In-situ modified carbon cloth with polyaniline/graphene as anode to enhance performance of microbial fuel cell

Lihua Huang\textsuperscript{a,b,c,d}, Xiufen Li\textsuperscript{a,c,d,*}, Yueping Ren\textsuperscript{a,c,d}, Xinhua Wang\textsuperscript{a,c,d}

\textsuperscript{a} Laboratory of Environmental Biotechnology, School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, PR China
\textsuperscript{b} School of Life Science, Linyi University, Linyi 276005, PR China
\textsuperscript{c} Jiangsu Key Laboratory of Anaerobic Biotechnology, Wuxi 214122, PR China
\textsuperscript{d} Jiangsu Cooperative Innovation Center of Technology and Material of Water Treatment, Suzhou 215009, PR China

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\textbf{Abstract}

The microbial fuel cell (MFC) technology has potential in recovering bioelectricity from different types of waste. However, the low power density, closely associated with anode performance, limits its practical application. In this study, polyaniline (PANI) together with graphene was chosen to in-situ modify oxidized carbon cloth (CC) by the aid of secondary bond forces (such as $\pi-\pi$ stacking, hydrogen bonds and electrostatic forces). The MFC reactor with PANI/graphene modified CC (PANI\textsuperscript{+}G\textsuperscript{+}CC) anode achieved the highest voltage with $573 \pm 37$ mV, and produced a peak power density of $884 \pm 96$ mW/m\textsuperscript{2}, which was 1.3 and 1.9 times of those with the CC control. Based on cyclic voltammetry (CV) scanning and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra, it was speculated that the weakly acidic microenvironment derived from bio-anode delayed or impeded the deprotonation of PANI and made PANI hold a certain level of conductivity for electron transfer. This study provided a simple and environment-friendly modifying method to enable the prepared PANI\textsuperscript{+}G\textsuperscript{+}CC anode to dramatically promote the performance of MFC.

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\textbf{Introduction}

Microbial fuel cell (MFC), being able to directly convert biomass to bioelectricity, has become a research hotspot in recent years. Exoelectrogens are prone to decomposing substrate and directly or indirectly transferring the released electrons towards the anode. The electrons are further transferred to the cathode via the external circuit, wherein ultimately combine with $H^+$ and electron acceptor (such as $O_2$) to form water [1–3]. Increasingly more attention has been paid to MFC in the background of energy crisis and huge environmental pressure [4,5]. At present, the low bioelectricity generation is one of the main bottlenecks limiting the practical application of MFC. The anode can directly affect microbial
attachment and electron transfer, and then further influence electrogenesis [6]. The efficient anode is highly desired.

Polyaniline (PANI), with conductivity, safe environment, low cost and ease of synthesis, has been studied in MFC as the anode modifier. It can switch from an insulator to a conductor by protonic acid doping [7]. Moreover, PANI carries positive charges in neutral environment, and is attractive to adhesion growth of negatively charged bacteria. Graphene is a kind of carbonaceous material possessing the stable chemical properties, anticorrosion, good conductivity and huge specific surface area, which also has been extensively applied in MFC electrode modification [8]. However, PANI presents smaller surface area and reluctant conductivity and graphene possesses poor biocompatibility, which resultanty influence the bacterial loading capacity and extracellular electron transfer (EET) efficiency. So, the combined modification by PANI and graphene would make best use of their advantages and bypass shortcomings above, and may be more suitable to serve as the MFC anode. The previous researches about modified anode with PANI and/or graphene in MFC system were summarized in Table 1. The MFC reactor with carbon cloth (CC) anode modified firstly by electrochemically reducing graphene oxide and then coating PANI nano-fibers outputted the maximum power density \( P_{\text{max}} \) of 1390 mW/m\(^2\) [9]. Zhao et al. fabricated a novel MFC anode by electro-depositing PANI networks onto graphene nanoribbons-coated carbon paper (CP/GNRs/PANI), and the maximum power density in MFC reactor equipped with the CP/GNRs/PANI anode was 856 mW/m\(^2\) [10]. Kumar et al. fabricated the graphene/PANI/Pt modified CC as anode of MFC via spray technique by the aid of 1 wt% polytetrafluoroethylene (PTFE) solution, generating the maximum power density of 2059 mW/m\(^2\) [11]. It was worth noting that the higher power densities above-mentioned were produced with ferricyanide as electron acceptor in cathode chamber. Further, the modification methods above brought about complex procedures, such as needing organic solvent, binder, or electrochemical facilities.

In this study, some secondary bond forces (such as electrostatic forces, \( \pi-\pi \) stacking and hydrogen bonds) were utilized to in-situ modify CC with PANI/graphene. Before modification, CC was soaked in 6 mol/L nitric acid for obtaining oxygen-containing groups (such as COOH, C=OH, C==O and C-O-C) [12] to form hydrogen bonds with graphene. Graphene was prepared by redox method and usually contained a few oxygen-containing groups due to incomplete oxidation. The spectra of an energy dispersive X-ray (EDX, Genesis Apollo X/ XL, EDAX) (Fig. S1), compared with the pristine CC (1.99%), which indicated that more oxygen-containing groups were introduced into the carbon backbone of CC due to nitric acid oxidation. The spectra of an attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet iS10, Thermo Fisher Scientific spectrophotometer) further demonstrated that some oxygen-containing groups joined into the carbon backbone of CC (Fig. S2). Next, the oxidized CC was immersed into 0.1 mol/L aniline monomer solution (prepared using 1 mol/L HCl) for 6 h till full infiltration, and then dripped with 25 mL pre-cooled 0.4 mol/L APS (prepared using 1 mol/L HCl) at ice-cooled and magnetic stirring conditions (400 r/m), wherein the polymerized PANI would tightly bonded with the oxidized CC by the aid of hydrogen bonds and \( \pi-\pi \) stacking [19,22]. Six hours later, the oxidized CC with PANI (PANI-CC, green in color) was taken out, rinsed by 1 mol/L HCl solution and deionized water in turn, and air-dried.

For the preparation of G+CC, the aqueous dispersion of graphene was prepared by the method described by Marcano et al. [38]. The oxidized CC was immersed into graphene aqueous solution (2 g/L) for 6 h with stirring (400 r/m) to obtain the G+CC via hydrogen bonds and \( \pi-\pi \) stacking. For the fabrication of PANI-G+CC, firstly the G+CC was prepared according to the procedure above-mentioned, and then the following process was the same as the PANI+CC.

Experimental

Preparation of modified anodes

Before modification, the non-watertight CC (thickness of 0.34 mm, HCP330N, Shanghai Hesen Electric Co., Ltd, China) was soaked in acetone and 1 mol/L HCl solution in turn for 12 h to remove impurities, rinsed with deionized water to neutral, and dried at 60 °C for 12 h. Then, the clean CC was oxidized in 6 mol/L nitric acid at 30 °C for 4 h to obtain oxygen-containing groups [12]. The atomic percentage of oxygen in the oxidized CC by nitric acid reached 3.23% based on the spectra of an energy dispersive X-ray (EDX, Genesis Apollo X/ XL, EDAX (Fig. S1)), compared with the pristine CC (1.99%), which indicated that more oxygen-containing groups were introduced into the carbon backbone of CC due to nitric acid oxidation. The spectra of an attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet iS10, Thermo Fisher Scientific spectrophotometer) further demonstrated that some oxygen-containing groups joined into the carbon backbone of CC (Fig. S2).

Setup and operation of MFC reactors

Air-cathode single chamber MFC reactors with an internal volume of 28 mL were used in this study as previously reported [39]. The cathodes were made by applying platinum (7 cm\(^2\), 0.5 mg/cm\(^2\) Pt, Hispec3000, Shanghai Hesen Electric Co., Ltd, China) and four diffusion layers (polytetrafluoroethylene, PTFE) on a 30 wt% wet-proofed CC (thickness of 0.40 mm, HCP330P, Shanghai Hesen Electric Co., Ltd, China) [40]. The MFC reactors were inoculated with effluent from the existing well-running MFC (originally seeded with Taihu Lake sediment, China) and culture medium solution at a ratio of 1:1. The culture medium solution contained 1 g/L NaAc, 2.77 g/L NaH\(_2\)PO\(_4\), 2H\(_2\)O, 11.40 g/L Na\(_2\)HPO\(_4\), 12H\(_2\)O, 0.31 g/L NH\(_4\)Cl, and 0.03 g/L KCl.
<table>
<thead>
<tr>
<th>Anodes</th>
<th>Modification method</th>
<th>Reactor configuration</th>
<th>Anode inoculum</th>
<th>Cathode or catholyte</th>
<th>$P_{\text{max}}$ (mW/m$^2$)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT/PANI/nickel foams</td>
<td>Mechanical coating</td>
<td>Single</td>
<td>E. coli</td>
<td>Electrolyte</td>
<td>42</td>
<td>2007 [18]</td>
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<td>PANI/CF</td>
<td>In situ polymerization</td>
<td>Dual</td>
<td>Anaerobic sludge</td>
<td>Electrolyte</td>
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<td>2011 [19]</td>
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<tr>
<td>PANI/GF</td>
<td>Electrochemistry</td>
<td>Single</td>
<td>Rice paddy soil</td>
<td>Air-cathode (Pt)</td>
<td>2300</td>
<td>2010 [20]</td>
</tr>
<tr>
<td>HSO$_4$ doped PANI/CC</td>
<td>Electrochemistry</td>
<td>Dual</td>
<td>Photosynthetic bacteria</td>
<td>CC (Pt)</td>
<td>5.16 W/m$^3$</td>
<td>2011 [21]</td>
</tr>
<tr>
<td>Tartaric acid doped PANI/CC</td>
<td>In situ polymerization</td>
<td>Dual</td>
<td>S. oneidensis</td>
<td>Ferricyanide</td>
<td>490</td>
<td>2015 [22]</td>
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<tr>
<td>PANI/SSFF</td>
<td>Electropolymerization</td>
<td>Dual</td>
<td>Domestic wastewater</td>
<td>Ferricyanide</td>
<td>360</td>
<td>2015 [23]</td>
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<td>PANI/CNT/GF</td>
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<td>Dual</td>
<td>S. putrefaciens</td>
<td>Ferricyanide</td>
<td>257</td>
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<td>MnFe$_2$O$_4$/PANI/CC</td>
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<td>Single</td>
<td>S. putrefaciens</td>
<td>Air-cathode (MnFe$_2$O$_4$/PANI/CC)</td>
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<td>PANI/CF</td>
<td>Layer by layer technique deposition</td>
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<td>Ferricyanide</td>
<td>460</td>
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<td>G/graphite</td>
<td>Electrochemistry</td>
<td>Dual</td>
<td>S. oneidensis</td>
<td>Air-cathode (Pt)</td>
<td>102</td>
<td>2015 [28]</td>
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<td>G/SSM</td>
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<td>Ferricyanide</td>
<td>2668</td>
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<td>G/C</td>
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<td>52.5</td>
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<td>Crumpled G/CC</td>
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<td>Ferricyanide</td>
<td>5.16 W/m$^3$</td>
<td>2012 [32]</td>
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<td>Nitrogen doped G/CC</td>
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<td>E. coli</td>
<td>Ferricyanide</td>
<td>1008</td>
<td>2015 [33]</td>
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<td>G/SnO$_2$/CC</td>
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<td>1624</td>
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<td>Dual</td>
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<td>Ferricyanide</td>
<td>335 A/m$^2$</td>
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<tr>
<td>G/PEI/CP</td>
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<td>S. oneidensis</td>
<td>Ferricyanide</td>
<td>368</td>
<td>2014 [36]</td>
</tr>
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<td>Dual</td>
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<td>Ferricyanide</td>
<td>768</td>
<td>2012 [37]</td>
</tr>
<tr>
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<td>Dual</td>
<td>S. oneidensis</td>
<td>Ferricyanide</td>
<td>1390</td>
<td>2013 [9]</td>
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<tr>
<td>PANI/graphene nanoribbons/CP</td>
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<td>Dual</td>
<td>S. oneidensis</td>
<td>Ferricyanide</td>
<td>856</td>
<td>2013 [10]</td>
</tr>
</tbody>
</table>

* G denotes graphene, MWCNT denotes multiwalled carbon nanotubes, PEI denotes polyethyleneimine, CP denotes carbon paper, CF denotes carbon felt, GF denotes graphite felt, SSFF denotes stainless steel fiber felt, and SSM denotes stainless steel mesh.
0.13 g/L KCl, 5 mL/L vitamins and 12.5 mL/L trace minerals, with a conductivity of ~7.0 mS/cm. Vitamins and trace minerals were prepared as previously described [41]. In the end, the pH of culture medium solution was adjusted to 7.0. When the outputted voltage of MFC reactors declined to below 20 mV across an external loading resistance of 1000 Ω, a complete cycle was over. All reactors were conducted in batch mode and the temperature was maintained at 30 ± 1 °C.

**Analysis**

Morphology and elemental composition were examined using a scanning electron microscope (SEM, S-4800, Hitachi) and EDX detector (Genesis Apollo X/XL, EDAX) [39]. The functional groups carried by prepared electrodes were analyzed with ATR-FTIR method. The functional groups of graphene were examined with FTIR [42]. The surface chemistry of newly formed electrodes was characterized on X-ray photoelectron spectroscopy (XPS, AXIS HIS 165 spectrometer, Kratos Analytical) equipped with a monochromatized Al Kα X-ray source (1486.71 eV photons). The background in XPS data was subtracted using Shirley method and XPS curve-fitting was performed according to Lorentzian–Gaussian function at high resolution. The spectra in C1s and N1s regions were deconvoluted by XPS PEAK 4.1 software to quantify the function groups [43].

Cell voltages (U) were automatically recorded using a data acquisition unit (34972A, Agilent) at a pre-determined sampling frequency (30 min) under an external resistance of 1000 Ω (R). The current was calculated using Ohm’s law: \( I = U/R \), the power was given by \( P = UI \), and the current density and power density were obtained by dividing anode area (7 cm²) from the current and power. Using oxygen as electron acceptor, coulombic efficiencies (CE) were calculated based on COD removal by a method described by Kim et al. [44].

Electrochemical analysis was performed using an electrochemical workstation (CHI660D, Shanghai Chenhua Instruments Co., Ltd, China) with a three-electrode model, wherein the anode served as the working electrode, Pt wire with a size of Ø 0.5 mm × 37 mm (Wuhan Gaoss Union Science and Technology Co., Ltd) as the counter electrode and saturated calomel electrode (SCE, type of 232, 0.224 V vs SHE, Shanghai Leici) as the reference electrode. The working terminals of electrochemical instrument were in-situ connected to the corresponding electrodes of the examined MFC reactors. In this study, all electrochemical tests were performed in MFC reactors, rather than in special electrochemical cells, for the purpose of making the testing results closer to the actual system. In abiotic system, cyclic voltammetry (CV) was conducted at open-circuit voltages over a frequency range of 10⁵ to 0.005 Hz with sinusoidal perturbation amplitude of 10 mV. Before starting each impedance measurement, the MFC reactors were operated at open-circuit voltages for over 1 h to attain static state [46].

COD was determined using the standard method [47]. The biomass densities of cultured anodes were determined by lipid-phosphorous method [48]. In this study, all potentials quoted were referred to SHE, unless otherwise stated. All tests were conducted in triplicate and the averages were presented.

**Results and discussions**

**Characteristics of bare anodes**

The difference of electrodes was visually observed by a change in color. Compared with the oxidized CC control, the color of G+CC changed lighter due to modification with graphene. The modification with PANI resulted in green coloration of the PANI+CC and PANI+G+CC electrodes, according with the previous report [49]. SEM examination showed that the oxidized CC electrode consisted of carbon fibers with a smooth surface (Fig. 1a), however, the G+CC electrode consisted of carbon fibers with a smooth surface (Fig. 1a), however, the G+CC electrode was covered with graphene (Fig. 1b) and a wrinkled graphene layer was observed on the surface of carbon fibers at high-resolution (Fig. 1c). For the PANI+CC, a layer of PANI matrix was densely packed on the oxidized CC (Fig. 1d and e). Similarly, a layer of PANI matrix was also observed on the surface of PANI+G+CC (Fig. 1f and g). Ding et al. presented a vertical aligned PANI nanowire array (with diameters of approximately 68 ± 5 nm) prepared by electrochemical polymerization on the Au substrate, which was conducive for adhesion growth of bacteria and extracellular electron transfer (EET) [50]. The three-dimensional (3D) structure resulting from PANI matrix increased the more available area of anode and enhanced local topographic interaction between PANI and cellular components, consequently bearing the larger bacteria loading and favoring the efficiency of EET.

The survey level XPS spectra showed the presence of C and O elements in the oxidized CC and G+CC, and the presence of C, O, N and Cl elements in the PANI+CC and PANI+G+CC (Fig. S3). The relative atomic percentages derived from corresponding photoelectron peak areas of XPS spectra were presented in Table S1. The atomic percentage in O1s region (2.77%) for the oxidized CC was smaller than that for graphene (4.43%) due to incomplete redox during graphene preparation [13]. The FTIR spectra also showed that some oxygen-containing groups existed in graphene (Fig. S4), which was favorable for graphene to easily attach onto the surface of oxidized CC via hydrogen bonds as well as π–π stacking. HCl was trapped in the polymer network of polymerized PANI for doping, leading to Cl element existing in the PANI+CC and PANI+G+CC. N element mainly came from the polymerized PANI. The deconvoluted C1s core level spectra of CC and G+CC
presented three different characteristics of carbon bonds. A large peak at 285.0 eV corresponded to C sp2, and the peaks at 287.3 eV and 288.5 eV corresponded to C sp3 and C sp2 [51]. The deconvoluted N1s core level spectra of PANI+CC and PANI+G+CC can be resolved into two primary peaks at 399.8 and 401.6 eV. The former was attributed to C sp3 and C sp2, and the latter was attributed to C sp3 and C sp2 [52,53]. The area ratios of different C or N species were also presented in Table S1.

The CV curves of bare oxidized CC and G+CC presented a straight line in 1 mol/L HCl solution, indicating that there was almost no response current (Fig. 2). However, a pair of significant redox peaks appeared in the bare PANI+CC (with formal potentials of 511 ± 19 mV (vs. SHE)) and PANI+G+CC (with formal potentials of 499 ± 23 mV (vs. SHE)). It was reported that the CV curves of PANI in acid solution generally presented two pairs of peaks, corresponding to redox transition of PANI (i.e., the leucoemeraldine emeraldine transition and the emeraldine pernigraniline transition, respectively) [54,55]. However, only one pair of peaks was observed in our study, possibly because the anode (7 cm²) served as working electrode had a relatively large area (compared with glassy carbon electrode) [55], leading to a large background current and superimposition of the two pairs of redox peaks. The redox peaks with the formal potentials of 511 ± 19 or 499 ± 23 mV (vs. SHE) were induced by redox reaction of PANI itself. In addition, the current densities of redox peaks in the PANI+G+CC were obviously larger than those in the PANI+CC. The graphene layer with good conductivity embedded between the oxidized CC and PANI enhanced the efficiency of electron transfer, equivalent to increasing the conductivity of electrode base. Additionally, the adhesion between graphene and PANI was more powerful than that between the oxidized CC and PANI due to the plane structure of graphene in nanometer range and higher content of oxygen element (contributing to hydrogen bonds) compared with the oxidized CC.
MFC performance

Over 24 reaction cycles, the profiles of cell voltages were shown in Fig. 3, suggesting that the PANI+G+CC reactor achieved the highest outputted voltages (V_{max}) with 573 ± 37 mV. From Table 2, the PANI+G+CC reactor also gained the highest open circuit voltages (OCP), indicating that the electrochemically active biofilm attached on the PANI+G+CC had the greatest capability of electron-generating. The maximum power densities for the PANI+G+CC reactor were 884 ± 96 mW/m² over 6 reaction cycles (after approximately 3 weeks of operation), which was 1.4, 1.5 and 1.9 times of those for the G+CC, PANI+CC and oxidized CC reactors (Fig. 4a and Table 2). The cathode potentials were similar in different MFC reactors, and the anode potentials changed greatly (Fig. 4b), suggesting that the anode potentials were responsible for the increment in power generation, and further demonstrating the superior performance of PANI+G+CC anode. After 2 months of operation, the PANI+G+CC reactor still produced the highest power densities of 732 ± 76 mW/m² (declined by 17%) (Fig. 4c), which was 1.2, 1.5 and 2.0 times of those in the G+CC, PANI+CC and oxidized CC reactors, demonstrating that the PANI+G+CC reactor had the capability of generating high sustainable power. Fig. 4d also showed that the difference in power generation was caused by the performance of anodes after long-time running.

To investigate the electron transfer ability of bio-anodes, a constant polarization potential was applied to the anodes after MFC reactors were stabilized for 1 h under open circuit condition, and the corresponding polarization current over time was recorded. The higher polarization potential was harmful to bio-anode activity, but the lower polarization potential produced a smaller corresponding polarization current. In view of the bio-anode potential of – 176 mV (vs. SHE) under open circuit condition, the constant polarization potential of 224 mV (vs. SHE) was selected. As can be seen from Fig. 5a, the polarization current densities tended to stabilize after 400 s with 0.18 ± 0.05 mA/cm² for the PANI+G+CC bio-anode, which was 1.7, 2.0 and 4.3 times of those for the G+CC, PANI+CC and oxidized CC bio-anodes. Under the same polarization condition, the larger the current density, the higher the efficiency of EET. EIS analysis revealed that the MFC reactors with the oxidized CC, PANI+CC, G+CC and PANI+G+CC anodes had similar ohmic resistances of ~15 Ω, whereas presenting a big difference in charge transfer resistances (R_{ct}) (Fig. 5b). R_{ct} with the oxidized CC control was 115 ± 13 Ω, and was 2.5, 3.1 and 8.2 times of those with the PANI+CC, G+CC and PANI+G+CC anodes. The smaller R_{ct} indicated the resistance between biofilm and anode was smaller, leading to the higher efficiency of EET and electricity production [56]. The PANI+G+CC anode presented the lowest R_{ct} of 14 ± 2 Ω, supporting the result of constant potential polarization. The linear area in low-frequency region represented diffusion resistance. The reactor with the oxidized CC control tended to present a larger linear area, while smaller linear areas were shown in low-frequency regions of the reactors with the PANI+CC, G+CC and PANI+G+CC anodes. Compared with the oxidized CC control, the surface area of G+CC increased due to modification by graphene, the 3D-PANI matrix modified anodes (both PANI+CC and PANI+G+CC) was rougher and presented the larger surface area, which enhanced the mass transfer efficiency and further alleviated the diffusion resistance for bioelectrochemical reactions [57].

The biomass density was 2.4 ± 0.3 µg/P/cm² on the PANI+G+CC, higher than 1.8 ± 0.2 µg/P/cm² on the G+CC, 2.1 ± 0.3 µg/P/cm² on the PANI+CC and 1.3 ± 0.3 µg/P/cm² on the oxidized CC control, demonstrating that the good biocompatibility of PANI contributed to higher bacterial loading. COD removal rate of 88.5 ± 8.6% and CE of 31.5 ± 4.4% were also the highest in the reactor with the PANI+G+CC anode (Table 2). The 3D-PANI matrix modified anodes (both PANI+CC and PANI+G+CC) was rougher and presented the larger surface area, which enhanced the mass transfer efficiency and further alleviated the diffusion resistance for bioelectrochemical reactions [57].

The graphene interlayer with good chemical active sites were developed and the efficiency of EET was enhanced [50]. The graphene interlayer with good conductivity between the oxidized CC and PANI enhanced the efficiency of electron transfer.

![Cell voltages (mV) vs. Time (h)](image)

**Fig. 3** Profiles of the outputted cell voltages in MFCs with the different anodes during operation.

<table>
<thead>
<tr>
<th>Anodes</th>
<th>R_{ct} (Ω)</th>
<th>V_{max} (mV)</th>
<th>OCP (mV)</th>
<th>P_{max} (mW/m²)</th>
<th>COD removal (%)</th>
<th>CE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>115 ± 13</td>
<td>454 ± 34</td>
<td>721 ± 45</td>
<td>454 ± 47</td>
<td>75.3 ± 8.9</td>
<td>22.6 ± 1.7</td>
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<tr>
<td>PANI+CC</td>
<td>47 ± 5</td>
<td>517 ± 45</td>
<td>756 ± 67</td>
<td>589 ± 38</td>
<td>81.6 ± 4.9</td>
<td>26.1 ± 2.4</td>
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<tr>
<td>G+CC</td>
<td>37 ± 4</td>
<td>534 ± 56</td>
<td>783 ± 45</td>
<td>634 ± 78</td>
<td>84.5 ± 9.4</td>
<td>27.8 ± 3.2</td>
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<tr>
<td>PANI+G+CC</td>
<td>14 ± 2</td>
<td>573 ± 37</td>
<td>836 ± 79</td>
<td>884 ± 96</td>
<td>88.6 ± 8.6</td>
<td>31.5 ± 4.4</td>
</tr>
</tbody>
</table>

*Bioelectrochemical parameters were obtained under an external loading of 1000 Ω, and values were given as mean values ± standard deviation (n = 3).*
Conductivity of PANI

PANI was generally prone to losing conductivity due to deprotonation in neutral or alkaline solution [58,59]. The existing investigations in MFC system demonstrated that PANI was conductive and had the capability of transferring electrons in anolyte of pH ~7 [19, 24, 37]. So far, this discrepancy was still not consolidated. Here, by comparing the structural change in PANI chain between the newly prepared, uncultured and cultured PANI+CC, the conductivity of PANI in neutral anolyte was explored and analyzed. CV scanning was applied to investigate the process of electron transfer between the PANI+CC anode and biofilm. Because the bioelectrochemical current was usually relatively small, a lower scanning rate of 1 mV/s was adopted to reduce the interference of background current (derived from electrochemical reaction) and the difficulty of screening redox peaks [60]. The cultured PANI+CC anode revealed a pair of obvious peaks with the formal potentials of 251 ± 21 mV (P₂), corresponding to the redox reaction of PANI itself (Fig. 6b). There also existed a pair of inconspicuous peaks due to interference by a large background current. After processing with the method of subtracting straight line by Origin 9.0, the peaks were shown obviously in the inset plot in Fig. 6b, with the formal potentials of −369 ± 24 mV (P₁). No redox peaks was observed in the CV curves of the bare PANI+CC anode in sterile anolyte (Fig. 6a), which demonstrated that the redox species corresponding to the formal potentials of −369 ± 24 mV came from microorganisms. According to the previous studies, the formal potentials for cytochrome c (Cyt c) involving in EET varied in a wide range of potentials (from 242 mV to −258 mV, vs. SHE) with different inocula, growth stages and operation conditions (e.g., pH, temperature, substrate, ion concentration) [37, 61–65]. Thus, the species at −369 ± 24 mV possibly was a type of Cyt c associated with EET, i.e., the CV curves of PANI+CC bio-anode displayed Cyt c peak current. Because the
mixed bacteria were applied as inocula in our study, it was unscientific to identify Cyt c types simply based on the formal potentials. Additionally, PANI displayed different formal potentials in CV curves under different conditions. For example, its formal potentials were 287 ± 19 mV in 1 mol/L HCl solution with abiotic system (Fig. 2), 175 ± 22 mV in anolyte with abiotic system (Fig. 6a), and 251 ± 21 mV with biofilm coating (Fig. 6b).

ATR-FTIR was used to further analyze the structure change between the newly prepared, uncultured and cultured PANI+CC anodes, and the results were shown in Fig. 7. The PANI+CC in MFC reactor containing sterile anolyte was regarded as uncultured. The absorption peak at 1060 cm⁻¹ corresponded to stretching vibration of sulfonate S=O in aromatic ring, where the sulfonate came from APS[42]. The absorption peak at 1590 cm⁻¹ corresponded to stretching vibration of quinone ring C=O in PANI chain. The stretching vibration of C=C in aromatic ring revealed peaks at 1500 cm⁻¹. The stretching bands at 1290 and 1160 cm⁻¹ belonged to C=N and N–Q–N (Q denoted the quinoid ring)[66]. The PANI+CC anode gradually deprotonated in pH = 7.0 anolyte, and the rearrangement of electrons led to transformation of aromatic rings to the quinone rings, reducing the ratio of aromatic rings to quinone rings. So, the intensity ratio of peaks at 1590 and 1500 cm⁻¹ represented the level of protonation in PANI chain or conductivity of PANI[67]. As can be seen from ATR-FTIR spectra, the intensity ratio of absorbance of aromatic ring (1500 cm⁻¹) to quinone ring (1590 cm⁻¹) was PANI+CC (newly prepared) > PANI+CC (cultured) > PANI+CC (uncultured), i.e., the deprotonation degree was in order of PANI+CC (newly prepared) < PANI+CC (cultured) < PANI+CC (uncultured), which indicated that the biofilm on the surface of PANI+CC alleviated deprotonation of PANI and played a promoting effect on maintaining PANI conductivity.

It was reported that PANI deprotonated and lost conductivity if soaked in neutral or alkaline solution[58,59]. On the other hand, the existing literature suggested that PANI modified anodes contributed to power output in MFC system (Table 1). Concerning PANI’s capability of transferring electrons in MFC anolyte (pH = 7), only one study suggested that the quinone ring of PANI chain was similar to quinone chemicals, and PANI played a role of electron transfer mediator instead of a conductor during EET. The CV curves of PANI modified Au anode displayed redox peaks of PANI rather than those of Cyt c, but its control only presented the redox peaks of Cyt c[50]. In our study, the internal structure of PANI chain analyzed by ATR-FTIR showed that the deprotonation of cultured PANI+CC anode fell between newly prepared and uncultured PANI+CC, suggesting that the cultured PANI+CC anode was not completely deprotonated and still maintained certain conductivity. In addition, SEM images showed that the surface of PANI+CC anode was completely covered by densely packed PANI layer (Fig. 1d and e), excluding the direct contact of microorganisms with the oxidized CC. That meant electrons were transferred to the oxidized CC base across PANI layer.

In abiotic electrochemical system, Li et al. suggested that PANI conductivity at high pH level was mainly due to the presence of protons near PANI chain, i.e., acidic microenvironment near PANI chain contributed to maintaining PANI conductivity[68]. In MFC system, exoelectrogens continuously produced protons accompanying the release of electrons during the oxidation of organic matter. The electrons were rapidly and sustainably transferred towards anode via conductive pili or Cyt c, whereas protons sank in biofilm and were delayed to diffuse out due to hindrance by biofilm layer, leading to a weakly acidic microenvironment within bioanode (Scheme S1). Franks et al. proved that pH level near
the graphite anode surface covered with Geobacter sulfurreducens strain PCA was as low as 6.1 compared to ~7 of the bulk solution [69]. Babauta et al. also observed that pH was 6.3 at the bottom of the G. sulfurreducens biofilms cultured on the glassy carbon [70].

Based on the analysis above, it was extrapolated that the weakly acidic microenvironment derived from biofilm attached on the PANI-CC delayed or impeded PANI deprotonation, and enabled PANI to hold a certain level of conductivity for electron transfer in the neutral anolyte of MFC. Due to the different experimental conditions (such as pH, inocula, electrodes and reactor configuration), this study did not rule out that PANI served as electron transfer mediator [50].

Conclusions

In this study, the secondary bond forces were utilized to in-situ modify oxidized CC with PANI/graphene. This modification procedure was characterized by simplicity, easy operation, and friendly environment. The as-received PANI-G-CC anode displayed a synergistic effect of graphene and PANI, achieving a peak power density of 884 ± 96 mW/m². A weakly acidic microenvironment derived from biofilm delayed or impeded the deprotonation of PANI, and thus PANI held a certain level of conductivity in the neutral anolyte of MFC for EET.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2016.05.048.

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