Electrochemically exfoliated graphene anodes with enhanced biocurrent production in single-chamber air-breathing microbial fuel cells

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ABSTRACT

Microbial fuel cells (MFCs) present promising options for environmentally sustainable power generation especially in conjunction with waste water treatment. However, major challenges remain including low power density, difficult scale-up, and durability of the cell components. This study reports enhanced biocurrent production in a membrane-free MFC, using graphene microsheets (GNs) as anode and MnOx catalyzed air cathode. The GNs are produced by ionic liquid assisted simultaneous anodic and cathodic electrochemical exfoliation of iso-molded graphite electrodes. The GNs produced by anodic exfoliation increase the MFC peak power density by over 300% compared to plain carbon cloth (i.e., 2.85 W m$^{-2}$ vs 0.66 W m$^{-2}$, respectively), and by 90% compared to conventional carbon black (i.e., Vulcan XC-72) anode. These results exceed previously reported power densities for graphene-containing MFC anodes. The fuel cell polarization results are corroborated by electrochemical impedance spectroscopy indicating three times lower charge transfer resistance for the GN anode. Material characterizations suggest that the best performing GN samples were of relatively smaller size (≈500 nm), with higher levels of ionic liquid induced surface functionalization during the electrochemical exfoliation process.

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1. Introduction

Microbial fuel cells (MFCs) are devices capable of electricity generation by employing bacteria to decompose organic compounds through a series of electrochemical reactions (Logan et al., 2006). Perhaps, the most relevant and immediate implication of MFCs lies in the wastewater treatment for simultaneous power generation and degradation of organic matter (Zhuang et al., 2012). Studies have shown that wastewater in a modern treatment plant may contain as much as nine times the energy used for its treatment (Shizas and Bagley, 2004). Such significant amount of stored energy could potentially be harnessed by MFCs. Although their power output has not yet reached levels required for self-sustaining a wastewater treatment plant, they could theoretically be used to offset energy consumption throughout the treatment process (Wang and Ren, 2013); especially considering the energy-intense nature of the conventional wastewater treatment operations such as aeration (Rabaey and Verstraete, 2005).

Under anaerobic conditions, a typical MFC process involves bacterial decomposition of organic matter, from organic-rich sources such as wastewater, which produces electrons that are passed on to the anode, and transferred through an external circuit towards an electron acceptor at the cathode, usually air or oxygen, where reduction occurs (Liu et al., 2004). There are several technical challenges that need to be addressed for practical uses of MFCs in large-scale renewable energy production. One aspect is the slow degradation kinetics of organic compounds, and consequently the much low power output of MFCs compared to hydrogen fuel cells (Bullen et al., 2006; Khera and Chandra, 2012).

Enhancing the MFCs’ performance requires further fundamental research on their design, and substrate composition along with exploring novel electrode materials (Chen et al., 2015), and better understanding the underlying mechanism of the anode-bacteria interactions (Yates et al., 2012). The majority of research has been devoted to studying the anode at the expense of the cathode and other components. This is mainly due to the fact that the organic matter oxidation on the anode is typically the main bottleneck, and any improvement on the anodic interactions translates into further increase in the overall efficiency (Schroder, 2012; Zhao et al., 2005).
Among the materials used as the anode for MFCs, carbon-based electrodes are the most promising due to their chemical inertness, high surface area, and low cost (Minteer et al., 2012). Synthesis of unique carbon nanomaterials such as porous carbons, fullerenes, carbon nanotubes (CNTs) and graphene has had a great impact on the research and development of high-performance energy storage devices (Candelaria et al., 2012). Particularly graphene is employed in many areas of energy and environmental research owing to its numerous unique properties (Kemp et al., 2013; Zhao et al., 2012). This includes large surface area, high charge mobility, strong mechanical properties, and excellent thermal conductivity, while showing remarkable biocompatibility with potential applications in biomedical engineering and biotechnology (Gurunathan et al., 2013; Song et al., 2016). More importantly, chemical treatment of graphene, which tailors its electronic and surface properties, significantly broadens its applications in cross-disciplinary areas with structural doping and surface functionalization (Chen et al., 2013; Machado and Serp, 2012). Thus, graphene can be a promising alternative to the traditional MFC anodes such as graphite rod, activated carbon and carbon cloth (Wang et al., 2013).

Despite the extensive research on MFC electrode materials, the development of graphene-based anodes is still in the early stages, and the reports on related studies are relatively sparse in literature. Liu et al. (2012) examined the electrochemical performance of a graphene-modified carbon cloth anode, and reported a 2.7-fold improvement in power density compared to a plain carbon cloth anode. Xiao et al. (2012) observed that graphene-modified anodes can produce twice as much power as activated carbon anodes. Zhang et al. (2011) attributed the increase in power generation to the high surface area of the graphene-based anode, and subsequent promotion of microbial loading on the anode compared to an unmodified stainless steel mesh. Hou et al. synthesized graphene composites with polyaniline (PANI) reporting a maximum power density of 1.4 W m\(^{-2}\), with a 40% improvement over reduced graphene oxide performance (Hou et al., 2013). Zhao et al. (2013) also modified graphene’s surface using ionic liquids, and reported beneficial effects with power densities exceeding 0.6 W m\(^{-2}\). Finally, Tang and coworkers obtained 1.7-fold higher power density with graphene sheets produced via graphite exfoliation in ammonium sulfate solutions (Tang et al., 2015).

We have recently introduced high-throughput graphite electrochemical exfoliation techniques for GN production using aprotic ionic liquids with prospects for in-situ functionalization of GNs (Taheri Najafabadi and Gyenge, 2014) along with simultaneous exfoliation of both anode and cathode (Taheri Najafabadi and Gyenge, 2015). Our approach offers a number of potential advantages including ease of operation, control over the entire synthesis process by optimizing the electrochemical cell voltage, elimination of harsh chemical oxidizers/reducers and fast exfoliation rates at ambient pressure and temperature. This created an opportunity to examine our GN products produced by simultaneous anodic and cathodic graphite exfoliation, as anode materials in MFCs.

Fig. 1a depicts the schematic diagram of the single-chamber MFC configuration used in the present work with biofilm growth on the graphene-based anode, and oxygen reduction on the air-cathode catalyzed by MnO\(_x\) along with images of the units constructed and tested (Fig. 1b and c). Removing the ion exchange membrane (Fig. 1) brings about substantial benefits by lowering the cost and improving the power density due to the overall lower ohmic voltage loss in the cell. Furthermore, the possibility of membrane fouling is also eliminated (Rismani-Yazdi et al., 2008). Employing non-precious metal catalysts, namely manganese oxide, for the oxygen reduction reaction (ORR), further contributes to cost reductions and improving the practical feasibility (Zhang et al., 2009).

2. Materials and methods

2.1. Graphene synthesis and characterization

GNs were synthesized using the simultaneous anodic and cathodic graphite electrochemical exfoliation procedure developed by us previously (Taheri Najafabadi and Gyenge, 2014, 2015). The exfoliation experiments were conducted in a divided H-cell separated by a semi-permeable ceramic frit (Pine Research Instrumentation – RRPG060), High-purity iso-molded graphite rods with 6.35 mm diameter and 4 cm effective length exposed to the electrolyte (Graphite Store – 99.99% purity), the electrochemical cell voltage, manganese oxide, for the oxygen reduction reaction (ORR), further contributes to cost reductions and improving the practical feasibility (Zhang et al., 2009).

![Fig. 1.](image_url)
The first signs of exfoliation occurred at potentials of around 10–12 V in the first minutes, and the extent of exfoliation increased with elevating the applied cell voltages. There is, however, a trade-off between current and the quality of exfoliated products. We found that applying higher cell voltages (> 20 V) leads to more IL reactivity and aggressive destruction of the graphitic rod, releasing unaffected graphite particles into the electrolyte; whereas voltages lower than 10 V failed to produce significant exfoliation yields during 2 h of experiments. Thus, an applied voltage of 15 V provides a balance between exfoliation rate and GN product quality. Further details about these effects have been provided in our previous publications (Taheri Najafabadi and Gyenge, 2014, 2015). Additional information regarding the controlled single-electrode exfoliation studies and the synergistic effect of simultaneous exfoliation are presented in the Supplementary Material (Fig. S1). Importantly, the sole purpose of using the divided cell here was to collect and analyze separately the anodic and cathodic exfoliates. Otherwise, as we explored in our previous work, the exfoliation could happen at voltages as low as 5 V in an undivided cell due to lower ohmic voltage losses (Taheri Najafabadi and Gyenge, 2015).

For characterization, transmission electron microscopy (TEM) images were obtained with a Tecnai G2 microscope at acceleration voltages of 20–200 kV. TEM grids were covered with 5 microliters of the diluted products in water/IPA mixtures to provide more facile evaporation and product visibility in imaging. Field emission scanning electron microscopy (FESEM) was performed using a Hitachi S-4700 FESEM at 3–30 kV. X-ray photoelectron spectroscopy (XPS) was done using Leybold Max200 for the surface elemental composition and binding energy analysis with five channeltrons, using an unmonochromated Mg Ka X-ray source (1253.6 eV). A Micromeritics surface area analyzer (ASAP2020) was utilized to determine the specific surface area of the electro-exfoliated flakes using nitrogen sorption isotherms at 77 K. The Raman spectra were recorded by a LabRAM ARAMIS Microscope Raman spectrometer with an argon-ion laser at an excitation wavelength of 632 nm. The samples deposited on aluminum substrates were exposed to the laser beam (1 μm diameter at the focus) with the exposure time of 1 s. The data was collected from 15 to 30 different regions of each sample to ensure the consistency of the results. All the measurements were taken at room temperature without special mention.

2.2. MFC construction and characterization

Fig. 3 shows the overview of the anode preparation and inoculation process. All bacteria cells in this experiment were derived from a commercial agar slant containing pure Escherichia coli B (Merlan Scientific – 155070), an easily available exoelectrogenic bacterium especially for carbonaceous substrates with polytetrafluoroethylene-based binders (Qiao et al., 2008; Zhang et al., 2007). A small sample of cells was removed from the source using an inoculum loop, previously sterilized under a Bunsen burner flame, and streaked onto a petri dish containing tryptic soy agar nutrient (Sigma-Aldrich – 22091). The petri dish was sealed and incubated at 310 K for 24 h. Further scale-up to a liquid culture was performed by transferring cells from the petri dish into a shake flask containing 50 ml of 30 g L⁻¹ sterile tryptic soy broth (Sigma-Aldrich – 22092). The cells were agitated at 310 K and 150 RPM for 24 h with consecutive dilutions via stock tryptic soy broth until an optical density reading of 0.8 at 600 nm (equivalent to approximately 6.2 × 10⁶ cell mL⁻¹) was achieved using a spectrophotometer. Subsequently, the assembled electrode inoculation brace was submerged into the solution, and incubated at 310 K for 18 h. The inoculated sample-modified anode was then used for MFC operations.

As for the anode preparation itself, GNs were dispersed in isopropanol (IPA), then mixed with 5 wt% Nafion solution (Sigma-Aldrich – 274704) as binder, and sonicated for 1 h to form a homogenous solution using a tabletop ultrasonic cleaner (VWR Scientific – B3500). The dry Nafion to carbon weight ratio on the electrode was set at 15 wt%. The ink solutions were subsequently deposited on carbon cloth (Fuel Cell Earth – CCP40) with a machine sprayer, and dried in the oven at 333 K for 24 h. For performance comparison, other MFC anodes with porous carbon black (Cabot Corporation – Vulcan XC-72), and CNTs (Sigma-Aldrich – 724769) were prepared with the same loading of 2 mg cm⁻² on carbon cloth (CC). For comparison, an unmodified CC anode was also tested in the MFC. All the chemicals were of the analytical grade, and double-distilled water was used during all of the preparation steps.

After anode inoculation, the membrane-less MFC with a 20 mL empty bed volume (EBV) was assembled. The inoculated anode was secured on one end of the cell with the circular exposed surface of 2.5 cm diameter. On the other side, the manganese oxide air cathode (Gaskatel – 82010) was sandwiched between two plates with the same exposed diameter of 2.5 cm diameter. The inter-electrode gap was 4 cm. Next, the nutrient solution was...
pipetted into the cell; which was prepared by mixing the following volumetric proportions of 50% tryptic soy broth (Sigma-Aldrich – 22092), 49.125% 0.1 M phosphate buffer solution with pH 7.4 (Sigma-Aldrich – P4417), and 0.875% vitamin/mineral solution mix (Sigma-Aldrich – K3129), along with powdered glucose added to reach a final concentration of 1 g L\(^{-1}\). The solution was purged with nitrogen for 15 min to ensure the complete oxygen removal, and the sampling ports were sealed with parafilm afterwards.

With the assembled MFCs (Fig. 1), electrochemical characterizations were carried out using a potentiostat/galvanostat (Princeton Applied Research-263A) equipped with electrochemical impedance spectroscopy (EIS) capability. Further details regarding the EIS analysis and modeling is discussed in the Supplementary Material, Section 2. Polarization curves were measured at 293 K with sweep rate of 5 mV s\(^{-1}\) starting from the open circuit potential (OCP). The power output and current were normalized

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**Fig. 3.** Graphical representation of the graphene anode preparation and inoculation process.

**Fig. 4.** MFC (a) Polarization and (b) power density curves for anodic and cathodic GNs compared to carbon cloth. Potential sweep rate of 5 mV s\(^{-1}\) starting from the open circuit potential. 293 K. Error bars represent the reproducibility over three-days of repeated testing.
either to the electrode geometric area \((W \text{ m}^{-2}, A \text{ m}^{-2})\) or the MFC empty bed volume \((W \text{ m}^{-3}, A \text{ m}^{-3})\). Galvanostatic polarization experiments were also performed to evaluate the longer-term durability of the MFC.

### 3. Results and discussion

Fig. 4 shows the MFC polarization and power density curves using the anodic and cathodic exfoliated GNs compared to the plain carbon cloth (CC) as MFC anodes. The error bars indicate the performance reproducibility in the course of three-day repeated testing. Both anodic and cathodic GN exfoliated products showed superior polarization performance compared to the conventional CC electrode. In terms of maximum current drawn from the MFC (Fig. 4a), the GN anodic product generated a maximum of \(10.25 \text{ A m}^{-2}\) at \(50 \text{ mV}\) while the cathodic GNs produced \(9.5 \text{ A m}^{-2}\) at \(50 \text{ mV}\). These currents are \(310\%\) and \(280\%\) higher than those produced on CC at the same potential (\(2.5 \text{ A m}^{-2}\) at \(50 \text{ mV}\)). Constant current MFC polarization experiments were also performed and repeated six to eight times in 20–30 min batches during three days of testing with good reproducibility (see Supplementary Material, Fig. S2). The cell voltage decrease of about \(1–1.5 \text{ mV h}^{-1}\) (Fig. S2) is due mainly to glucose consumption during batch operation mode.

As for the maximum power density (Fig. 4b), the anodic GN exfoliates emerged as the most promising candidate with \(2.85 \text{ W m}^{-2}\) at \(530 \text{ mV}\) compared to the cathodic GNs (\(2.3 \text{ W m}^{-2}\) at \(450 \text{ mV}\)). The maximum power densities were \(330\%\) and \(250\%\) higher with the GN electrodes compared to the bare CC electrode (\(0.66 \text{ W m}^{-2}\) at \(506 \text{ mV}\)), respectively. It is inherently difficult to compare the present results with literature reported power densities due to different experimental conditions and cell designs (e.g., divided vs. undivided cell). Considering this caveat, the maximum power densities reported in Fig. 4b are significantly higher than literature results using diverse graphene based anodes. For example, Liu et al. (2012) used a dual-chamber MFC construction separated by a Nafion membrane, and reported a maximum of \(2.7\)-fold improvement in power density for reduced graphene oxide (rGO) anode compared to a plain carbon cloth anode. Similarly, while Hou et al. (2013) aimed at improving the rGO performance by adding PANI (\(40\%\) improvement), their maximum power density of \(1.4 \text{ W m}^{-2}\) in a two-chamber MFC is still less than half of what we achieved in our work. More comprehensive comparison with literature is provided in Table S1 (Supplementary Material).

In order to explain the differences between the MFC polarization performance using the anodic and cathodic GN exfoliates, the surface chemistries of the two types of graphene must be considered. The anode surface chemistry influences the electron...
transfer rate and biofilm development (Santoro et al., 2015). Inducing hydrophilic functional groups on the electrode surface has been shown to improve the MFC performance by enhancing bacteria attachment to the electrode, and further promoting electron transfer from bacteria to the surface (Cheng and Logan, 2007). Recently, Pinto et al. (2015) also reported improved biocompatibility for graphene compounds with higher oxidation levels and smaller particle sizes.

Here, based on extensive characterization of the exfoliation products presented in Fig. 5, we found distinctive differences in functionalization/disorder between the anodic and cathodic GNs, which could explain the MFC performance trends shown in Fig. 4. It can be inferred from both XPS survey scans (see Supplementary Material, Fig. S3a) and C 1 s narrow scan analyses (Fig. 5a and b) that the anodic products contain relatively higher oxygen contents and remnants of the intercalating ions (O/C: 10%, F/C: 3%); whereas the cathodic products are less functionalized/oxidized (O/C: 4%, N/C: 2%). Particularly, the two carbon–oxygen peaks (C–O and C=O) located at 286.5 eV and 287.9 eV, respectively (Phaner-Goutorbe et al., 1994), had a total contribution of 32% to the C1s peak at the anode (Fig. 5b) while this number was significantly lower for the cathodic products (18%, Fig. 5a).

TEM imaging (Fig. 5c and d) showed that the cathodic exfoliates are relatively defect-free (demonstrated by the Moire Fig. 6. Characterization of anodic GN and Vulcan XC-72 MFC anodes, respectively. (a) MFC polarization and power density curves, respectively. 293 K. Sweep rate of 5 mV s⁻¹ starting from OCP. (b) EIS spectra at OCP in the frequency range of 10⁵–10⁻³ Hz. (d) Nitrogen adsorption isotherms at 77 K. (e) SEM images of the MFC anodes: anodic GN/CC, Carbon cloth (CC) and Vulcan XC-72/CC.
patterns in Fig. 5c (Miller et al., 2010)), and larger in size (~800 nm compared to ~500 nm for the anodic products based on 500 images – more in Fig. S3b, Supplementary Material. Furthermore, the anodic products contained more irregularities, including multiple structural curls and edge deformations (resembling intensified edge folding patterns in Fig. 5d (Meyer et al., 2007)). This was also reflected by the lower G/D peak ratios for the anodic products in Raman analysis (Fig. 5e) which generally represents the disorder level in graphene (Ferrari, 2007).

Consequently, the aforementioned physicochemical differences between the two types of exfoliates demonstrate that the more IL-functionalized and relatively smaller anodic products exhibit better MFC polarization performance compared to the cathodic exfoliates (Fig. 4). It is also worth noting that our characterizations, including the 2D peak (second order of D peak) patterns (Fig. 5f), also confirm the successful production of ultrathin GNs. The evolution of the 2D peak is a clear indication of structural transformation to graphene via electrochemical exfoliation of the graphitic electrodes (Ferrari et al., 2006).

Lastly, to compare the electrochemical performance of the GN-modified anodes with other porous carbon materials, Vulcan XC72 supported on CC was also tested under similar conditions (see Section 2.2). Fig. 6a compare the MFC polarization and power density curves using anodically exfoliated GN and Vulcan XC72 anode, respectively. The OCP of the cell with Vulcan XC72 anode was only 0.67 V vs. over 0.9 V for GN. The excellent bioelectrocatalytic activity of the anodic GN compared to Vulcan is also reflected in the approximately 90% higher MFC power density (Fig. 6a). This observation is corroborated by the Nyquist plot (Fig. 6b) obtained in the frequency range of 10^2–10^1 Hz. The Supplementary Material (Section 2) provides further details regarding the recording and analysis of the EIS data. It is noted that the plot reveals only the anode impedance spectra due to the comparatively much faster charge transfer on the air cathode catalyzed by MnOx. Furthermore, the charge transfer resistance of the Vulcan/CC anode was over three times higher compared to the anodic GN exfoliate/CC (i.e., 2830.9 Ω cm^2 vs. 897.8 Ω cm^2).

Additionally, in the low frequency region the straight line of the impedance spectra is characteristic for a diffusion-limiting step (referred to as the Warburg impedance). Nutrient diffusion within the porous anode structure could also constitute a limiting factor at higher current densities corresponding to cell voltages lower than 0.4 V. In this regard, the morphology differences between the GN/CC and Vulcan/CC electrodes are important. A comparison of the N_2 absorption isotherms (Fig. 6c) reveal different morphologies for the two investigated anodes. The type IV isotherm for Vulcan XC72 is characteristic for its well-known meso and micro-porous structure, whereas the isotherm for the anodic GN exfoliate is consistent with multilayer adsorption on a non-porous surface. The SEM images in Fig. 6d show the GN flakes (with wrinkles/irregular shapes) coated on CC, contrasting the dense, compact coating of the Vulcan spherical particles. Thus, it is plausible to assume that the more open morphology of the GN/CC anode could have an impact as well on the MFC polarization behavior with regard to nutrient diffusion into the porous anode structure and cell growth.

4. Conclusions

Graphene microsheets (GNs) produced by simultaneous anodic and cathodic electrochemical exfoliation of iso-molded graphite, were investigated as anodes in microbial fuel cells. The anodically and cathodically exfoliated GNs deposited on carbon cloth were comparatively studied in an undivided (i.e., without membrane or any other type of separator) MFC, paired with an MnOx catalyzed air cathode. The mildly functionalized GNs produced by the anodic exfoliation, increased by almost 330% the peak power density compared to the plain carbon cloth (i.e., 2.85 W m^-2 vs. 0.66 W m^-2 or 73 W m^-3 vs 17 W m^-3 on empty cell volume basis, respectively). The less functionalized, cathodically synthesized GNs, also displayed a 250% improvement in power density over carbon cloth. These results can be attributed to the desirable surface chemistry of the mildly-functionalized graphene which could enhance bacterial attachment to the electrode and facilitate the electron transfer. Electrochemical impedance results demonstrated much lower charge transfer resistance for the GN/carbon cloth anode. The encouraging power densities obtained with the electrochemically exfoliated GN anodes, in conjunction with the simple undivided cell design and precious-metal free oxygen electrode catalysts employed, warrant further investigations of this system especially with regard to long-term durability in continuous operation mode.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.02.054.

References
