Facile in-situ fabrication of graphene/riboflavin electrode for microbial fuel cells

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Abstract

A novel graphene/riboflavin (RF) composite electrode was developed and its potential application as microbial fuel cell (MFC) anode was demonstrated. Graphene layers were first grown on the surface of graphite electrode by a one-step in-situ electrochemical exfoliation approach. Then, noncovalent functionalization of the graphene layers with RF was achieved by a simple spontaneous adsorption process. The graphene/RF electrode was extensively characterized by transmission electron microscopy, Fourier transform infrared spectroscopy, Raman analysis, and cyclic voltammetry analysis. Remarkably, when applied as the anode of Shewanella oneidensis MR-1 inoculated MFCs, the graphene/RF electrode significantly decreased charge transfer over-potential and enhanced cell attachment, which in turn delivered about 5.3- and 2.5-fold higher power output, when compared with that produced by the bare graphite paper electrode and graphene electrode, respectively. These results demonstrated that electron shuttle immobilization on the electrode surface could be a promising and practical strategy for improving the performance of microbial electrochemical systems.

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1. Introduction

Microbial fuel cell (MFC) is an emerging and promising technology for energy-saving wastewater treatment because it could convert the chemical energy of organic chemicals into electrical energy, and thus has the potential to recover energy from wastewater [1–4]. As a promising environmental biotechnology for wastewater treatment and renewable energy production, MFC has attracted much attention in the past decade [5]. It has made great progress and various potential applications such as self-powered biosensor [6,7], remote/low density power supply [8,9], and wastewater treatment have been demonstrated [10,11]. However, low power output becomes the main bottleneck of MFC and limits its practical and large-scale applications.

Therefore, great efforts have been made to improve the power output of MFC with electrode modification during the past decades. For example, electrode modification with various nanomaterials such as graphene, carbon nanotube, metal oxide nanoparticles, and conductive polymers, have been used as electrode modifiers, which significantly improved the power output of MFCs [12–16]. In particular, graphene, a single atomic layer of carbon atoms arranged in a hexagonal lattice, showed immense potential in improving the performance of MFCs owing to its extraordinary electrical and physiochemical properties [17]. For example, by electrochemical reduction of chemically synthesized graphene oxide and depositing it on carbon cloth or other electrode through different methods, the power output of MFC was significantly enhanced [18–20]. Although these findings demonstrated the potential of graphene modification in improving MFC performance, the graphene synthesis and modification procedures are quite complicated and time-consuming. Recently, a one-step in-situ approach for the growth of graphene layers on the surface of graphite paper (GP) was developed, which showed significant advantages over the above-mentioned strategies [21,22]. Besides, electron shuttle manipulation is another powerful strategy to improve the power output of MFCs by enhancing the extracellular electron transfer (EET) efficiency [23]. Electron shuttle, such as quinone, thionin, riboflavin (RF), methyl viologen, and neutral red, can effectively facilitate EET between bacteria and electrode, and thus boost power output. However, addition of these mediators directly into the electrolyte may lead to their washing out in
continuous operation and also pose potential environmental problems. Therefore, immobilization of mediators onto the surface of anode may solve these problems. It has been reported that immobilization of anthraquinone-2,6-disulphonicdisodium salt on the electrode with covalent grafting onto the graphite surface improved the power output to about 94% [24,25]. Besides, copolymerization of electron shuttle with conductive polymers on the electrode surface is another interesting strategy [26]. However, these strategies require complicated grafting or polymerization process. As graphene presents excellent noncovalent adsorption property towards different low molecular weight compounds, it might be a promising carrier for electron shuttle immobilization with simple adsorption procedure [26].

In this study, we demonstrated a facile in-situ approach to fabricate RF immobilized graphene/RF (GO/RF) composite electrode (Fig. S1). The composite electrode was characterized in detail with scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), Raman analysis, and cyclic voltammetry (CV) analysis. Furthermore, the MFC performances, including voltage output, power output, and anodic biofilm with the composite electrode, were compared in detail with those of other conventional electrodes.

2. Materials and methods

2.1. Electrode fabrication

GP (Jinglong Special Carbon, Beijing, China) was first immersed in chloroform overnight, washed with absolute ethanol to remove possible impurities, and dried at room temperature. The pretreated GP (1 × 2 cm) was then connected to titanium wire as the GP electrode. The GP electrode was further electrochemically exfoliated in 0.1 M sulfuric acid with direct current (DC) power supply (10 V) to allow in-situ formation of graphene on its surface [24]. This electrochemically fabricated graphene electrode was denoted as the GO electrode.

RF solution was prepared by dissolving RF (analytical grade) in aqueous solution (95 μM). The GP and GO electrodes were immersed into the RF solution for 48 h (designated as GP/RF, GO/RF, respectively) to facilitate physical absorption. The prepared GP/RF and GO/RF composite electrodes were washed thrice with distilled water to remove the unadsorbed RF and dried at 30 C. The composite electrodes were characterized and used as MFC anodes.

2.2. Electrode characterization

The electrochemical properties of different electrodes were characterized by CV analysis using CHI660E electrochemical workstation (CHI, Shanghai, China). A three-electrode system, comprising saturated calomel electrode (SCE, +0.243 vs. SHE) as reference electrode and platinum wire as counter electrode, was constructed in 0.1 M PBS (pH = 6.8). After purging nitrogen into the electrolyte for 15 min to remove the dissolved oxygen, the different electrodes (before and after MFC operation) were subjected to CV analysis between −0.7 and −0.2 V with a scanning rate of 2 mV/s. FTIR was conducted using Nexus 470 FTIR (Thermo Fisher Nicolet, USA) from 400 to 4000 cm⁻¹. Raman spectra were measured from 500 to 3500 cm⁻¹ via DXR Raman Microscope.
(Thermo Fisher) equipped with 523-nm argon laser. The surface morphologies of different electrodes were characterized using Thermal Field Emission Scanning Electron Microscope JSM-7001F (Joel, Japan).

2.3. Bacteria cultivation, MFC setup, and operation

*Shewanella oneidensis* MR-1 was aerobically cultivated in LB broth at 30 °C with shaking (200 rpm) for 16 h. The cell cultures were centrifuged at 5000 rpm for 5 min and resuspended in mixed medium (95% M9 salt medium [28], 5% LB broth, and 18 mM sodium lactate) to reach an optical density (OD600) of 1.0. This bacterial suspension (30 ml) was added into the MFC anode chamber as the anolyte.

Dual-chamber MFCs (inner size of 2 × 4 × 4 cm for each chamber) separated by Nafion117 membrane (3 × 3 cm, DuPont, USA) were used in this study [29]. Graphite felt electrodes (2 × 3 cm) were used as cathodes, and the catholyte consisted of the following: 50 mM K3Fe(CN)6, 50 mM KCl, 22 mM KH2PO4, and 50 mM Na2HPO4. All the MFCs were operated under batch mode with 2-KΩ external resistance. All the experiments were performed in triplicate to ensure reproducibility. During MFC operation, the output voltage (V) across the external resistance was recorded (1-min intervals) using a digital data collector. Polarization curve was obtained by changing the external resistances as described in a previous work [17].

3. Results and Discussion

3.1. GO/RF composite electrode preparation and characterization

Electrochemical exfoliation with DC has been proved to be an efficient way for graphene preparation from GP [30,31]. Accordingly, this method was adopted in this study to enable in-situ formation of graphene on the GP electrode (denoted as GO electrode). As shown in Fig. 1a, obvious morphological changes on the electrode surface could be observed after DC exfoliation. In addition, surface exfoliation was also confirmed by the release of a large amount of graphene-like sheets from the GP during the preparation of GO electrode. SEM analysis showed that the GP electrode had a smooth surface with large flakes of graphite (Fig. 1b). After DC exfoliation, layered graphene-like structures with rougher surface were observed (Fig. 1c). Moreover, TEM image of the surface materials of the GO electrode indicated the formation of few transparent layered graphene (Fig. 1d).

Raman and FTIR spectra were used to further characterize the graphene formed on the GO electrode surface. Raman analysis revealed two well-defined G band (81580 cm⁻¹) and D band (81350 cm⁻¹) for the GO electrode after DC exfoliation (Fig. 2a), while only G band could be identified for graphite electrode [23,31]. The occurrence of D band demonstrated the presence of defective graphite in the GO electrode, which can be ascribed to the functional groups and graphene layer nanostructures formation on the graphite surface. Further analysis by FTIR spectroscopy indicated the presence of hydroxyl (83400 cm⁻¹) and epoxy (81100 cm⁻¹) groups, suggesting that the graphene formed on the GO electrode was in oxidized state, which is in accordance with previous reports that demonstrated the graphene formed by DC electrochemical exfoliation contained low content of oxygen [21,22]. The presence of oxidized functional groups could provide the potential active site for further modification.

RF is a typical redox active molecule secreted by bacteria, and has been proved to be an effective electron shuttle for the model exoelectrogen *S. oneidensis* [30]. Therefore, RF was selected as the model electron shuttle and *S. oneidensis* MR-1 was used as the model exoelectrogen to test the electron shuttle immobilization strategy and its application in MFC. For RF immobilization, GO and GP electrodes (control) were immersed in 100 μM RF solution for 48 h (named as GO/RF and GP/RF, respectively). During the absorption process, the residue RF concentration in the aqueous solution gradually decreased (Fig. S2), indicating the adsorption of RF onto the electrodes. After adsorption, the residue RF in different systems was also analyzed. As expected, the GO electrode adsorbed much more RF than the GP electrode as evidenced by lower residue RF concentration was detected. The result is reasonable as graphene is superior to graphite for organic adsorption [32]. After 48 h, the GO electrode adsorbed 86% of RF in the aqueous solution, while the GP electrode adsorbed only 43% of RF (Fig. S2).

The adsorption of RF onto the GO electrode was further confirmed by FTIR spectroscopy and Raman analysis. According to the FTIR spectra (Fig. 2a), the GO/RF electrode showed the featured peaks of the GO electrode, along with the extra peaks associated with RF, which suggested the presence of RF. Furthermore, the Raman spectra (Fig. 2b) for the GO/RF electrode revealed obvious D and G bands similar to the GO electrode after RF modification. However, RF mostly influenced the intensity ratio of I_D/I_G, indicating that the modification of RF significantly affected the electron density distribution of the graphene nanosheets. The noncovalent π–π interaction between graphene or graphene oxide and aromatic molecules is dominant and quite stable owing to the highly extended π system of graphene or graphene oxide. Further analysis revealed an obvious G-band shift from 1599 to 1584 cm⁻¹ after RF modification, suggesting a π–π interaction between graphene and hybridized RF [33]. The non-covalent π–π interaction between graphene and aromatic bodied molecules is quite stable due to the highly extended π system of graphene or graphene oxide, suggesting the GO/RF composite electrode should be robust enough for practical application.

CV analyses were conducted to assess the electrochemical activity of the GO/RF composite electrode. There was no obvious
redox peaks in the CV curves of the bare GP and GO electrodes, while the CV curves of the RF composite electrodes (GP/RF and GO/RF) demonstrated a couple of well-defined redox peaks centered at about −0.47 V (vs. SCE) (Fig. 3a), which corresponded to the redox reaction of RF on the electrode surface. This result further confirmed the successful fabrication of RF-modified composite electrode. The GO/RF electrode showed about 1-fold higher peak current than the GP/RF electrode (Fig. 3a), which is in good agreement with the fact that the amount of RF adsorbed onto the GO/RF electrode was about 2-fold higher than that adsorbed onto the GP/RF electrode (Fig. S2). Furthermore, higher redox peaks and larger CV graph area of the GO/RF composite electrode, when compared with those of the GP/RF and bare electrodes, indicated its higher electrocatalytic activity and fara-discharge capacity, which could be attributed to the increased electroactive surface area and accelerated mass transfer on the GO/RF electrode.

Moreover, de-adsorption is another important factor with respect to electron shuttle immobilization. The electrodes with RF immobilization (GO/RF and GP/RF) were immersed in phosphate buffer solution with continuous stirring for 15 days to test the stability of RF immobilization. Notably, no detectable RF was released into the aqueous solution from these electrodes as analyzed by HPLC. Similarly, the CV curves of these electrodes after 15 days' desorption test (Fig. 3b) or MFC discharge (Fig. S3) showed no significant difference from that of the fresh electrodes (0 day). Strong non-covalent π-π interaction between graphene backbone and riboflavin isosalloxazine ring (Fig. S1) was assumed to explain the stable riboflavin immobilization.

3.2. Improved MFC performance with GO/RF composite electrode

The composite electrode (GO/RF) was used as the anode in the S. oneidensis MR-1 inoculated MFC. For comparison, different bare electrodes (GP and GO) and GP/RF composite electrode were used as the control. As shown in Fig. 4a, after bacterial inoculation, the voltage output of all the MFCs reached the steady plateau within about 10 h. The GP and GO electrodes equipped MFC exhibited the worst performance, with maximum voltage output reaching only 72 mV (180 mA/m²) and 110 mV, respectively. However, the GO electrode showed much better performance than the GP electrode probably owing to the graphene layers formed on the electrode surface, similar to those reported in previous studies [27]. After RF modification, the electrodes (GP/RF and GO/RF) significantly enhanced the voltage output of MFCs, when compared with the bare electrodes (GP and GO). Interestingly, MFC with the GO/RF electrode delivered 320 mV (800 mA/m²) maximum voltage output, which was about 3.4-fold higher than that from the bare GP electrode, and outperformed all the other electrodes (GP, GO, and GP/RF).

![Fig. 3. CV analysis of different electrodes in oxygen-free phosphate buffer (0.1 M, pH = 6.8) at a scan rate of 2 mV/s for confirmation of the presence of RF (a) and examination of RF stability (b).](image1)

![Fig. 4. Performance of MFCs equipped with different electrodes. V-t curves (a), power density curves (b), and polarization curves (c) of MFCs with different electrodes.](image2)
The maximum power density of MFC with different anodes was determined with P–j curve (Fig. 4b). It was found that the maximum power density output for MFC equipped with GP, GO, GP/RF or GO/RF was 41, 74, 148 and 257 mW/m², respectively. Impressively, the maximum power density of GO/RF electrode was about 5.3-, 2.5-, and 0.7-fold higher than that of the GP, GO, and GP/RF electrodes, respectively. These results substantiated that in-situ graphene exfoliation and spontaneous RF modification of the graphite electrode significantly improved MFC performance.

When compared with covalent immobilization, electrode modification through physical absorption in the MFCs has been seldom explored, which could probably be owing to the notion that physical absorption is relatively unstable and the adsorbed electron shuttles are easy to desorb with MFC operation. However, RF, as an endogenous electron shuttle secreted by Shewanella spp., could be efficiently absorbed onto the biofilm and electrode interface, especially when the electrode is modified with nanomaterial. Interestingly, no obvious change was observed after 15 days of desorption (Fig. 3b) and MFC discharge (Fig. S3). The stable RF immobilization could be explained by the enhanced noncovalent π–π interaction between the graphene backbone and RF isoalloxazine ring (Fig. S1). In the present study, the large surface area and unique graphene layers led to the excellent performance of MFC.

### 3.3. RF immobilization enhanced EET and bacterial attachment

To further analyze the effect of RF immobilization on EET between the cells and electrode, the V–j curves of MFCs equipped with different electrodes were analyzed. As shown in Fig. 4c, the V–j curve of the GP and GO electrodes showed similar trend, which was characterized by low open circuit potential (OCP) (381 and 259 mV, respectively), followed by a linear potential drop between 250 and 40 mV. According to a previous study, the linear potential drop in the V–j curve of Shewanella MFC exhibited an apparent ohmic resistance and over-potential for increased charge transfer rate at the Shewanella and electrode interface [31]. A shorter slope in the V–j curve of the GO electrode, when compared with that of the GP electrode, indicated reduced over-potential for charge transfer, suggesting that graphene formation on graphite effectively improved the EET efficiency of S. oneidensis MR-1 and further enhanced the MFC performance. With regard to the MFC with GO/RF electrode, increased OCP was observed (637 mV vs. 381 mV for the GP electrode and 259 mV for the GO electrode), which was similar to that of the GP/RF electrode. As the MFC OCP is principally determined by the redox potential of the final electron carrier at the anode and cathode, the increased OCP indicates that Shewanella could efficiently utilize the redox species with more negative standard redox potential for EET. As RF has more negative standard redox potential than Shewanella OM cytochromes, which are generally recognized for direct electron transfer (~ –0.47 V vs. –0.2—0.1 V), it is convincible to postulate that increased OCP suggests that Shewanella could effectively use the immobilized RF to facilitate EET when the RF-absorbed electrode is adopted as the MFC anode. The above-mentioned postulation was further validated by analyzing the potential drops in the V–j curve. Unlike the potential drop in the V–j curve of the GP and GO electrodes, that of the GO/RF electrode exhibited two distinct linear parts separated by the inflection point at around 220 mV. Consistent with the findings of a previous work, the linear potential drop above 220 mV indicated RF participation in EET in this identical system, while the potential drop under 220 mV could be ascribed to the direct electron transfer between Shewanella OM cytochrome. Furthermore, the linear potential drop in V–j curve of Shewanella MFC reflects the charge-transfer resistance between Shewanella and the electrode [31]. Slower slope in the V–j curve of GO electrodes (GO, GO/RF) compared with GP indicates GO growth on the electrode surface reduced over-potential for charge transfer and thus improved the EET efficiency. These results suggested that graphene formation on the graphite surface, together with RF immobilization, improved the EET between cells and electrode and

![Fig. 5](image-url) **Fig. 5.** SEM images of the GP electrode (a), GO electrode (b), GP/RF electrode (c), and GO/RF electrode (d) after MFC discharge. Red arrows indicate the presence of bacterial cells.
might be the underlying mechanism for MFC performance enhancement by the GO/RF electrode.

Cell loading on electrode is another important factor that determines the power output of MFC. Accordingly, cell attachment on these electrodes after MFC discharge was further analyzed by SEM (Fig. 5). When compared with the bare GP and GO electrodes, the GP/RF and GO/RF composite electrodes had a higher number of bacteria attached onto the electrode surface, respectively. In particular, the GO/RF electrode possessed the highest number of bacterial cells on the surface, which was consistent with its highest performance in the MFC. Thus, these results indicated that RF could improve the formation of a thick biofilm on the electrode surface, which may also contribute to the improvement in the power output of MFC.

4. Conclusions

A novel and facile strategy for fabrication of GO/RF composite electrode was developed. The noncovalent interaction between graphene and RF was quite stable and significantly improved the EET between the S. oneidensis MR-1 cells and electrode. By applying the GO/RF electrode as the anode, the maximum power density output of the MFC was about 5.3-fold higher than that of the bare GP electrode. Further analyses indicated that improved EET and bacterial attachment might be the underlying mechanism for the performance improvement by this composite electrode. These findings demonstrated that noncovalent immobilization of electron shuttles with graphene might be a simple, efficient, and practical method for MFC electrode modification.

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References