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Anolyte *in-situ* functionalized carbon nanotubes electrons transport network as novel strategy for enhanced performance microbial fuel cells

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HIGHLIGHTS

- Functionalized CNTs are exploited to construct anloyte *in-situ* network in the MFC.
- The proposed network can compile the electrons from the anode-away microorganisms.
- Superior power (1.1 W m^{-2}) and current (4.1 mA m^{-2}) densities were obtained.
- The functionalization process and the CNTs content should be optimized.

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ABSTRACT

Anolyte *in-situ* electrons transport network from functionalized multi-walled carbon nanotubes is introduced as a novel strategy to compile the electrons from the anode-away microorganisms. The strategy was investigated by a single-chamber air-cathode microbial fuel cell with *Escherichia coli*. Based on our best knowledge, for the utilized MFC configuration, unprecedented output power (1.1 W m^{-2}) and current density (4.1 A m^{-2}) were obtained when 6.06 mg ml^{-1} autoclave-treated MWCNTs (in tryptone medium at 121 °C) was used. Moreover, after 6 h working time, the observed current density was almost duplicated in case of using the best sample compared to the carbon nanotubes-free cell. FTIR and Bio-TEM analyses indicated that the proposed hydrothermal treatment leads to functionalize the carbon nanotubes by nitrogenous groups that strongly enhances the attachment with the bacterial cell wall and improves the biocompatibility. EIS measurements confirmed the good adhesion as a small charge-transfer resistance was observed; 33 Ω. Besides the treatment temperature of the carbon nanotubes, which should be 121 °C, the concentration in the anolyte should be optimized; 6.06 mg ml^{-1} reveals the best performance compared to 1.21, 3.63, 8.48 and 10.9 mg ml^{-1}. On the other hand, due to formation of the carbonyl group, acid treatment converts the carbon nanotubes to have antibacterial activity toward the *E. coli* that decreases the cell performance drastically.

1. Introduction

Microbial fuel cell (MFC) is a system in which the organic matters are biologically oxidized by microorganisms, which in turn transfer electrons to the anode. These electrons flow to the cathode through an external load to produce an electrical current [1]. One of the most unique advantage of using MFC is the metabolic diversity of the microorganisms which enables using of various substrates for electricity generation [2]. However, insufficient performance obstructs the practical use [3]. Up to date, the electron transfer rate by the exoelectrogenic bacteria (i.e. the current generation) and the electron transfer proportion (i.e. the Columbic yield) are still the major bottlenecks for the performance of MFCs and their applications [4,5]. To increase the electron transfer rate and consequently the generated power, various approaches were attempted including utilizing different types of microorganisms [6], media (fuel) [7,8], materials/sub-materials for electrodes [6], membrane [9], and cell configurations [10,11]. The aforementioned strategies focus on increasing the delivered electrons to the anode by increasing the theoretical produced bacterial electrons rate or decreasing the transfer resistance or maximizing the

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number of the anode-attached microorganisms or facilitating electrons diverting to the anode. However, the achieved success is still not satisfactory.

Development the anode can distinctly enhance the MFC performance due to the direct and fast response on the generated power. In this regard, the reported efforts have been done for two main purposes: (1) increasing the anode surface area to provide an enough space for the biggest number of microorganisms to contact the anode [12,13], and (2) functionalizing the anode material by new groups to improve the cell attachment [14,15]. These two goals are mainly focusing on increasing the number of the anode-attached microorganism. However, whatever the anode surface area, the number of anode-attached microorganisms is small compared to those cannot reach to the anode surface and are considered out of service [1]. Therefore, development of a novel methodology to compile the produced electrons from the anolyte-suspended microorganisms can strongly enhance the generated power. In other words, utilizing of long nanowires having high conductivity, interconnecting, biocompatibility, and good tendency to bind with the bacterial cell wall features can effectively transfer the electrons from a huge number of the microorganisms in the MFC medium. Most of the aforementioned requirements can be found in the multi-walled carbon nanotubes (MWCNTs). Moreover, the MWCNTs can be functionalized to add specific active groups for enhancing the bonding with the cell wall proteins [16–18].

The main purpose of this study was investigating the feasibility of exploiting MWCNTs as an anolyte *in-situ* electrons transporter system, and study the influence of the content and the type of functionalization process on the generated power. For a proper evaluation, a single and widely used culture model (*Escherichia coli; E. coli*) with a simple single chamber air-cathode MFC were utilized. The results were interesting as amide groups-containing MWCNTs at a specific content showed unprecedented power and current densities with the utilized microorganism and cell design.

2. Materials and methods

2.1. MWCTS functionalization and anolyte in-situ MWCNT-ET preparation

MWCNTs with an average diameter of ~15 nm (Carbon Nano-material Technology Co., Ltd., Korea) were functionalized by two different procedures (acid and hydrothermal treatments) to examine effect of the functionalization method on the performance of MWCNTs as an electrons transport system. Acid treatment functionalization method was carried out as described previously [19]. Briefly, the MWCNTs were sonicated for 4 h in a 1:3 mixture of concentrated nitric and sulfuric solutions, followed by precipitation and rinsing with distilled water. Functionalization by the hydrothermal treatment method was following the procedure reported by Ozden et al. [18]. In this case, the MWCNTs were autoclaved with the Tryptone Sucrose media at 121 °C and 180 °C to investigate the effect of autoclaving temperature degree. The process was carried out for 20 min with a steam sterilizer (Autocalve, AC-60, Hanyang Scientific Equipment Co., Ltd., Korea), while, for the 180 °C, a 200 ml Teflon-lined stainless-steel autoclave was used in the oven for 6 h [20].

2.2. Inoculum

Escherichia coli 0157 NCCP-14541 was grown aerobically at 37 °C overnight in the tryptone sucrose media which containing (for 1 L), tryptone (10 g), yeast extract (5 g), K_2HPO_4 (17.418 g) and sucrose (2 g) as the carbon energy source [21].

2.3. MFC configuration and operation

Single chamber air–cathode MFC with anode chamber volume of 110 cm^{-3}, was used. The anode (carbon felt, Alfa Aesar) was soaking in pure acetone and then dried in advanced [22]. The cathode was prepared by applied Pt/C (0.5 mg cm^{-2}) as catalyst on a water-facing side of the carbon felt (3.18 mm, Alfa Aesar) as described by Feng et al. [23]. The MFC was assembled as described in our previous work [24]. The cathode was sandwiched between CEM (CMI-7000, Membrane International Inc., NJ, USA) as proton exchange membrane and current collector (316 Stainless steel plates of 1 mm thickness) in one side. The anode was in the other side attached to current collector with a space of 4 cm for cathode side. The assembled MFC was sterilised by UV (CHC LAB Co., Ltd, Korea), while the culture media and the glassware adopted in this study were autoclaved at 121 °C for 20 min before operation with a steam steriliser (Autocalve, AC-60, Hanyang Scientific Equipment Co., Ltd., Korea). The MFC was operated in a batch mode at 37 °C. A 10 ml from the cell suspension plus 100 ml of media containing the functionalized MWCNTs were inoculated in the MFC, and cultivated under anaerobic conditions by blocking the access of oxygen [25].

Besides evaluate the functionalized method, the effect of functionalized MWCNTs concentration (1.21, 3.63, 6.06, 8.48 and 10.9 mg ml^{-1}) was examined to determine the preferable conditions for MFC performance. For comparison purpose, An MFC was operated without MWCNTs (*E. coli* with medium only) as a control.

2.4. Data acquisition and electrochemical characterization

The MFCs electrochemical analysis were obtained using HA-151A potentiostat (HA-151A POTENTIOSTAT/GALVANOSTAT, Japan). Cyclic voltammetry (CVs) was performed at a scan rate of 10 mV s^{-1} using a three-electrode set up with the anode as a working electrode (WE) and a Pt wire and an Ag/AgCl as counter and reference electrodes (CE and RE), respectively. Linear sweep voltammetry was carried out to determine polarization curves at scan rate of 1 mV s^{-1} with a two-electrode system where the cathode was used as WE and the anode as both CE and RE [19,26]. Power (P) was obtained as $P = IV$. Power density was normalized to the anode surface area (6.25 cm^{-2}). The electrochemical impedance spectroscopy (EIS) data was obtained to study the electron transfer behavior in the frequency range of 0.01 Hz–100 kHz. EIS data was measured in a two-electrode mode, using the anode as the working electrode and the cathode as both the counter and reference electrodes. The voltage was recorded using a GL220 midi-logger. The morphology of the MWCNT-ET was characterized with Biological Transmission Electron Microscope (Bio-TEM, HITACHI 7650), where, the samples were freeze-dried in sterilized conical tubes (15 ml) then, serially diluted with ethanol and finally loaded on a carbon film, 400 mesh copper grades (CF400-CU, Electron Microscopy Sciences) for scanning. Fourier transform infrared spectroscopy (FT-IR) studies were conducted with a Frontier FTIR spectrometer (Perkin Elmer, USA), where, the functionalized MWCNTs were carefully washed from the nutrient media and then each sample (3 mg) was ground and mixed in a KBr matrix (100 mg). A pellet was formed by compressing the mixture at 167 MPa. Then, the pellets were placed in the oven at 40 °C for 24 h to remove the moisture. Finally, the pellets were applied for FTIR measurement. The spectra were measured with an average of 50 scans and a resolution of 4 cm^{-1} [27]. The MWCNTs biocompatibility toward the *E. coli* was examine by Bauer et al. (1966) agar disk-diffusion method [30]. Briefly, plain filter paper discs were sterilized by autoclaving at 121 °C for 20 min, after cooling the samples were kept on the discs by micro titer pipette with sterile tips. The discs were then applied on the agar surface containing *E. coli* biofilm. The results were obtained after 24 h of inoculation at 37 °C [28].

3. Results and discussion

3.1. Target and MWCNTs functionalization

Geobacter and *Shewanella* species are among the best performance microorganisms in the microbial fuel cells due to exploiting self-