Sustainable Energy Recovery in Wastewater Treatment by Microbial Fuel Cells: Stable Power Generation with Nitrogen-doped Graphene Cathode

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ABSTRACT: Microbial fuel cells (MFCs) recover energy sustainably in wastewater treatment. Performance of non-noble cathode catalysts with low cost in neutral medium is vital for stable power generation. Nitrogen-doped graphene (NG) as cathode catalyst was observed to exhibit high and durable activity at buffered pH 7.0 during electrochemical measurements and in MFCs with respect to Pt/C counterpart. Electrochemical measurements showed that the oxygen reduction reaction (ORR) on NG possessed sustained activity close to the state-of-art Pt/C in terms of onset potential and electron transfer number. NG-MFCs displayed maximum voltage output of 650 mV and maximum power density of 776 ± 12 mW m⁻², larger than 610 mV and 750 ± 19 mW m⁻² of Pt/C-MFCs, respectively. Furthermore, long-time test lasted over 90 days, during which the maximum power density of NG-MFCs declined by 7.6%, with stability comparable to Pt/C-MFCs. Structure characterization of NG implied that the relatively concentrated acidic oxygen-containing groups improved such long-time stability by repelling the protons due to the same electrostatic force, and thus the C–N active centers for ORR were left undestroyed. These findings demonstrated the competitive advantage of NG to advance the application of MFCs for recovering biomass energy in treatment of wastewater with neutral pH.

INTRODUCTION

Great efforts have long been exerted in harnessing renewable energy in a simultaneous response to both the energy crisis and environmental pollution. Microbial fuel cells (MFCs) suit such a sustainable paradigm to recover biomass energy in the form of electricity in wastewater treatment by destroying organic matter.¹⁻⁵ Although biodegradation of organic matter occurs on anode, the performance of cathode, where the electron acceptors are present, also determines the cell power production.⁶ Among the electron acceptors including nitrate, chromium, ferricyanide, etc., ambient O₂ is the most attractive due to its ubiquity, low cost, and high standard equilibrium potential.⁶,⁷ O₂ is electrocatalytically reduced on cathode by scavenging the electrons coming from the anode side, and thus the oxygen reduction reaction (ORR) efficiency determines the overall cell performance.

Basically, ORR proceeds favorably via 4-electron pathway on platinum-group metals (PGMs) as catalyst,⁸ or 2-electron pathway on non-noble materials such as activated carbon, carbon black, etc.⁹,¹⁰ Cell voltage can be maximized via the former. Also, the cost of cathode materials accounts for as high as 47–75% of the MFC capital.¹¹ Consequently, to minimize the cost, employment of any non-noble cathode catalyst, instead of PGMs with extremely high cost and scarcity on the Earth, remains essential for the large-scale application of MFCs.¹¹⁻¹⁵ Among non-noble ORR catalysts, carbon-based materials are appealing owing to their low cost and ubiquity. Some
chemically modified carbon materials are capable of catalyzing ORR via 4-electron pathway in MFCs.\textsuperscript{2,16–18} Nitrogen-doped graphene (NG), a newly emerging nitrogen-doped ORR catalyst, has been reported to have a lower cost than PGMs once it is synthesized through a chemical route.\textsuperscript{17} So far, the performance of NG toward ORR has been widely verified to be comparable to PGMs in alkaline fuel cells.\textsuperscript{19–25} It is attractive that the N atoms incorporated into graphene provide active centers for 4-electron ORR. As a result, NG may find its application in MFCs as cathode catalyst by bringing high performance of NG toward ORR. However, reports associated with this issue are limited.\textsuperscript{17,18}

On the other hand, concern arises about the longevity of NG toward ORR in MFCs. The MFC systems, commonly operated in neutral pH medium, have quite a different solution condition from alkaline fuel cells. Such difference impacts the long-time activity of NG toward ORR. It has been evidenced that the durability of NG toward ORR becomes worse as the proton concentration in the solution decreases.\textsuperscript{24} Thus, the long-time performance of NG toward ORR must be further assessed in MFCs prior to its utilization. Meanwhile, the mechanistic explanation of the long-time stability has not been reported yet.

The long-time stability of NG as electrocatalyst is commonly assessed by means of electrochemical measurements. Unfortunately, these measurements are completed in quite a short period, during which the solution condition is able to remain constant. By contrast, the bulk solution of real MFCs inevitably experiences a variation in proton concentration in a long period.\textsuperscript{7} Undoubtedly, the results of electrochemical measurements are far from adequate in predicting the long-time performance of MFCs. Therefore, following the electrochemical measurements of NG, long-time assessment in MFCs system is indispensable.

For the mechanistic understanding of the NG performance, the structure characterization is always a powerful tool. Investigations on correlation between the surface structure and the instant ORR activity of NG in MFCs are available. Evidence shows that the surface nitrogen species, such as pyridinic and pyrrolic N, ensure the instant ORR activity of NG.\textsuperscript{25} However, investigation on the correlation between the surface structure and the long-time ORR activity of NG in MFCs is not available.

In this work, a lab-made NG through a chemical method was employed as the cathode catalyst of MFCs. The performance of NG was initially evaluated in terms of the ORR activity in neutral pH medium by electrochemical measurements, and then in terms of a 90 day power output of real MFCs. For comparison, the performance of a commercially available Pt/C was also assessed. Besides, a conceptual explanation of the long-time performance of NG for stable power generation of MFCs was proposed based on structure characterization of NG.

\section*{MATERIALS AND METHODS}

\textbf{Preparation of NG and Electrode.} Graphene oxide (GO) was synthesized as the precursor of NG using a modified Hummers’ method as previously described.\textsuperscript{26} A typical chemical approach for NG synthesis is briefly described in Scheme S1 and Section 1 of Supporting Information (SI). Electrode preparation for electrochemical measurements can be found in Sections 2 of the SI.

\textbf{Electrochemical Measurements.} Electrochemical measurements were conducted on a 2273 plus 263A Princeton workstation (Princeton Applied Research, Oak Ridge, TN) assembled with an MSR electrode rotator (Pine Research Instrumentation, Durham, NC). A conventional three-electrode cell was used. Besides the working electrode, it also contained Ag/AgCl (KCl, saturated) as a reference electrode and platinum mesh as a counter electrode. Cyclic voltammetric (CV) experiments were performed in 0.1 M phosphate-buffered saline (PBS, pH 7.0) after sparged with N\textsubscript{2} and O\textsubscript{2} for 15 min at a potential sweep of 50 mV s\textsuperscript{-1}. Linear polarization experiments on rotary ring disk electrode (RRDE) were carried out in the same PBS with saturated O\textsubscript{2}. The potentials varied from −0.6 V to +0.6 V vs Ag/AgCl at a potential sweep of 5 mV s\textsuperscript{-1} and varied rotating speeds from 400 to 2400 rpm. The ring potential was set at 0.6 V vs Ag/AgCl. Potential cycling measurement was utilized to predict the durability of catalyst by means of CV in certain potential range with potential sweep of 50 mV s\textsuperscript{-1}. Calculations of electron transfer number of ORR can be found in Sections 3 of the SI.

\textbf{MFC Configuration and Measurements.} H-shaped MFCs were constructed by channeling the two 100 mL chambers through a 20 mm diameter tube. To fabricate the cathodes, NG or Pt/C was brushed onto carbon paper (projected area 9 cm\textsuperscript{2}) using a binder of Nafion and isopropyl alcohol based on a previous method\textsuperscript{27} with loadings of 2.0 mgNG /cm\textsuperscript{2} and 0.5 mgPt /cm\textsuperscript{2}, respectively. The anode was carbon cloth (projected area 9 cm\textsuperscript{2}, Cetech Co., Ltd.), which was pretreated by a previously reported method.\textsuperscript{28} The anode and cathode were connected with an external resistor of 1000 Ω via titanium wire, and all exposed metal surfaces were sealed with nonconductive epoxy. Anode and cathode chambers were separated by Nafion membrane (Nafion 117, Dupont) after pretreatment.\textsuperscript{29}

Activated sludge from a municipal sewage treatment plant (Lijiatuo, south of Chongqing, China) was used as inoculum. The anode chamber was filled with mixture\textsuperscript{30} of 0.1 M PBS (pH 7.0) containing NaAc (1.0 g L\textsuperscript{-1}), KCl (0.13 g L\textsuperscript{-1}), NH\textsubscript{4}Cl (0.31 g L\textsuperscript{-1}), a mineral solution (12.5 mL L\textsuperscript{-1}), and a vitamin solution (5 mL L\textsuperscript{-1}). Before inoculation, the anode feed solution was sparged with N\textsubscript{2} to maintain the anaerobic condition. The feed solution was replaced once the cell voltage dropped below 10 mV, which was noted as a complete cycle of electricity generation. The cathode chamber was filled with the same mixture media but without NaAc, mineral solution, and vitamin solution. The cathode chamber was continuously aerated at a flow rate of 100 mL/min to maintain oxygen saturation. The dissolved oxygen (DO) concentration of catholyte was monitored by means of a portable DO meter (Hach, HQ40d) at the end of each cycle of power generation, and then the catholyte was replaced for further cycle.

The cell voltage across the external resistor was measured using a multimeter equipped with a data acquisition system (Advantech, PCI-1747U). The maximum power densities of cells were obtained from the polarization curves by varying the value of external resistor from 10 Ω to 5000 Ω as the performance of MFC approached a steady state. Data were recorded as <1 mV variation in voltage. All experiments were conducted at room temperature (30 ± 1 °C) in duplicate. The power density was calculated based on the anode area as follows:

\begin{equation}
P = \frac{E_{\text{cell}}^2}{A_{\text{An}} R_{\text{ext}}}
\end{equation}
where $E_{\text{cell}}$ is the cell voltage, $R_{\text{ext}}$ is the external resistance, and $A_{\text{An}}$ is the anode project area. Coulombic efficiency over a period of time was calculated as

$$\varepsilon_{\text{Cb}} = \frac{8}{n} \int_0^{\Delta t} f_0 n_\text{An} \Delta \text{COD} \, dt$$

where $n_\text{An}$ is the volume of liquid in the anode chamber and $\Delta \text{COD}$ is the change in COD over time $t$.

**Structure Characterization of NG.** The NG structure was characterized by means of scanning electronic microscopy (SEM), transmission electronic microscopy (TEM), X-ray diffraction (XRD), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) and Fourier transformed infrared spectroscopy (FTIR), as described in Section 4 of the SI.

**RESULTS AND DISCUSSION**

**Performance of NG Assessed by Electrochemical Methods.** In electrochemical measurement system with O$_2$-saturated/free 0.1 M PBS solution, ORR activity and the associated pathway and durability of NG as cathode catalyst were investigated in comparison with Pt/C counterpart.

CV and linear polarization experiments were carried out to understand the occurrence of ORR on NG cathode. According to Section 5 of the SI, the results shown in SI Figure S1 suggested that the as-prepared NG was able to catalyze the ORR in neutral pH medium. The linear polarization experiments as shown in SI Figure S2 confirmed this suggestion.

Further, the onset potential of ORR on NG observed from the ORR curves was 0.23 V vs Ag/AgCl as displayed in SI Figure S2a. It was more negative than that on Pt/C with value of 0.36 V vs Ag/AgCl (shown in SI Figure S2b), while more positive than the reported value ($-0.04$ V vs Ag/AgCl). As described in Section 6 of the SI, when both the anode composition and cell configuration are fixed, the onset potential that represents the performance of cathode would determine the performance of overall cell system. Therefore, the more positive onset potential of as-prepared NG would result in a larger cell voltage.

The pathway of ORR was explored also on the basis of the polarization curves. The inset of SI Figure S2 profiled the corresponding K-L plots, which demonstrated the inverse current density ($j^{-1}$) as a function of the inverse of square root of the rotation rate ($\omega^{-1/2}$) at different potential values. Unexceptionally, the K-L plots had good linearity with parallelism, suggesting a first-order dependence of O$_2$ kinetics. Meanwhile, the electron transfer number for ORR was calculated to be 3.87 ± 0.04 (Section 3 of the SI), which demonstrated that ORR on NG cathode dominantly proceeded through a 4-electron reaction pathway. This result appeared to be different from the previous reported value of 3.69 of NG, which indicated that ORR on NG proceeded via a combined pathway involving 2-electron and 4-electron processes. The number of electron transfer of state-of-art Pt/C was calculated to be 3.93 ± 0.03, which was slightly larger than that of NG.

Potential-dependent H$_2$O$_2$ formation during ORR process is complementary to K-L plots for assessing the reaction pathway within the full potential range. The results of polarization curves of RRDE tests are illustrated in Figure 1. Although the onset potential of NG was more negative than that of Pt/C, the current was relatively close (Figure 1a). According to the ring current shown in the upper half of Figure 1a, the H$_2$O$_2$ yield in percentage as a function of potential was displayed in Figure 1b (the right axis). Clearly, only a trace amount of H$_2$O$_2$ was formed on both Pt/C and NG. The corresponding numbers of electron transfer for ORR based on the yield percentage of H$_2$O$_2$ were calculated and are shown in Figure 2b (the left axis). The potential-coursed curve of the number of electron transfer of Pt/C depicted a 4-electron reaction for ORR as expected. The number of electron transfer for NG was also...
close to 4.0 (over 3.83 in the whole potential range), which indicated similar ORR activity to Pt/C.

Durability test of NG was performed by means of potential cycling in the range from $-0.3$ to $0.1$ V vs Ag/AgCl in the presence of O$_2$. Figure 2a shows that after 5000 potential cycles, the current density of ORR on NG slightly declined, but the onset potential still remained. According to the polarization curves in Figure 2b and the corresponding K–L plots shown inset, the number of electron transfer of ORR on NG after the durability test of 5000 cycles stood at 3.79 $\pm$ 0.03, showing that NG potentially remained a 4-electron reaction for ORR.

**Performance of MFC Equipped with NG Cathode.**

Following the electrochemical measurements, the performance of NG as cathode catalyst was further evaluated in real MFCs in terms of power generation (cell voltage and power density). For comparison, the same investigation was carried out on the MFCs equipped with Pt/C.

To differentiate the performance of cathode and anode in MFCs, the variation of electrode potential with current density was further monitored and the results were displayed in Figure 3a. It was shown that the anode potentials for both NG-MFC and Pt/C-MFC were almost the same as each other due to the same electrode materials and inoculum used in anode chamber, suggesting that the power generation of MFCs was determined by the performance of cathode. Furthermore, the profiles of cathode potential showed similar tendency depending on the current density for both NG and Pt/C. Besides the nature of catalysts, many parameters including DO concentration could influence the ORR. The DO concentration of catholytes for both NG-MFCs and Pt/C-MFCs were monitored and illustrated in SI Figure S3. The same concentration level of saturated DO indicated that it did not limit the ORR activity in the present system. Hence, the results of Figure 3a suggested that the NG cathode possessed a comparable performance with Pt/C cathode. Therefore, it could be presumed that the performance of NG-MFCs would be comparable with Pt/C-MFC.

The results of power density NG-MFCs and Pt/C-MFCs are shown in Figure 3b. Polarization data revealed that the maximum power density generated was 776 $\pm$ 12 mW m$^{-2}$ in NG-MFCs, whereas 750 $\pm$ 19 mW m$^{-2}$ in Pt/C-MFCs. The Coulombic efficiencies in NG-MFCs and Pt/C-MFCs were calculated to be 76.32 $\pm$ 1.67% and 70.72 $\pm$ 0.92%, respectively. It could be seen that both maximum power density and Coulombic efficiency of NG-MFCs were larger than those of Pt/C-MFCs.

The results of long-time stability of the two MFCs are presented in Figure 4. Prior to the sharp deterioration in each cycle as shown in Figure 4a, the voltage value of NG-MFC was stable, decreasing quite slowly from 650 mV to 500 mV with an average value of 590 mV. Similarly, the voltage value of Pt/C-MFC decreased gradually from 610 mV to 570 mV with an average value of 580 mV. It appeared that the NG-MFCs generated a competitive voltage to the Pt/C-MFCs. Furthermore, Figure 4a shows the time course of voltage output of NG-MFCs and Pt/C-MFCs within seven cycles spanning 2200 h (over 90 days). The voltage production cycles were reproducible within seven feeding cycles for both NG-MFCs and Pt/C-MFCs, suggesting an excellent stability of NG used as cathode in MFCs for a long period. Figure 4b shows the variation in the maximum power density through the 90 day operation. The maximum power density of NG-MFCs decreased from 776 mW m$^{-2}$ to 717 mW m$^{-2}$ after seven cycles, corresponding to 7.6% deterioration. A decrease of 8.8 $\pm$ 0.8% of maximum power density of NG-MFCs was reported after 70 day operation in previous study. Likewise, the maximum power density of Pt/C-MFCs declined from 750 mW m$^{-2}$ to 700 mW m$^{-2}$ after seven cycles, corresponding to 6.7% deterioration. Therefore the NG-MFCs displayed comparable performance to Pt/C-MFCs in terms of cell
Besides providing active sites for O\(_2\) adsorption and electron transfer, the O species also probably influence the long-time stability of NG in neutral pH medium. The result of FTIR spectroscopy of NG is shown in Figure 5b. It illustrates that N and O species were present in the forms of C–N groups and O–H groups, respectively. The C–N groups are basic with negative electrons, whereas the O–H groups, acidic with positive electrons. The improvement of long-time stability of NG can be well understood simply from the properties of electrostatic force brought by the two types of surface groups.

As illustrated in Scheme 1, a conceptual explanation of the effect of O–H groups on the long-time stability of NG was proposed. When the NG with low content of O–H groups is immersed in proton-rich medium, the basic C–N groups, with negative electrostatic force, would be favorably attacked by the protons with opposite electric charges because of electrostatic attraction. As a result, the C–N groups that are widely considered as active centers for ORR are destroyed in a long run, and thus the long-time activity gradually declines. By contrast, the NG with high content of acidic O–H groups potentially improves the proton-tolerance ability during a long-time operation. It can be seen from Scheme 1 that, as the acidic O–H groups are densely distributed around the C–N groups, the attack of proton to C–N group can be obstructed owing to the repelling force between the O–H groups and the protons with the same positive electrostatic forces. Hence, the C–N groups are protected by the O–H groups around them and left unattacked.

**Scheme 1. Scheme for the Improvement of Long-Time Activity of NG by O–H Groups**

![Scheme 1. Scheme for the Improvement of Long-Time Activity of NG by O–H Groups](image-url)
undestroyed. Obviously, the present NG is capable of keeping its activity after a longer time in proton-rich medium to well prolong the stability of NG cathode in MFCs.

**REMARKS**

This study has demonstrated that the electrochemical measurements serve to provide valuable thermodynamic and kinetic information of ORR on NG cathode, and are suggestive of its desirable performance as cathode catalyst in MFCs, while the performance assessment in real MFCs is confirmative and determinate. A combination of the performances during electrochemical measurements and in real MFCs has demonstrated the stable power generation of NG-MFCs, which serves to guarantee the feasibility using NG as cathode catalyst in MFCs for potential large-scale application. Also, the present findings provide a clue for further improvement of NG catalysts by adjusting the content of N species and O species.

Since the performance of NG as cathode is established in neutral media, it is of significance for sustainable recovery of biomass energy in wastewater treatment by means of MFCs. A large amount of domestic wastewater is generated through the world. For example, over 46 billion cubic meters in the U.S. and 40 billion cubic meters in China are released each year. Due to the neutral pH value of domestic wastewater, it is great promising in fueling MFCs. In turn, the electricity recovered from the wastewater by MFCs is believed to significantly contribute to the electricity recovered from the wastewater by MFCs. Thus, the success in this study in demonstrating the high and sustained performance of NG at neutral pH aids to advance the MFCs toward the recovery of biomass energy from the domestic wastewater in a sustainable manner.

**ASSOCIATED CONTENT**

* Supporting Information
Sections 1–7, Scheme S1, Figures S1–S6, Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

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**REFERENCES**


