frac{RT}{(1-\alpha)nF} \ln v \tag{2}$$

where α is the electron-transfer coefficient, v is the scan rate, and $E^{o'}$ is the formal potential.

Figure 3c shows the plot of E_P vs ln v of the CNT hydrogel-modified electrode in PBS (pH 7.0). E_P could be estimated as the middle between the cathodic and anodic peak potentials at a low scan rate.[35] At a high scan rate, the plot of E_P vs ln v was linear. The equations of the straight lines are as follows:

$$E_{pc} = -0.1995 - 0.0961 \ln v \tag{3}$$

$$E_{pa} = -0.8009 + 0.1261 \ln v \tag{4}$$

From the above equations, the k_s value for the cathodic reaction was calculated to be 0.1 s^{-1}, and that for the anodic reaction was 0.09 s^{-1}, suggesting that the rate-determining step in the reduction process might be different from that in the re-oxidation process.[34] For an MFC bioanode, the electron transfer from bacteria to electrode is a major rate-limiting step. Interestingly, the k_s value for the anodic reaction obtained in this study is of a similar level to that of biological electron shuttles, such as riboflavin or quinone, adsorbed on an electrode (k_s < 0.7 s^{-1}).[36] These results indicate that the CNT hydrogel could facilitate the electron transfer at the solution/electrode interfacial, resulting in the high efficiency of the modified electrode as an MFC anode.

Performance of the CNT Hydrogel in Microbial Power Generation. The CNT hydrogel had stable electrochemical behavior. To evaluate the microbial electricity generation of the CNT hydrogel-modified electrode under defined conditions, it and the raw carbon paper electrode were respectively poised as the working electrode at a constant potential (−100 mV versus a Ag/AgCl reference electrode) with a potentiostat. The raw carbon paper was used for control experiment. Figure 4a shows the current density profiles. The current density at the CNT hydrogel-modified electrode started to increase 20 h after inoculation, which was much faster and reached a higher value (500 mA/m^2), compared with the raw carbon paper (150 mA/m^2). The Coulombic efficiency of the poised potential cells with the CNT hydrogel-modified electrode and the carbon paper were calculated to be 32% and 19%, respectively. The higher current density in our study offers solid evidence that the CNT hydrogel facilitated the current generation.

Figure 4b shows the start-up and long-term performance of the MFCs with two different materials as anode. The current of the MFC with the CNT hydrogel increased sharply at hour 90, while that of the control MFC increased nearly 100 h behind. The current density (across a 1000 Ω resister) of the MFC with the CNT hydrogel during the stable period was 320 mA/m^2, which was substantially higher than that with the carbon paper (260 mA/m^2). The open circuit voltage was 566 mV for the MFC with the CNT hydrogel-modified electrode, much higher than that for the MFC with the raw carbon paper anode (480 mV). With the CNT hydrogel-modified electrode, a maximum current density of 132 mW/m^2 was generated (Figure 5).

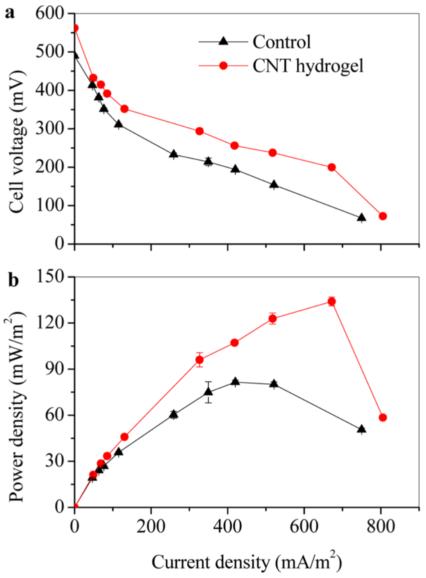


Figure 5. Polarization curve (a) and power output (b) of the MFCs with different anodes.

The increase in current with the CNT hydrogel-modified electrode might be attributed to the multi-layered biofilm because of the large surface area of the CNT hydrogel. After the experiments, biomass standardized to the whole proteins on the two electrodes was measured and found to be nearly equivalent to 16.3 μg of proteins. Thus, the biomass amont attached onto the electrode was not responsible for the increased current at the CNT hydrogel.

Electrochemically active bacteria mainly have two distinct pathways for electron transfer: direct electron transfer through outer membrane c Cyts and indirect transfer mediated by electron shuttles.[37,38] Our recent work has revealed that the use of CNT could facilitate the electrode to reach closer to the active center of c Cyts. The decrease in c Cyts-CNT distance suggests that the CNT could accelerate the microbial electron transfer. Furthermore, the use of CNTs could reduce the thermodynamic barrier for electron transfer from bacteria to CNTs.[27] Because of the direct contact between bacteria and CNT (Figure 6), which could enhance the communication between c Cyts and CNT and accelerate the electron transfer at the bacteria/electrode interface, the MFC with the CNT hydrogel had a higher current density than that with the raw carbon paper.

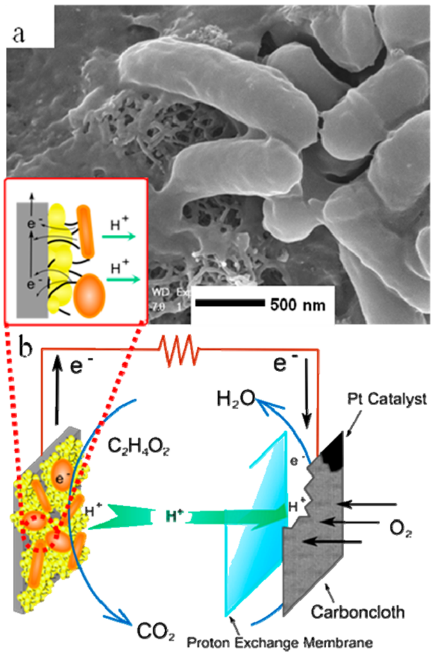


Figure 6. Biofilms attached on the CNT hydrogel-modified carbon paper (a) and the working principles of the MFC bioanode (b).

In addition, the quinone structure on the surface of CNT could act as a redox mediator, as evidenced by XPS. It was reported that activated carbon rich in quinone surface groups was used as an immobilized redox mediator for the reduction of azo dye by facilitating microbial electron transfer rate.[39] The shift between quinone and hydroquinone of the carbonyl group could be response for the highest current and power density of the MFC with the CNT hydrogel anode. To examine the stability of the anode material, after autoclaving the electrodes with biofilms, the MFC was restarted up (SI Figure S2). The better performance of the MFC with the CNT hydrogel demonstrates that this material was stable.

Significance of this Work. BES has been extensively investigated recently. Various configurations of BESs have been developed with a volume varying from microliters to liters.[10,40] Therefore, design and application of novel electrodes with patterned structure in a facile and flexible manner are of a significance to improve their current generation and meet the BES configuration requirements. Also, it is beneficial for elucidating the mechanisms of microbial electron transfer.[41,42] In this work, a new anode material was prepared for BES application and the prepared CNT hydrogel was demonstrated to be an effective and stable anode to generate electricity in BESs. The use of such a nano-structured material as BES anode could significantly enhance their current density, which is originating from the increased microbial electron transfer rate. The operating results show that the MFC with such a CNT hydrogel anode has a better stability, compared with the control. In addition, the use of electrochemical deposition to fabricate this porous CNT hydrogel is flexible and easy to handle, without using complicated device, such as chemical vapor deposition. This enables the possibility of BES for its cost-effective scaling up for wastewater treatment. Our work not only offers a straightforward and effective route to enhance the MFC current generation but also provides an easy way to prepare appropriate MFC electrodes.

CONCLUSIONS

A new CNT hydrogel was developed through electrodepositing both CNT and chitosan onto a carbon paper electrode and used as an effective MFC anode material. Both the power generation and the maximum power density of the MFC with the CNT hydrogel increased substantially, compared with the control. The great conductivity of CNT and large content of functional groups on their surface were response for the improvement of electron transfer and the enhanced power generation of the MFC with the hydrogel anode.

ASSOCIATED CONTENT

Supporting Information

Results on XPS and MFC performance in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors wish to thank the National Basic Research Program of China (2011CB933702), the Program for Changjiang Scholars and Innovative Research Team in University of the Ministry of Education of China and the National Synchrotron Radiation Laboratory, Hefei, China, for the partial support of this work.

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