WO₃ nanorods-modified carbon electrode for sustained electron uptake from *Shewanella oneidensis* MR-1 with suppressed biofilm formation

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1. Introduction

The capabilities of many microorganisms in electrode-dependent respiration have drawn intensive interest and led to the rapid development of BES in recent years [1–4]. The efficiency of the extracellular electron transfer (EET) at the microorganism-electrode interface is one of the critical factors limiting the performance of these BES [5]. Generally, there are three different EET routes between the exoelectrogenic bacteria and electrode [6]: (a) direct transfer through surface-bound cytochromes [7], (b) indirect transfer via redox mediators [8], and (c) through conductive nanowires [9]. Interestingly, unlike many extracellular bacteria, which mainly transfer electrons through a direct pathway that relies heavily on the formation of biofilm [10,11], other environmentally ubiquitous bacteria including *Shewanella Oneidensis* MR-1 accomplish EET predominantly via redox mediators.

Recent studies revealed that up to 95% of the electron transfer by *Shewanella Oneidensis* MR-1 in the BES proceeded through the riboflavin-mediated EET route [8,12]. Although the development of biofilm is favorable for direct electron transfer route [9], the formation of a dense biofilm would, on the contrary, significantly decrease the indirect EET rate due to the inefficient diffusion of mediators across the biofilm [13,14]. Since the mediated EET efficiency is considerably decreased upon formation of the biofilm, we hypothesize that a biofilm-free electrode would be beneficial for efficient and continuous stable operation of BES especially those operated by the indirect EET. Thus, it is of interest to prepare suitable electrode materials that could suppress the growth of biofilm and response sensitively to redox mediators.

In this study, we achieved this goal by modifying a carbon paper electrode with WO₃ nanorods. WO₃ nano-materials have widely been used in the biosensor, bio-imaging and BES. This is due to their good biocompatibility and electric conductivity [15–17]. Recently, we have reported the use of WO₃ nanorods as sensitive probe for electrochemically active bacteria including *Shewanella* species [18,19]. In this work, we revealed an interesting feature of this material, i.e., it could effectively suppress the establishment of biofilm. This makes it an ideal option of electrode material for...
promoting mediated EET. Our results demonstrated the superior performance of C–WO3 nanorods as an anode electrode over C–WO3 nanoparticles and pure carbon paper. In addition, the mechanisms of EET promotion and biofilm suppression were also elucidated.

2. Experimental section

2.1. Electrode preparation

Carbon paper was purchased from GEFC Co., China. Commercial WO3 nanoparticles were purchased from Sigma–Aldrich and used as received. WO3 nanorods were synthesized using a hydrothermal process with Na2WO4•2H2O as the precursor [20]. In brief, 0.825 g of Na2WO4•2H2O and 0.290 g of NaCl were dissolved in 20 mL of deionized water. Then, 3 M HCl was slowly added under stirring until pH reached 2.0. The solution was transferred into a 45 mL Teflon autoclave and heated at 180 °C for 24 h in an oven. After cooled down to room temperature, a white powder of WO3 nanorods was obtained. The powder was washed thoroughly with deionized water, and then filtered through a 0.45 μm membrane and the solid was collected. The structure of the prepared crystalline WO3 nanorods and the commercial WO3 nanoparticles was characterized using X-ray diffraction (XRD; X’Pert PRO, Philips Co., Netherlands), and the morphology of the prepared crystalline WO3 nanorods was observed using a scanning electronic microscope (SEM; JSM-6700F, JEOL Co., Japan).

The C–WO3 nanorods were prepared by painting the nanorods on a carbon paper. WO3 nanorods powder was at a weight ratio of 0.2% was added to 100 mL of ethanol and ultrasonicated for 30 min. The mixture was then uniformly spread on a carbon paper (2 × 4 cm) and maintained at 120 °C in an oven to evaporate off the ethanol. The C–WO3 nanoparticles were prepared as follows. A mixture of WO3 nanoparticles powder and poly(ethylene-glycol) at a weight ratio of 10% was added to 100 mL of ethanol and ultrasonicated for 30 min. The C–WO3 nanoparticles were then obtained following the same procedures as described above. After evaporation of ethanol, the sample was heated to 450 °C in a muffle furnace for 2 h to completely remove poly(ethylene glycol).

2.2. Microbial culture

Shewanella oneidensis MR-1, kindly provided by Prof. K.H. Nealson at the University of Southern California [21], was cultivated aerobically in Luria–Bertani (LB) broth. Cultures were grown at 30 °C and agitated at a rate of 150 rpm until the late stationary phase. The cells were collected by centrifugation at 5000 rpm for 5 min, washed three times with sterile sodium lactate minimal salt medium and re-suspended in sterile sodium lactate minimal salt medium. The optical density at 600 nm (OD600) was used to determine the concentration of planktonic bacteria. The mineral medium contained: lactate, 20 mM; HEPES, 50 mM; NaOH, 7.5 mM; NH4Cl, 28 mM; KCl, 1.3 mM; NaH2PO4•H2O, 4.3 mM; NaCl, 100 mM; and 10 mM/L of each of vitamin solution, amino acid solution, and trace mineral stock solution [22].

2.3. MFCs construction and operation

Double-chamber MFCs, with H configuration and a potassium ferricyanide cathode, were used in this work. Each electrode chamber had a volume of 100 mL. C–WO3 nanorods or C–WO3 nanoparticles with a uniform dimension of 2 × 4 cm2 was used as the anode, whereas carbon felt with a dimension of 2 × 4 cm2 was used as the cathode. The anode and cathode were separated by a proton exchange membrane, and connected through external circuit with a fixed external resistance of 1000 Ω to allow current measurement. Before experiments, the MFCs components were sterilized in autoclave at 121 °C for 20 min. The S. oneidensis MR-1 culture was inoculated into the MFCs chamber containing 100 mL of sodium lactate minimal salt medium to an initial OD600 of 0.2. The MFCs were operated in a batch mode. At the end of one cycle, the anodes were removed and used for SEM analysis.

2.4. Electrochemical measurements

A data acquisition system was used to record the voltage across the 1000 Ω resistor per 10 min. Current density was normalized by the projected area of the two sides of anode (16 cm2). The electrochemical performances of C–WO3 nanorods and C–WO3 nanoparticles were analyzed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in electrochemical probe solution (5 mM Fe(CN)6 3−/4− in 1/15 M phosphate buffer saline, pH 7.0). CV and EIS measurements were performed using a CHI 660 electrochemical workstation (CH Instruments Inc. USA), with an Ag/AgCl (3 M) reference electrode and a Pt counter electrode. The CV was initially scanned from a high potential to a low one, then followed by a reversed scanning. For EIS tests, the open circle potential was used as the initial potential, a frequency ranging from 10 K to 0.1 Hz was utilized with a potential amplitude of 5 mV. A nitrogen atmosphere of the solution was maintained during the measurements.

2.5. SEM analysis

The C–WO3 nanorods and C–WO3 nanoparticles electrodes before and after the MFCs operation were imaged using scanning electron microscopy (SEM). Prior to the imaging, the electrode samples were fixed for 2 h in 2.5% glutaraldehyde in sterile cultivation solution, rinsed thrice with phosphate buffer saline (pH 7.0, 50 mM), dehydrated by a graded ethanol series (30%, 50%, 70%, 80%, 95% and 100%), and then vacuum dried. Samples were coated with Au prior to the SEM observation.

3. Results

3.1. Structure characteristics of crystallize WO3 nanorods and commercial WO3 nanoparticles

Fig. 1A shows the XRD pattern of the synthesized WO3 products. All the diffraction peaks can be indexed to the hexagonal phase of WO3 structure (JCPDS 85-2460). The sharp peaks indicate that the prepared sample was well-crystallized. This was further confirmed by the SEM imaging, which clearly reveals the morphology and microstructure of the crystals and the formation of uniform WO3 nanorods. The average sizes of nanorods were 2 μm in length and 60 nm in width (Fig. 1B). The XRD pattern of commercial WO3 nanoparticles is shown in Fig. S1, the diffraction peaks can be indexed to the monoclinic phase of WO3 structure (JCPDS 83-0950).

3.2. Electrochemical characteristics of the modified electrodes

The C–WO3 nanorods and C–WO3 nanoparticles electrodes were characterized by CV and EIS in electrochemical probe solution (Fig. 2). All the electrochemical tests were conducted in a 20 mL beaker. The CV scanning rate was 5 mV/s. During the experiments, the positions of all the electrodes were kept unchanged to ensure a good comparability. The CVs of Fe(CN)6 3−/4− recorded with C–WO3 nanorods and C–WO3 nanoparticles electrodes both show a couple of well-defined redox

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peaks, with the reductive peak at 180 mV or 190 mV and the oxidative peak at 260 mV or 250 mV vs. Ag/AgCl (3 M). These peaks are ascribed to the electrochemical redox reactions of Fe(CN)$_6^{3-}$/Fe$^{4+}$ (Fig. 2A). However, very weak to almost no redox couple was observed in the CV when C electrode was used (Fig. 2A). Comparing these cyclic voltammograms, the highest current in the electrochemical redox couple of Fe(CN)$_6^{3-}$/Fe$^{4+}$ was recorded with the C–WO$_3$ nanorods electrode. The better catalytic activity of the C–WO$_3$ nanorods was further evidenced by ohmic resistance ($R_s$) and charge-transfer resistance ($R_{ct}$) data obtained from the EIS analysis [23]. In general, a smaller $R_{ct}$ value indicates a faster electron-transfer between electrode and electrolyte [23]. As shown in Fig. 2B, a substantial decrease of $R_{ct}$ was noted for electrode modified with WO$_3$-nanorods, suggesting a more efficient electron transfer process between the electrode and electrolyte. Thus, the CV and EIS characterizations both suggest that the C–WO$_3$ nanorods had a better performance and activity towards Fe(CN)$_6^{3-}$/Fe$^{4+}$ redox reactions than the other two electrode materials.

### 3.3. Electricity generation in MFCs

To evaluate galvanic current due to the electrode discharges, current evolution in fuel cells without *S. oneidensis* MR-1 inoculum was investigated. For all the fuel cells operated without *S. oneidensis* MR-1, very weak currents were initially observed, and quickly dropped and stabilized at around zero within about 4 h (Fig. S2). Electricity was generated in all the MFCs immediately upon inoculation with *S. oneidensis* MR-1. The different current responses for cells operated without *Shewanella* corroborate the crucial role of biological activity of *Shewanella*, which can be attributed to bacterial catalyzed lactate oxidation, in the electricity generation in these MFCs. After the current increased to the maximum value, only the system constructed with C–WO$_3$ nanorods electrode sustained a stable performance over a long time operation. In contrast, for the other MFCs with C- or C–WO$_3$ nanoparticles electrodes, the current density of the generated electricity started to drop after about 100 h of operation (Fig. 3). These trends were observed in two different sets of independent experiments, confirming the reproducibility. Comparing the three MFCs, MFCs with C–WO$_3$ nanorods anode produced the highest total charges of 61 C than the other two MFCs (44 C for MFCs with the C–WO$_3$ nanoparticles anode and 41 C for MFCs with the C anode). After the current of the MFCs system with C–WO$_3$ nanorods dropped to near zero,
supplement of a concentrated lactate solution into the anodic chamber could immediately restore and further maintain the current to its maximum level (Fig. S3).

3.4. Responses to riboflavin

Each aliquot of 15 mL sterile mineral medium, which contained 5 μM riboflavin as a redox mediator, was added into a 20 mL beaker. Before the electrochemical test, nitrogen was bubbled for 10 min to deoxygenate the mineral medium. The potential scanning was from −0.6 V to −0.2 V, with a scan rate of 50 mV/s. The cyclic voltammograms recorded using the C–WO3 nanorods electrode showed a well-defined redox couple with a reductive peak at −420 mV and oxidative peak at −370 mV vs. Ag/AgCl (3 M) (Fig. 4A), which is the characteristic electrochemically redox peaks of riboflavin, while the related reductive and oxidative peaks were observed at −470 mV and −350 mV, respectively, when the C-electrode was used. The much narrower peak-to-peak separation (20 mV vs. 120 mV) and higher peak current recorded with the C–WO3 nanotubes electrode are suggestive of more electrochemical reversibility of riboflavin. With the C–WO3 nanorods electrode, the peak current increased with the scan rate (Fig. 4B). In contrast, related electrochemical redox process of riboflavin was not observed in the cyclic voltammograms (Fig. 4A) when using the C–WO3 nanoparticles electrode. Similar results were also observed when glassy carbon was used as the working electrode (Fig. S4).

3.5. SEM analysis

The SEM morphologies of C, C–WO3 nanoparticles and C–WO3 nanorods electrodes before and after using in the MFCs are shown in Fig. 5. The deposition of WO3 nanorods on the carbon paper is illustrated in Fig. 5(a)B1. The WO3 nanoparticles on the C–WO3 nanoparticles electrode displayed a cracked-mud structure, which increased the actual electrode surface area [Fig. 5(a)C1]. After using the electrodes for MFCs operation, compact biofilm was formed on the surfaces of both the C electrode [Fig. 5(b)A2 and 5(b)A3] and C–WO3 nanoparticles electrode [Fig. 5(b)C2 and 5(b)C3]. In contrast, almost no bacteria were attached on C–WO3 nanorods electrode [Fig. 5(b)B2 and 5(b)B3].

Fig. 4. A) CV responses of different electrodes to riboflavin B) CV responses of C–WO3 nanorods electrode to riboflavin at different scanning rates.

Fig. 5. SEM images of electrodes (a) before and (b) after the MFCs operation. (A) C electrode; (B) C–WO3 nanorods electrode; (C) C–WO3 nanoparticles electrode.
4. Discussion

In BES, the EET efficiency of S. oneidensis MR-1 is subjected to gradual decline over time due to the formation of a dense biofilm on electrode, which hinder the diffusion of an electron mediator (e.g. riboflavin). As demonstrated in this study, such limitation can be circumvented by using a C–WO3 nanorods anode, where no biofilm was formed even on prolonged operation (Fig. 5). Because the C–WO3 nanorods electrode exhibited a strong response to riboflavin (Fig. 4), and did not affect the diffusion of riboflavin at the electrode surface over a continuous operation. As a consequence, a more sustained electricity generation process was achieved in this biofilm-free C–WO3 nanorods system over the dense-biofilm control (Fig. 3).

The interesting phenomenon of suppressed biofilm formation on WO3 nanorods surface is most likely associated with the unique surface properties of this material. Since the carbon paper surface was almost completely covered by the WO3 nanorods, the original adsorption sites of the carbon paper (i.e., functional groups such as carboxylic acids, alcohols, and quinines) for the bacterial cells was decreased dramatically [24]. On the other hand, the small size and good crystallinity of the nanorods decreased the roughness of the electrode surface and thus adsorption affinity for bacteria. Further, the nature of the hexagonal-structured tungsten oxide after receiving electrons make it even harder for bacteria to attach [25,26]. Interestingly, distinct formation of biofilm on electrode surface and thus adsorption affinity for bacteria. Further, the nature of the hexagonal-structured tungsten oxide after receiving electrons make it even harder for bacteria to attach [25,26]. Interestingly, distinct formation of biofilm on WO3 nanoparticles surface. The difference between the nanorods and nanoparticles in biofilm growth might be caused by two reasons. Firstly, the commercial nanoparticles had a smaller size of 50 nm, which led to a larger surface tension than the nanorods [27]. In addition, the cracked-mud structure of the C–WO3 nanoparticles may result in higher surface roughness than the nanorods [17]. These factors favored the bacterial adsorption.

As shown in this study, the C–WO3 nanorods electrode exhibited a better electrochemical response to riboflavin over the C–WO3 nanoparticles and pure C electrode (Fig. 2). Such enhancement can be attributed to the hexagonal-structured WO3 nanorods, which has an unique triangular and hexagonal tunnel structure built up from WO6 octahedra to act as an efficient intercalation host for cations and electrons. In contrast, the monoclinic phase of WO3 nanoparticles function as a semiconductor [18] and has poorer electron intercalation ability. When the C–WO3 nanorods electrode was used, it had a lower overpotential and a higher peak current for the redox of the riboflavin (Fig. 4A) than using the pure C electrode. The significant higher current recorded by C–WO3 nanoparticles electrode (Fig. 4A) was attributed to the catalytic current for hydrogen production from water electrolysis [28].

In light of the good electrochemical properties, biofilm-suppression nature of the C–WO3 nanorods and the uniqueness of mediated EET process in natural environment, this unique material could serve as a robust, biofueling-resistant electrode material with potential applications in a broad spectrum of electrochemical processes.

5. Conclusions

This work demonstrates the excellent performance of C–WO3 nanorods as an electrode material in maintaining a stable EET process for S. oneidensis MR-1 during continuous operation. The C–WO3 nanorods electrode exhibited better electrochemical performance and higher catalytic activity to riboflavin than both pure C

and C–WO3 nanoparticles electrodes. More importantly, it completely suppressed the formation of biofilm. These two features, together, contributed to a more sustained and efficient mediated EET process than other materials as evidenced by the continuous stable electricity generation in the C–WO3 nanorods electrode system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.electacta.2014.11.103.

References