Graphene/carbon cloth anode for high-performance mediatorless microbial fuel cells

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1. Introduction

Microbial fuel cells (MFCs) are a promising clean energy source to convert organic fuels including organic wastes to electricity by microorganisms (Mohan and Chandrasekhar, 2011; Qiao et al., 2010, 2009; Qiao and Li, 2011; Weld and Singh, 2011; Xiang et al., 2009; Zhang et al., 2011a); however, the relatively low power density and poor energy conversion efficiency of conventional MFCs, resulting from sluggish electron transfer between bacteria and electrode, limit their practical applications. Extensive efforts have been devoted to improve the electron transfer rate at the bacteria/electrode interface. Small diffusive electron mediators such as thionine, quinone and neutral red have been used in MFCs to pass electrons from electron donors to the anode for oxidation (Lovley, thionine, quinone and neutral red have been used in MFCs to pass electrons from electron donors to the anode for oxidation (Lovley, 2006); however, the exogenous mediators are expensive, toxic and incur operational losses. Therefore, direct electron transfer-based mediatorless MFCs have been investigated to improve power density (Chung et al., 2011; Erable et al., 2010; Wang et al., 2011). It has been reported that physical contact between unique nanostructures of electrode and outer-membrane cytochromes or/and conductive pili of bacteria can enable direct electron transfer, and that endogenous electron mediators generated from some bacteria also facilitate fast electron transfer between bacteria and electrode in a pseudo-direct electron transfer process (Logan, 2009; Qiao et al., 2008b).

Pseudomonas aeruginosa (P. aeruginosa) produces pyocyanin (PYO), a phenazine based soluble redox metabolite, that can function as mediator for the transfer of electrons between bacteria and anode in a MFC. Pseudomonas species produce other phenazine derivatives such as phenazine-1-carboxamide (PCN) and phenazine-1-carboxylic acid (PCA) which have similar mediating effects on electron transfer (Pham et al., 2008a; Rabaey et al., 2005). Although the P. aeruginosa MFCs involve an efficient “direct” electron transfer process, the energy conversion efficiency and power density are still too low for practical applications.

Nanostructured materials have been applied to anodes to significantly improve MFC output power density (Feng et al., 2010; Qiao et al., 2008a, 2007; Sun et al., 2010). Graphene is a new carbon nano-material whose two-dimensional lattice made of sp²-hybridized carbon atoms, extended honeycomb network, and long-range π-conjugation yield electrocatalytic properties for potential applications in energy conversion and storage systems such as fuel cells, batteries, ultracapacitors, sensors and solar cells (Guo et al., 2010a). Zhang et al. (2011b) used a porous graphene/PTFE paste electrode to improve the power density of an Escherichia coli-catalyzed MFC, but only the graphene on the paste surface was exposed to the electrolyte and most of graphene in the paste was not fully used. In the present study, carbon cloth, which has been used as MFC anodes due to its good conductivity, high stability and commercial availability, was used as a substrate to fabricate a graphene-functionalized P. aeruginosa anode for a MFC. The...
bioelectrocatalytic behaviors of the graphene/carbon cloth anode in *P. aeruginosa* MFC were investigated and the mechanism for the graphene-enhanced performance was studied. The results demonstrate that the graphene/carbon cloth anode provides very promising potential for high power MFCs and good energy conversion efficiency.

2. Methods

2.1. Bacteria cultivation and pretreatment

*P. aeruginosa* (ATCC 9027) was cultured with shaking at 37 °C for 12 h to a concentration of ~2 × 10^8 colony forming units per milliliter (cfu/ml) in flasks containing 150 ml standard glucose medium, which was composed of 10 g glucose, 5 g yeast extract, 6.8 g NaHCO₃, 8.5 g NaH₂PO₄ per liter. The culture was transferred to the anodic chamber of the MFC to perform discharge. After discharge for 300 h, the bacteria (referred as pretreated) were collected and cultured on a LB agar. A single colony picked from the agar plate was cultured in glucose medium and further used for the follow-up MFC.

2.2. Preparation of graphene/carbon cloth anode

Graphite oxide was synthesized by using the solution-based route as described by Guo et al. (2010b). Graphene oxide (GO) was prepared by dissolving the graphite oxide in DI water at a concentration of 0.2 mg/ml, followed by ultrasonication under ambient condition (Elmasonic S80H, 150 W) for 30 min. In a two-electrode cell with a Pt sheet as the counter electrode, the negatively charged GO was electrophoretically transported to the carbon cloth (E-TEK, B1D, plain, 1 cm × 2 cm) electrode surface by using 0.3 mA/cm² anodic current for 10 min, followed by 0.6 mA/cm² cathodic current for 90 s to reduce GO to graphene (An et al., 2010). The optimization of the deposition time is described in Supplementary information (Fig. S1). The graphene/carbon cloth electrode was air-dried and UV-sterilized for 3 h in biological safety cabinet (Gelman BH Class I) for 90 s to reduce GO to graphene (An et al., 2010). The optimization of the deposition time is described in Supplementary information (Fig. S1). The graphene/carbon cloth electrode was air-dried and UV-sterilized for 3 h in biological safety cabinet (Gelman BH Class I) and used as the control electrode.

2.3. Material characterization

Raman spectra were recorded from 500 to 3500 cm⁻¹ with an integrated confocal Raman microscopy system (CRM 200, WITEC, Germany) using a 633 nm He–Ne laser beam. Field emission scanning electron microscopy (FESEM, JSM-6700, JEOL, Japan) was used to investigate the morphology of as-prepared electrode materials. To prepare the bacteria-absorbed anode samples for FESEM measurements, discharged bacteria-absorbed anodes were immersed in 5% glutaraldehyde for 15 min and sequentially dehydrated with ethanol (30%, 40%, 50%, 60%, 70%, 80%, 100%). The samples were dried before the FESEM experiments.

2.4. Electrochemical characterization

Cyclic voltammograms (CVs) and electrochemical impedance spectroscopy (EIS) experiments were performed by using a PCSTAT302 Autolab system (Ecochemie, Utrecht, Netherlands) in a three-electrode cell that consisted of a working electrode, a saturated calomel electrode (SCE), and a platinum foil counter electrode. All tests were conducted at room temperature.

2.5. MFC setup and operation

A dual-chamber MFC with two equal rectangular Perspex frames (with a volume of 180 ml) was constructed, separated by a proton exchange membrane (PEM, Nafion 117, DuPont, Wilmington, DE). The anode was graphene/carbon or plain carbon cloth. The cathode was made from a plain carbon cloth (4 cm × 4 cm). For MFC operation, the bacterial culture was inoculated in the anodic compartment and purged with nitrogen to remove dissolved oxygen. The cathodic compartment contained 150 ml potassium hexacyanoferrate (50 mM K₃[Fe(CN)₆] and 0.1 M KCl). The external load resistor for the discharge experiments was 1.96 kΩ, which was selected based on published reports (Xiang et al., 2009; Zhang et al., 2006) for performance comparison. The voltage across the external resistor was recorded using a bench-top digital multimeter (ESCORT 3146A). The polarization and power output curves were measured by varying the output load resistor over a range from 1.5 to 30 kΩ to monitor the MFC steady-state current. The current and power output were normalized to the surface area of the anode for analysis. Glucose concentration in the anodic compartment was measured using glucose assay kit (Sigma).

2.6. Spectrum analysis of supernatant

The electrolyte in the anodic compartment was collected from a working MFC operated under its maximum output current density, centrifuged at 4 °C (6000g, 5 min), and the supernatant was analyzed using a UV–Vis spectrophotometer (Varian Cary 5000, USA) with a scan range from 200 to 800 nm. The supernatant was freeze-dried and examined by Fourier transform infrared (FT-IR) spectroscopy (Nicolet MAGNA-IR 560, USA) in potassium bromide, recording from 4000 to 400 cm⁻¹.

3. Results and discussion

3.1. Structural properties and electrochemical behavior of graphene/carbon electrode

The Raman spectra (Fig. S2a) showed two peaks at ~1349 and ~1589 cm⁻¹ corresponding to the D and G bands of carbon cloth due to disordered and graphic phases, respectively, and thus indicated a microcrystalline graphite structure (Nemanich et al., 1988). The graphene/carbon cloth displayed a D band around 1350 cm⁻¹ and a G band around 1582 cm⁻¹, which is in good agreement with the well-documented D and G bands of graphene (An et al., 2010). The intensity ratio of the D to G bands can also provide further evidence of the existence of graphene (Gomez-Navarro et al., 2007). The Ig/I₀ ratio of graphene/carbon cloth is close to 1, while the ratio of plain carbon cloth is significantly larger than 1, suggesting a successful graphene coating on carbon cloth.

FESEM imaging (Fig. S2b) showed that plain carbon cloth had a relatively smooth surface whereas the graphene/carbon cloth (Fig. S2c) had a wrinkly surface as previously (Guo et al., 2010b,c). The graphene nanosheets/carbon cloth showed good contact between the graphene and carbon fiber. Furthermore, the graphene/carbon cloth electrode was stable as it could be used repeatedly for more than one month (Fig. S3), which is a favorable property for MFC applications.

The electrochemical behaviors of both plain carbon cloth and graphene/carbon cloth electrodes were investigated in 10 mM K₃[Fe(CN)₆] + 1 M KCl solution. Cyclic voltammograms (CVs) of both electrodes exhibit a pair of well-defined [Fe(CN)₃]⁻ / [Fe(CN)₃]⁺ redox waves (Fig. 1a). The peak current density of graphene/carbon cloth electrode was 1.367-fold higher than that of plain carbon cloth, indicating that the deposited graphene increased the electroactive surface area, likely due to providing a higher specific surface area than the densely packed carbon fiber. In addition, the anodic and cathodic peak separation of the
3.2. Bioelectrocatalysis of graphene/carbon–P. aeruginosa anode

Bioelectrocatalytic behaviors of *P. aeruginosa* on both graphene/carbon cloth and carbon cloth electrodes were investigated. Before testing, the *P. aeruginosa* culture was anaerobically activated for 24 h in glucose-free medium. CVs at 30 mV/s show two pairs of well-defined redox waves (−0.6 V, denoted as Peak 1, and −0.51 V, denoted as Peak 2) on both graphene/carbon cloth and carbon cloth electrodes (Fig. 2a, after background subtraction). No redox peak was observed in buffer without *P. aeruginosa* cells (Fig. S4). For Peak 1, the anodic peak current versus square root of scan rate showed a linear relationship (Fig. 2c), suggesting that the electrochemical reaction is subject to a diffusion-controlled process. However, for Peak 2, the anodic peak current was proportional to the scan rate (Fig. 2d), indicating a surface reactant-controlled process. In fact, the anodic and cathodic potential exhibiting almost the same value for Peak 2 on both electrodes (Fig. 2a) is strong evidence for the surface-absorbed reactant-controlled nature of the reaction at Peak 2. Very interestingly, when the activated *P. aeruginosa* cells were rinsed and resuspended in PBS buffer for immediate CV measurements, CVs illustrated only Peak 2 (data not shown). *P. aeruginosa* cells are electrochemically active on both electrodes; however, the two redox waves (Peaks 1 and 2) represent different electron transfer mechanisms, in which the Peak 1 reaction very likely resulted from the cell-excreted accumulated mediators-enabled electron transfer, a pseudo-direct electrochemistry process between bacteria and electrode as reported by Qiao et al. (2008b). The surface-reactant controlled electrode reaction at Peak 2 clearly indicates that the *P. aeruginosa* cell has surface redox species which enable direct electron transfer between cell and electrode. After adding 0.5% glucose (Fig. 2b), the anodic peak current of both Peaks 1 and 2 increased, but the cathodic peak current remained almost unchanged. Apparently, the larger anodic current was due to electrocatalytic glucose oxidation. The increased anodic current at Peak 1 was catalyzed by cell-excreted mediators, while the increased anodic current at Peak 2 was catalyzed by the surface-redox species. For the carbon cloth electrode, the anodic peak current of Peaks 1 and 2 increased by 5.58 and 1.38 μA/cm², respectively; for graphene/carbon cloth electrode, the anodic peak current increased by 9.89 and 9.4 μA/cm² for Peaks 1 and 2, respectively. For the carbon cloth electrode, the oxidation current delivered by the direct electrochemistry process at Peak 2 was only 37% of that delivered by the excreted redox molecules-mediated process at Peak 1, which shows that the former contributed much more to electricity generation. For the graphene-modified carbon cloth electrode, both Peaks 1 and 2 had a much higher catalytic glucose oxidation currents than those of the carbon cloth electrode (Fig. 2), which shows that the graphene modification on the carbon cloth electrode can significantly enhance the bioelectrocatalytic process. Nevertheless, the graphene/carbon cloth electrode delivered a 1.77-fold higher oxidation current through the mediation process (Peak 1) and a 6.81-fold higher oxidation current through the surface-redox species-catalyzed direct electrochemistry process (Peak 2) than that of the plain carbon cloth, implying that the graphene modification has a stronger effect on the direct electron transfer than the mediated electron transfer process. This proposed parallel electron transfer mechanism for *P. aeruginosa*-catalyzed MFC not only provides new fundamental insights into the MFC reactions, but could also offer new approaches to improve the electrode kinetics in MFC for high performance.

To clearly understand the mechanism of the bioelectrocatalytic effect of the graphene/carbon cloth electrode, the surface morphologies of the carbon cloth and graphene/carbon cloth anodes were examined immediately after discharge in a dual-chamber MFC system. Only a few *P. aeruginosa* cells were scattered on the carbon cloth electrode surface, while large numbers of bacteria cells adhered to the graphene/carbon cloth surface to form a thick biofilm (Fig. S5), thus demonstrating that graphene modification increased the biocompatibility as reported by Chen et al. (2008). Thus, the increased number of *P. aeruginosa* cells on the graphene/carbon cloth electrode likely contributed to the bioelectrocatalytic
enhancement for the Peaks 1 and 2 reactions (Erable et al., 2010; Logan, 2009).

*P. aeruginosa* cells are reported to excrete phenazine-like pigments such as PYO, PCA and PCN as electron shuttles in MFCs (Pham et al., 2008a,b; Rabaey et al., 2005). To verify the possible presence of these soluble electron mediating molecules in graphene/carbon cloth and carbon cloth-based MFCs, the anodic supernatant was isolated for spectrum analysis. UV–Vis spectra (Fig. S6) displayed absorption peaks at \(\lambda \approx 300\) and \(\lambda \approx 400\) nm which suggests a heterocyclic aromatic structure of a phenazine compound (Wang and Newman, 2008), whereas the FT-IR spectrum of the supernatant (Fig. S7) showed high similarity with that of commercial PYO (Sigma) treated with culture medium. In comparison to the standard curve of PYO (Fig. S8), the absorbance peak at 300 nm of the graphene/carbon-anodic supernatant has an estimated concentration of 11.4 \(\mu g/ml\), which is much higher than that of plain carbon cloth (3.8 \(\mu g/ml\)). This result may indicate that the excreted electron mediators are mainly generated from the surface-adhering bacteria. It is noted that in the UV–Vis spectrum, the intensity ratios of the two absorption peaks in the supernatants obtained from the carbon cloth and graphene/carbon cloth electrodes were different. This outcome is possibly caused by the different bioelectrocatalytic mechanisms of the carbon and graphene/carbon cloth electrodes, since the latter exhibits parallel electron transfer paths that possibly result in additional metabolic products with absorption near the second peak of the mediator. In fact, the second adsorption for the graphene/carbon cloth clearly showed overlapping absorption peaks. Further investigations will be conducted to identify the exact chemical structures of the compounds released from *P. aeruginosa* cells.

### 3.3. Discharge performance of graphene/carbon anode-based MFC

The performances of both plain carbon cloth and graphene/carbon cloth anode-based MFCs evaluated by measuring the output current profiles at a constant-load (Fig. 3a) showed that the fresh cultures with 0.5% glucose solution required an initial activation and enrichment period (12 h) to have a significant discharge rate (solid line), but the inoculums without glucose could not deliver a significant current output (dashed line). Further, the graphene/carbon and plain carbon cloth-based MFC took 70 and 85 h, respectively to achieve the maximum plateau discharge rates of 24.5 and 15.8 \(lA/cm^2\). Most importantly, with the same 0.5% glucose solution, the graphene/carbon cloth anode based MFC had a much longer discharge life (330 h) than that utilizing plain carbon cloth (230 h), during which the bacteria density in the anodic chamber remained unchanged (Fig. S9). The sharply dropping discharge current when no fresh glucose solution was added might have been caused by complete consumption of glucose. In fact, when the discharge current sharply dropped, no glucose in both anodic compartments could be detected (Fig. S10).

The power output performance curves (P–I curves) in Fig. 3b illustrate that the graphene/carbon cloth anode delivered a maximum power density of 52.5 mW/m\(^2\), which is 2.7-fold higher than that of the plain carbon cloth anode (19.5 mW/m\(^2\)) and is also
much better than that report using P. aeruginosa as the anode biocatalyst in the MFC (1.2 mW/m²) (Rabaey et al., 2005).

The total electrical energy, E = IVt converted from the same 0.5% glucose electrolyte was calculated from the discharge curves in Fig. 3a as 5.34 and 1.75 J for the graphene/carbon cloth and plain carbon cloth anode-based MFCs, respectively. This finding shows that the graphene modification improved the energy conversion efficiency by 300%. The polarization curves (V–I plots) in Fig. 3b reveal that the graphene/carbon cloth anode had a much lower polarization for better energy conversion efficiency. This apparently can be attributed to the modified graphene layer, which greatly reduced the Faraday resistance and electron transport resistance at the bacteria/electrode interface for high energy conversion efficiency and high power density.

4. Conclusion

In a P. aeruginosa mediatorless MFC, a graphene-modified carbon cloth anode delivered a 2.7-fold higher power density and a 3-fold higher energy conversion efficiency than a plain carbon cloth anode. A parallel bioelectrocatalytic mechanism of simultaneous direct electron transfer and cell-excreted mediator-enabled electron transfer pathways is proposed for the P. aeruginosa MFC. The biocompatible and conductive graphene significantly promotes bacteria growth for more direct electron transfer activation centers while producing more mediating molecules leading to a higher electron transfer rate. This work may provide a low cost manufacturing process to fabricate high power MFCs for practical applications.

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.biortech.2012.02.116.

References


