In-situ growth of graphene/polyaniline for synergistic improvement of extracellular electron transfer in bioelectrochemical systems

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ABSTRACT

Graphene composite has been widely used in various bioelectrochemical systems (BES). However, it is suffered from tedious fabrication procedure and ambiguous mechanism for its effect on BES. Here, a one-step and in-situ strategy for simultaneously graphene exfoliation and aniline polymerization was developed for fabrication of graphene/PANI composite electrode (GO/PANI)_OS. This GO/PANI_OS outperformed graphite paper (GP), GP with PANI (GP/PANI) and GP with electrochemical exfoliated graphene (GOH2SO4) in Shewanella oneidensis MR-1 inoculated BES (improved the power density output, i.e., 24, 3.4 and 5.7 times of GP, GP/PANI and GOH2SO4, respectively). Further analysis revealed a synergistic improvement on both direct and mediated extracellular electron transfer of S. oneidensis MR-1 by GO/PANI_OS contributed to its performance enhancement in BES. This work not only provided a simple strategy for graphene composite fabrication, but also unveiled the underlying mechanism for its stimulation on BES, which promises new opportunity of graphene composite application in various bio-systems.

1. Introduction

Bioelectrochemical systems (BES) have attracted vast research interest during the past decades as they combined pollutants biodegradation, bioremediation together with energy harvesting and biochemical production (Harnisch and Schroeder, 2010; Logan, 2009; Wang and Ren, 2013; Yong and Zhong, 2010). The distinguished characteristic of BES is that they utilize electroactive microorganism with versatile strategies for extracellular electron transfer (EET) between cells and the electrode, enabling anodic respiration or cathodic reduction. The well recognized EET strategies are generally categorized into two groups: direct electron transfer (DET) via cell membrane associated compounds and mediated electron transfer (MET) via soluble electron shuttles (Baron et al., 2009; Corby et al., 2009; Marsili et al., 2008; Rabaey et al., 2005; Strycharz et al., 2011). Since electroactive bacteria have to overcome the spatial and thermodynamic barrier between the intracellular metabolism and extracellular electrode for successful EET, develop suitable electrode would be of great significance both for mechanism study and applications.

Up to now, most of the efforts focused on electrode surface engineering, including surface treatment and surface modification with metal nanoparticles, carbon nanotube, conductive polymers and even electron shuttle (Deng et al., 2010; Feng et al., 2010; Li et al., 2011; Peng et al., 2010). Graphene and its derivatives, with basic one-layer atom of graphite structure, have been widely used in versatile electrochemical devices due to its excellent mechanical and electrochemical properties (Chen et al., 2010), and have been proved to be an effective electrode modifier to promote BES performance (Liu et al., 2012). However, the tedious physical and chemical preparation procedures limit its application, which includes complicated chemical graphene synthesis (chemical oxidation and exfoliation, chemical vapor deposition, etc.) and electrode surface modification. Thus, development of fast and simple preparation strategies proves significant values (Parvez et al., 2014; Tang et al., 2015).

Meanwhile, graphene is feasible for composite material formation, which further improves its performance and extends its applications (Bai et al., 2011; Kemp et al., 2013; Kuilla et al., 2010; Wu et al., 2012). The graphene composite electrode with nanotube and nanoribbon shows great potential in BES as these nanostructures would significantly enhance the EET efficiency between bacteria outer-membrane cytochromes (OMCs) and electrode.
Conductive polymers especially polyaniline (PANI) are another promising candidates for electrode modification in BES due to its moderate preparation condition, good conductivity and biocompatibility (Liao et al., 2015; Qiao et al., 2007; Wang et al., 2013). When the morphology and structure of PANI was carefully controlled, EET efficiency would be significantly improved with the interaction between PANI nanostructure and electroactive bacteria surface (Ding et al., 2012; Zhao et al., 2010). Thus, PANI would be an ideal choice for graphene composite electrode in BES, with anticipation for synergetic effect of graphene and PANI (Hou et al., 2013; Huang et al., 2016). However, multi-step and time-consuming composite fabrication procedures related to graphene exfoliation, PANI polymerization and electrode modification became the key technique bottleneck and largely hindered its application. More importantly, the fundamental issue about the underlying mechanism for its stimulation on BES is still unrevealed.

In this work, a novel and simple in-situ electrochemical fabrication strategy for one-step preparation of graphene/PANI composite electrode from graphite paper was developed. The composite electrode was detailed characterized and then applied as the anode of microbial fuel cells (MFC, a typical BES). The performance of MFC with this composite electrode was investigated, detailed electrochemical analysis was conducted and a synergetic improvement on bacterial EET which underlies the mechanism for MFC performance enhancement was also explored.

2. Materials and methods

2.1. Electrode fabrication

Graphite paper (Jinglong Special Carbon, Beijing, China) was first immersed in chloroform overnight, washed with absolute ethanol and finally dried in room temperature for use. The treated graphite paper was then cut into to small pieces with geometry size of 1 cm×2 cm and connected to titanium wire. These prepared graphite paper electrode (GP) was then either directly used as MFC anode (the control) or further electrochemically exfoliated with DC power supply (schematic as Fig. 1a) to fabricate the composite electrode as follows.

To fabricate graphene/PANI composite electrode, two-electrode system composed of GP electrode (working electrode, anode) and platinum wire electrode (counter electrode) was constructed. Cylindrical borosilicate glass bottle was used as the electrolytic cell and aqueous solution containing 0.1 M sulfuric acid and 0.05 M aniline was used as electrolyte (15 ml). The graphene exfoliation and aniline polymerization were simultaneously achieved by applying 10 V voltage to the two-electrode system for 4 min (2 min for each side) by using a DC supplier. The electrolysis time for electrode preparation was optimized based on the MFC performance with the resulting electrode by preliminary experiment. This one-step electrochemical fabricated graphene/PANI composite electrode is nominated as GO/PANI\(_{\text{chem}}\) electrode. GP electrode with graphene modification (nominated as GO\(_{\text{H2SO4}}\)) was prepared in two-electrode system similar to GO/PANI\(_{\text{chem}}\) electrode. GP electrode modified with chemically synthesized PANI (GP/PANI) were obtained by chemical polymerization of PANI on GO\(_{\text{H2SO4}}\) electrode and GP electrode, respectively. In brief, GO\(_{\text{H2SO4}}\) electrode or GP electrode was immersed in the aqueous solution contains aniline (0.05 M), ammonium persulfate (APS, 0.075 M) and H\(_2\)SO\(_4\) (0.1 M) in ice bath (12 h) for PANI in situ polymerization.

2.2. MFC setup and operation

*Shewanella oneidensis* MR-1 stored at –70 °C was recovered in Luria–Bertani (LB) broth overnight and transferred to aerobically cultivation in LB broth for 16 h at 150 rpm and 30 °C. The bacteria cells were then harvested by centrifugation and re-suspended in mixed medium containing M9 salt medium (Tian et al., 2002), 5% LB broth and 10 mM sodium lactate to an optical density of 1.5 (OD600). The bacteria suspension was further bubbled with nitrogen and employed as anode inoculum.

Dual chamber MFC (inner size of 4 cm×4 cm×2 cm for both chamber) separated by proton exchange membrane (Nafion 117) was used in this work. Graphite paper and four kinds of fabricated composite electrodes (GP, GP/PANI, GO\(_{\text{H2SO4}}\), GO/PANI\(_{\text{chem}}\) and GO/PANI\(_{\text{G2S}}\)) were used as anode, with the bacteria suspension as anolyte. Graphite felt electrode (2 cm×3 cm) was used as cathode and catholyte consists of 50 mM K\(_2\)Fe(CN)\(_6\), 50 mM KCl, 22 mM KH\(_2\)PO\(_4\) and 50 mM Na\(_2\)HPO\(_4\). All the MFC were operated under batch mode with 2 kΩ external resistance. Three parallel MFCs were set up for each condition. Abiotic MFCs with respective electrodes were also set up to study the influence of abiotic discharge during MFC operation.

2.3. Analytical and electrochemical techniques

During operation, MFC output voltage across the external resistance was recorded by a 15B digital multimeter (Fluke, USA) with MPS-010602 data collector (Qiauag, Beijing, China). When the output voltage reached steady state, MFC was open circuit and polarization curve was measured via change external resistance, all the current density and power density are calculated based on the anode projected area unless otherwise indicated. Cyclic voltammetry (CV) analyses were also conducted when the output voltage reaches the stable state with CHI660E electrochemical workstation (CHI, Shanghai, China) in three-electrode system, slow scanning rate of 1 mV/s was chosen to observe the potential catalyzed electrochemical behavior of *Shewanella* biofilm formed on individual electrodes. Saturated calomel electrode (SCE, +0.243 vs. SHE) was used as reference electrode and platinum wire as counter electrode.

Raman spectra were measured from 500 to 3500 cm\(^{-1}\) via DXR Raman Microscope (Thermo-Fisher, USA) with 523 nm argon laser. FTIR was conducted in Nexus 470 FTIR (Thermo Fisher Nicolet, USA) from 400 to 4000 cm\(^{-1}\). X-ray photoelectron spectroscopy (XPS) was analyzed under Escalab 250Xi XPS (Thermo Scientific, USA). Electrode wettability was compared with YIKE-360B Contact Angle Meter (Yike, Chengde, China). The morphology of graphene and graphene/PANI composite formed on the surface of electrode were observed with high resolution transmission electron microscopy (HRTEM, FEI Tecnai F30 Field Emission Gun Transmission Electron Microscope, FEI, USA) and atomic force microscopy (AFM, Bruker Multimode V SPM, Bruker, USA). The morphology of prepared electrode and biofilm formed on the electrode after MFC operation were characterized with Thermal Field Emission SEM JSM-7001F (Joel, Japan). SEM sample was prepared as previous work (Liu et al., 2015).

3. Results and discussion

3.1. In-situ growth of graphene/PANI composite on graphite electrode

Electrochemical exfoliation with DC is an efficient way to prepare graphene from GP (Parvez et al., 2014; Tang et al., 2015). Meanwhile, electrochemical polymerization is a simple and fast
method for PANI synthesis. Therefore, a novel and simple electrochemical procedure for fabrication of graphene/PANI composite with DC was developed here. A DC voltage (10 V) was employed to two-electrode system with graphite paper as sacrificial electrode in aqueous solution of sulfuric acid (0.1 M) and aniline monomer (0.05 M) (Fig. 1a). The DC applied to working electrode was controlled to 4 min (2 min of each side) to enable graphene partially exfoliation. As expected, layered graphene like and PANI structures were formed on graphite electrode (GO/PANIOS) (Fig. 1d). Without aniline monomer addition (GOH2SO4), only layered graphene like structure formed on the surface of GP (Fig. 1c). TEM and AFM analyses of the surface layers of GOH2SO4 electrode further confirm that few layered graphene (with thickness of about 1.2 nm) formed (Fig. 2). The selected area electron diffraction (SAED) pattern exhibits typical hexagonal symmetric diffraction, indicating the single crystalline nature of as prepared graphene (Fig. 2b). As reported previously, the π-π stacking force between the phenyl of aniline and basal planes of graphene is beneficial to PANI polymerization on the surface of graphene layers (Xu et al., 2010). In accordance, the TEM image of GO/PANIOS (Fig. 2c and d) disclosed an intertwined PANI nanowire structure anchored on the surface and edges of the graphene layers. The as-formed PANI nanostructure is very suitable for BES application since they substantially increase the chance for the interaction between the material and nanostructured electrochemical active proteins on the bacteria outer-membrane surface (Ding et al., 2014). These results demonstrate the successful preparation of graphene/PANI composite on the GP surface.

Raman spectra, FTIR and XPS analyses (Fig. 3) were further conducted to characterize the element composition and chemical bonding state of the prepared electrodes. For Raman spectra (Fig. 3a), peak around 1580 cm⁻¹ is corresponding to the well documented G band of graphite (Ferrari et al., 2006). The increased D band in GOH2SO4 and GO/PANIOS around 1350 cm⁻¹ demonstrates the defected graphite occurred after electrochemical exfoliation, which probably derived from the formation of functional group due to oxidation of graphite layers and formation of graphene layer structure. FTIR analyses indicate the presence of hydroxy and epoxy (peaks at 3434 and 1226 cm⁻¹) in GOH2SO4 (Fig. 3b), indicating oxidized status of graphene formed (Wang et al., 2009; Zhao et al., 2012). Meanwhile, XPS analyses (Fig. 3c and d) demonstrate that 15.22% and 18.18% oxygen present in GOH2SO4 and GO/PANIOS and the corresponding C/O ratio (5.1 for GOH2SO4 and 4.1 for GO/PANIOS) is comparable to those of chemical reduced graphene oxide (Parvez et al., 2013). The oxygen functional group not only improves the hydrophilicity of the electrode surface and making it more appealing for bacteria attachment (Ding et al., 2015), and more importantly, can act as the anchor site and enables aniline polymerized on the graphene surface and edges (Xu et al., 2010).

The PANI formation in GO/PANIOS and its oxidization status is further investigated by XPS, FTIR and UV–vis spectra. The XPS (presence of -NH– and -N=, Fig. S1f) and FTIR (Fig. 3b, presence of benzenoid at 1302 and 1496 cm⁻¹ (reduced state), and quinoid ring at 1139 and 1580 cm⁻¹ (oxidized state)) analyses confirmed the formation of PANI and indicate the PANI synthesized here consists of reduced and oxidized repeat units, which is in the partially oxidized conductive emeraldine salt form (Qiao et al., 2007; Wang et al., 2013). Compared to GOH2SO4, the UV–vis absorption of GO/PANIOS consists of two new adsorption bands at 380–480 nm and 700–850 nm (Fig. S2), which could be assigned to the polaron transition of PANI (the typical characterization of conducting state PANI (Xu et al., 2010; Yang et al., 2007)). These results confirm the PANI within GO/PANIOS is partially oxidized...
and in the conductive status.

Wettability and cell attachment were investigated for its potential application in BES. The smaller water contact angles (68°, 50° and 30° for GP, GOH_{2}SO_{4} and GO/PANI_{0.05}, Fig. S3) of GO/PANI_{0.05} indicates better wettability compared to GP, which might facilitate EET of cells (Ding et al., 2015). SEM images shows Shewanella cells attached on the surface of GO/PANI_{0.05} with direct contact to PANI nanostructure (Fig. S4), which provides the spatial feasibility for potential EET enhancement of Shewanella.
3.2. MFC performance improved with graphene/PANI composite anode

Then, the one-step in-situ fabricated composite electrode (GO/PANIOS) was applied in MFC. Different electrodes (GP, GP/PANI, GOH2SO4, and graphene/PANI composite electrode prepared with chemical aniline polymerization (GO/PANIchem)) were used for performance comparison (Fig. 4 and Fig. S5).

Fig. 4a shows the voltage output of MFC operated under batch mode (V-t curve). It is not surprising to find that GP electrode equipped MFC had the worst performance, only 81 mV (203 mA/m²) steady state voltage was reached. MFC with GP/PANI and GOH2SO4 outperformed GP, with 225 mV (563 mA/m²) and 165 mV (413 mA/m²) steady state voltage achieved. The above result is consistent with previous works that PANI and graphene modification were reported to improve MFC performance as these modifications increase the surface area (Fig. S6) and electrochemical activity of electrode (Huang et al., 2016, 2011; Lai et al., 2011; Liu et al., 2012). Further improvement in maximum steady state output voltage was achieved with graphene/PANI composite electrodes, which was 296 mV (740 mA/m²) for GO/PANIchem and 319 mV (798 mA/m²) for GO/PANIOS. The maximum power density of MFC with different anode was estimated through plotting P-J curve (power density vs. current density) (Fig. 4b), which was 16, 112, 67, 328 and 381 mW/m² for GP, GP/PANI, GOH2SO4, GP/PANIchem and GO/PANIOS, respectively. The achieved maximum power density of GO/PANIOS was about 24, 3.4 and 5.7 times of GP, GP/PANI and GOH2SO4. Compared with “two-step” fabricated GO/PANIchem and other conventional or chemically prepared graphene/PANI composite electrodes, one-step in-situ electrochemically fabricated graphene/PANI composite (GO/PANIOS) developed here not only has the unique advantage of easier fabrication procedure, but also enables better MFC performance (regardless of pure or mixed culture) (Tables S1 and S2).

The V-J curve (voltage vs. current density) reflects how electrode (both anode and cathode) overpotential varies with current density increase. Since ferricyanide was used as the cathode electron acceptor in this study, the cathode overpotential variation during the polarization curve measurement was relative small, meaning the potential drop in V-J curve mainly derived from increased anode overpotential (Liu et al., 2015). The V-J curve of GP and GOH2SO4 shows similar trend (Fig. 4b). Low open circuit potential (OCP) is observed (334 and 324 mV, respectively) and follows linear potential drop until 50 mV. The slower slope in the V-J curve of GOH2SO4 compared with GP indicates smaller apparent ohmic resistance, which means that graphene formation on graphite effectively improved the EET efficiency of S. oneidensis MR-1 and further enhanced MFC performance (Liu et al., 2015; Yu et al., 2015). For MFC with GP/PANI electrode, increased OCP was observed (452 mV vs. 334 mV for GP and 324 mV for GOH2SO4), which is probably the result of PANI modification.

As with GP/PANI, higher OCP (600 mV for GO/PANIchem and 524 mV for GO/PANIOS) was achieved in MFC with GO/PANIchem or GO/PANIOS electrode (Fig. 4b). These results imply that PANI enables Shewanella to facilitate EET at more negative anode potential via EET pathways that is inapplicable for the GP and GOH2SO4. An
inflation point around 250 mV in the V-j curve can be observed in GP/PANI, GO/PANIchem and GO/PANIOS (arrowed in Fig. 4b), dividing the linear part into two sections with different slope, which indicates multiple EET modes between bacteria and these composite electrodes (Yu et al., 2015). It implied PANI modification activated multiple EET pathways of Shewanella, which is the unique advantage of this composite.

3.3. Synergistic improvement of direct and mediated EET

In order to investigate how graphene and/or PANI would influence the EET between the bacteria and specific electrode, S. oneidensis MR-1 was adopted as model electroactive bacterium in this study due to its well-recognized EET pathways. The EET pathways of S. oneidensis MR-1 include DET (outer-membrane cytochromes (OMCs) associated, located between the range of $-0.3V$ and $-0.1V$ vs. SCE under CV analysis, which usually varied along with the microenvironment) and MET (flavins mediated EET, located at $-0.46V$ under CV analysis), that could be clear discriminated in high resolution CV curve (Eggleston et al., 2008; Hartshorne et al., 2007; Marsili et al., 2008; Meitl et al., 2009; Okamoto et al., 2013). The turnover current of corresponding EET pathway in S. oneidensis MR-1 could be further individually studied since the CV curve of well-catalytic electroactive biofilm exhibits typical sigmoid shape at slow scan rate (described by Monod kinetics) and is easy to be recognized (Torres et al., 2008; Yu et al., 2015). Therefore, CV with low scanning rate ($1mV/s$) were conducted to detailed investigate how Shewanella interact with individual electrode when the output voltage was at steady state (Fig. 5 and Table 1).

For GP electrode, the main peak centered at $-0.31 V$ belongs to DET of OMCs, while no obvious peak corresponding to MET (around $-0.4$ to $-0.6 V$) was observed (Fig. 5). The large peak separation (173 mV) of OMCs with GP electrode (Fig. S7, Table 1) indicates that electroactivity of the cytochrome on this electrode is very poor. Moreover, only weak turnover current ($i_{DET}$) can be identified (25 μA/cm²) (Fig. 5c, Table 1). When graphene formed on electrode surface (GO$_{H2SO4}$), the peaks attributed to flavins (MET) and OMCs (DET) can be identified at about $-0.48 V$ and $-0.26 V$, respectively (Fig. 5a). However, the flavin mediated turnover current ($i_{MET}$) is negligible (Fig. 5b, Table 1), indicating that Shewanella could not effectively utilize the soluble flavin.
mediated MET with GOH2SO4 electrode. Compared to that with GP (173 mV), the peak separation of OMCs decreased about 140 mV (Fig. S7, Table 1), with corresponding turnover current (iDET) increased 72\% to 43 \( \mu A/cm^2 \) (Fig. 5c, Table 1). The reduced OMCs peak separation evidences the enhanced electron transfer efficiency between the OMCs and electrode surface (Deng et al., 2010; Peng et al., 2010; Zhao et al., 2013). The active surface area is greatly increased when graphene structure formed (Fig. S6) and the abundant oxygen functional group improves the wettability of graphene structure. This finding would broad the electrode recipe of BES and provide new idea for fabrication of versatile graphene composites.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.037.

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