Facile reconstruction of microbial fuel cell (MFC) anode with enhanced exoelectrogens selection for intensified electricity generation

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A B S T R A C T

The present work emphasized on the enhancement of microbial fuel cell (MFC) anode through the utilization of conductive polymer. The conductive polymer, poly(3,4-ethylenedioxythiophene) (PEDOT) was coated with varied concentrations onto graphite felt base anodes. The findings demonstrated that the optimum loading of 2.5 mg/cm² recorded maximum current density of 3.5 A/m² and coulombic efficiency of 51%. Higher loading of PEDOT enhanced the electrochemical characteristics of the anodes but exhibited unfavorable functionality. The charge transfer resistance of the modified anodes, $R_a$ decreased significantly compared to the control anode after biofilm formation. The successful application of palm oil mill effluent (POME) wastewater as substrate indicates that the optimum anode was effective in degrading high organic wastewater. Exoelectrogens were found to be distributed mainly on the anodic biofilm. The microbial diversity of the anodes varied greatly from the inoculum and Geobacter was identified as the prevailing exoelectrogen responsible for the power generation.

Introduction

Industrial effluents have been the sources for major aquatic contaminations persistently. These contaminations drastically limit the availability of the fresh water bodies and led to water scarcity. This underlines the need for more sustainable and efficient wastewater treatment technology. Wastewater with very high organic loading are recklessly discharged due to the rapid growth in agro-based industries. Though they are not as lethal as other industrial waste, this wastewater heavily burden the treatment processes. These organic compounds are usually complex and hard to treat due to its diverse constituent. Palm oil mill is one such massive agriculture industry that produces wastewater with resilient fibrous organics (~50,000–100,000 mg/L COD), with significant presence of oil and grease [1,2]. Conventionally, such organics are handled successfully through anaerobic digestion method where it eliminates ~90% of the organic content and simultaneously delivers energy-rich methane gas. However, the remaining 10% of organic load are released into the water bodies as pollutant. Hence, the industry is keen in developing an alternative method that could overcome this longstanding problem with a feasible solution. Microbial fuel cell (MFC) is among the best available alternative foreseen for handling these effluents.

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MFC is a prospective technology that accommodates both treatment and energy recovery. They are capable of transforming chemical energy stored in the pollutants into electrical energy. Their key advantages are viability in utilizing low organic content in anaerobic condition, low sludge generation and the ability to operate at lower temperature compared to conventional biological practices [3]. These essential features make MFC a strong nominee as supplementary treatment after anaerobic digester [4,5]. However, development in MFC is hindered due to its limited power production and high installation cost. Many researchers are motivated to find a better approach to overhaul these limitations. Most of their attempt concentrated on reactor configuration, optimization of operational parameters, materials etc. [6—9]. When these limitations were perceived intrinsically, it is irrefutable that MFC components like anodes, separators and cathodes are crucial in deciding its efficiency. The anode plays a vital role in electricity generation as they provide a platform for biofilm cultivation and effective electron transfer. In order to facilitate strong biofilm formation, the materials should possess characteristics like biocompatibility, conductivity and durability. Graphite felt was conventionally employed due to its high surface area and open structure, leading to improved biofilm formation and electrochemical reactivity in addition to their cost-effectiveness [10,11]. Nevertheless, the performance of these materials is not flawless and therefore needs continuous improvement [12].

Numerous alterations or modifications were carried out by researchers in the past to escalate anode functionality [13]. Heat and acid treatment, metal and metal oxide and composite materials are some of the most common methods of enrichment that has been successfully executed. Coating with conductive polymer is also one of the growing trend that instigates the application of polypropylene, polyanilines and their composites as anode enhancer [14—17]. The sulfur-containing conductive polymer, poly(3,4-ethylenedioxythiophene) (PEDOT) is considered as an emerging alternative for the improvement of the MFC anode [18,19].

Previous researches have successfully applied electro-polymerized and vapor-phase polymerized PEDOT onto MFC anodes [18—20]. However, this method is only suitable for thin or two-dimensional (2D) anode materials [21]. Hence, in order to study the incorporation of PEDOT onto three-dimensional (3D) anodes, the present study focused on synthesizing and applying in-situ polymerized PEDOT onto graphite felt. 3D anodes have higher surface area that facilitates better biofilm growth and adhesion. The contribution of PEDOT towards the MFC performance was evaluated from various aspects in terms of electrochemical reactivity, electricity generation and efficiency. Additionally, the distribution of exoelectrogens and biofilm community that governs the performance of MFC system were presented in this study.

Material and methods

Electrode synthesis

Graphite felts of 4 x 4 cm dimension were used as base anodes throughout this study. Graphite felts (Hao Shi Carbon Fiber Co. Ltd, China) were cleaned by soaking in 0.1 M HCl to remove loose fibres and surface impurities. The synthesis of PEDOT and anode modifications was detailed in our previous report [22]. Briefly, the synthesized PEDOT and a surfactant, dioctyl sulfo succinate sodium salt (SDBS, 1%) were sonicated in deionized water (20 mL) for 2 h. The amount of PEDOT was varied to produce three different concentration of PEDOT that corresponds to the loading of 2.5, 5.0 and 7.5 mg/cm². A dip-dry approach was applied to load the PEDOT onto the graphite felts. The cut graphite felts were dipped into the dispersed PEDOT and dried at 80 °C. The processes were repeated until the PEDOT solution was completely absorbed by the anodes. A base anode without PEDOT was maintained as a control in subsequent experiments and denoted as GF. The corresponding modified anodes for the loading of 2.5, 5.0 and 7.5 mg/cm² of PEDOT are designated as GF-P, GF-2P and GF-3P respectively.

Characterizations of modified anodes

Field Emission Scanning Electron Microscope (FESEM) (Quanta 450 FEG, FEI) was employed for identifying the surface morphology and PEDOT attachment onto the anodes. The electrochemical reactivity of the modified anodes were analyzed with both cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) using three-electrode mode in an electrochemical cell (AUTOLAB PGSTAT280N, Metrohm B.V., Netherlands). The synthesized anode electrodes, Pt wire and Ag/AgCl were used as working, counter and reference electrode respectively. The scan rate was set at 10 mV/s for CV while the EIS measurements were conducted between 100 kHz and 1 mHz and a stimulus of 10 mV was employed at open circuit potential. The electrolyte used for both analysis was 2.5 mM ferricyanide/ferrocyanide ([Fe(CN)6]3-/4-) in 0.5 M potassium nitrate (KNO3) as supporting electrolyte.

MFC setup and analyses

A dual-chamber MFC reactor with effective volume of 150 mL each was used throughout this research. The chambers were separated by a Nafion 117 proton exchange membrane (Sigma–Aldrich, USA). The control and modified anodes were applied as the anode while a carbon cloth was used as cathode for all the experiments. The dimensions for both anodes and cathode were fixed at 4 cm x 4 cm. The MFC circuit was completed by an external resistor of 1 kΩ. The anodes were inoculated with sludge obtained from an anaerobic pond of palm oil mill effluent treatment (POME) plant (Seri Ulu Langat Palm Oil Mill, Dengkil, Malaysia). The anode chambers were initially filled with 1:1 ratio of sludge and sodium acetate tri-hydrate (1 g/L) in 0.1 M phosphate buffer solution (PBS) while the catholyte comprised of 0.1 M potassium ferricyanide (K3[Fe(CN)6]) in PBS. The voltage across the resistor was recorded and the electrolytes were replaced once the voltage dropped below 10% of its peak voltage. This step was repeated until significant voltage generation across the resistor was achieved. The anolyte was then replaced with acetate solution in PBS without the sludge. The MFC experiments were carried out in fed-batch mode and the voltage across the resistor was...
recorded using a digital multimeter with data acquisition module (UNI-T 61D, Uni-Trend Group Ltd., China). Both electrolytes were replenished after the voltage dropped below 50 mV. The degradation of carbon content corresponding to the voltage generation was examined through the concentration of chemical oxygen demand (COD). COD analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater [23]. The coulombic efficiency (CE) was computed based on the ratio of electrons converted to electricity to the theoretical available electrons in the anolyte. The expanded formula for CE calculation in a fed-batch condition is shown in following equation.

\[
CE(\%) = \frac{M \int_{0}^{t} Idt}{F \Delta COD} 
\]

where \( I \) is current produced (A), \( M \) is molecular weight of the oxygen, 32 (g/mol), \( F \) is Faraday’s constant, \( b \) is the number of electrons exchanged per mole of oxygen, 4, \( v_{an} \) is volume of liquid in the anode compartment (L), \( \Delta COD \) is change of COD concentration (g/L) at time \( t \) (s).

After three repeatable cycles of voltage generation, linear scanning voltammetry (LSV) was carried out at a scan rate of 1 mV/s to verify the maximum current and power density of the system. The potential of individual electrodes were obtained through the external resistance method. External resistors in the range of 100 k\( \Omega \) were connected to the terminals of the electrodes for a period of 10 min in order to achieve pseudo-steady state condition. The potential of anode and cathode were then recorded against Ag/AgCl reference electrode for each resistor.

The internal resistance components of the MFC systems were obtained through EIS analyses under the two-electrode mode. The analyses were carried out under a fixed external resistance of 1 k\( \Omega \) to emulate the MFC under working conditions and to ensure the stability of the systems [12,24]. The acquired plots were then fitted into the appropriate equivalent circuit using the NOVA 2.0 software (Metrohm, Autolab) as shown in Fig. A1 [25]. \( R_{ohm} \) denotes the ohmic resistance that includes the resistance of both anolyte and catholyte as well as the membrane. \( W \) symbolizes the Warburg impedance and is related to the semi-finite diffusion of the anode component. The CPE represents the constant phase element that arises from the double layer capacitance due to the charged species accumulating near the anodes [26]. The charge transfer resistance of the anode and cathode were expressed as \( R_{t,an} \) and \( R_{t,cat} \) respectively. Meanwhile, \( C_{c,an} \) and \( C_{c,cat} \) conveyed the capacitance of the cathode and diffusion respectively. \( R_{d} \) represented finite diffusion resistance. The total internal resistance, \( R_{int} \), were computed by summation of all the resistance components. A \( R_{ext} \) of 1 k\( \Omega \) is inserted in the equivalent circuit corresponded to the fixed external resistance connected to the MFC during analysis.

**High organic wastewater as substrate for MFC**

The adaptability of the modified anode towards wastewater with complex organics as substrate was examined by introducing diluted POME with an initial COD of ~350 mg/L as anolyte. The optimum anode was acclimatized with POME by gradually increasing the POME to acetate concentration (50%, 75% and 100% of POME). The performances and efficiencies were then compared between pure acetate and POME substrates.

**Determination of exoelectrogens distribution**

A simple experiment was carried out to reveal the distribution of exoelectrogens in both anodic biofilm and planktonic cells as planktonic cells are retained in fed-batch mode but not in continuous mode [27,28]. Hence, it is important to distinguish the distribution of exoelectrogens in order to determine the feasibility of this research in a continuous MFC system. The electron transfer from the anodic biofilm was explored by inserting an anode with established biofilm into a reactor containing fresh anolyte. Conversely, a new anode without biofilm was inserted into the reactor with the used electrolyte to determine the contribution from planktonic cell. The obtained voltage by these reactor systems were monitored for 48 h. Finally, 1% glutaldehyde (Sigma–Aldrich), a biocide was added into the reactor to distinguish the role of microbes and abiotic factor on the voltage generated by the system.

**Characterization of biofilm**

The attachments of biofilms on the anode were examined using FESEM. Samples of GF and GF-P with stable biofilm were extracted and fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4, Sigma–Aldrich) for more than 4 h. The pre-treated samples were then soaked overnight in osmium tetroxide solution (OsO4, Sigma–Aldrich). This was followed by ethanol dehydration through a series of concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 95, and 100%). Finally, the ethanol in the samples was substituted with acetone through gradual dilution. The samples were dried at critical point and gold sputtered before viewed in FESEM.

The biodiversity analysis was done by extracting DNAs from pieces of GF and GF-P anodes as well as from the inoculum using the PowerBiofilm™ DNA Extraction Kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA). DNAs’ integrity and quality were evaluated by agarose-gel electrophoresis and quantified using fluorometer (Qubit® 2.0, Thermo Fisher Scientific). The samples were sequenced on Illumina MiSeq sequencer (Illumina Inc., San Diego, USA). Operational taxonomic unit (OTU) clustering was performed using UCLUST algorithm and the percentage of similarity within the OTU was set to be ~97%. Taxonomy was then classified using RDP classifiers with the Greengenes Database release May 2013.

**Results and discussion**

**FESEM analysis**

Fig. 1 showed all the sample anodes experienced changes in morphology attributed to the addition of PEDOT. GF consists of disorganized web of fibers with diameter ranging from 10 to 14 \( \mu m \). For the GF-P sample, most particles are confined between the fibers while minority of them was directly immobilized on the fibers as discussed in our previous report [22].
However, at higher loading of PEDOT (GF-2P and GF-3P), the particles were found to be very closely integrated with fibers of GF. The high amount of PEDOT led to the individual graphite fibers to form a bunch, wrapping most of the pores and restricted the open structure of the GF.

**Electrochemical analysis**

Fig. A2 shows the obtained CV analysis for the control and modified anodes. The incorporation of PEDOT onto felt anodes surged the electrochemical activity drastically and resulted in larger peak current and area under the curve. This demonstrated that PEDOT aid in amplifying the electrochemical reactivity of these materials. It can be inferred that the optimum anode is the GF-3P as it exhibited the largest peak current and area under the curve. The CV also exhibited that PEDOT was well anchored onto the anodes and improved the electrochemical reactivity of the modified anodes. Redox peaks denoting graphite was also inferred on the voltammogram for all the tested anodes [29]. However, the redox peak separation intensified with higher PEDOT loading. These shifts are attributed by lag in current response against applied voltage. This incident illustrates that the presence of PEDOT particles inhibits the diffusion of the electrolyte into the felts and the effect is more prominent with the increase in PEDOT loading [30].

EIS analyses were performed to evaluate the internal resistance components of the anodes. Bode plots presented in Fig. A3(a) and (b) were fitted into a modified Randle’s equivalent circuit. It is evident from the plot that the GF anode demonstrated highest charge-transfer resistance, \( R_{ct} \) (18.3 \( \Omega \)) and subsequent addition of PEDOT reduces its internal resistance significantly. Both GF-P and GF-2P recorded a drop of 36.1% and 86.9%, reducing their \( R_{ct} \) to 11.7 \( \Omega \) and 2.4 \( \Omega \) respectively. Further increase in PEDOT loading (GF-3P) caused the \( R_{ct} \) to rise to 5.6 \( \Omega \). This shows that the lowest \( R_{ct} \) among the anodes are GF-2P and further increase in PEDOT loading resulted in an adverse condition by increasing the charge transfer resistance of the anode.

**MFC performance**

**Current and power density**

From the electrochemical analysis, the most enhanced electrochemical surface is GF-3P while GF-2P has the least charge-transfer resistance. However, the actual performance of the anodes can only be determined by applying them in a MFC system. Polarization and power density curves were computed to obtain a more comprehensive analysis of these anodes’ performance and are presented in Fig. 2. The control anode (GF) recorded a maximum current density of 0.48 \( A/m^2 \). On the other hand, the least addition of PEDOT (GF-P) drastically surged the current density to 3.50 \( A/m^2 \), which is ~7 times greater than the control anode. Subsequent increase of PEDOT loading further improve the current density to 4.11 \( A/m^2 \) and 4.57 \( A/m^2 \) for GF-2P and GF-3P respectively. The

![Fig. 1 – FESEM images of felt anodes with different PEDOT loadings: (a) GF, (b) GF-P, (c) GF-2P and (d) GF-3P (single column).](image-url)
maximum power density achieved by all the modified anodes (GF-P, GF-2P and GF-3P) are almost similar; ranging from 1.56 W/m² to 1.62 W/m². This signifies an immense improvement compared to control (0.52 W/m²). Hence, it is evident from the obtained trend that with minimal addition of PEDOT delivers superior performance of the anode in MFC.

Individual electrode potential
The individual polarization curves for both anode and cathode recorded to establish the relationship between current density and the potential were displayed in Fig. A4. The figure clarifies that potential of GF plunged when reaching high current density, revealing that anode is the limiting factor in that particular system. This effect is less apparent in modified anodes due to the improved electron transfer capabilities instigated by the addition of PEDOT. The cathode potential remained stable throughout the range of current density for all tested system.

Voltage generation of tested anodes
The voltage generation across the external resistor was recorded for three cycles to ensure repeatability and stability of the anode materials as well as biofilm is presented in Fig. 3. The maximum voltage generated by GF anode is 637.0 ± 25.9 mV while modified anodes achieved higher voltages of 710.0 ± 2.6 mV, 711.0 ± 3.6 mV and 705.3 ± 10.3 mV for GF-P, GF-2P and GF-3P respectively. However, although all three modified anodes recorded almost similar voltages, the duration of their cycle differs greatly. GF-P proved to be the most stable as it was able to maintain the voltage for ~6 days while GF-2P and GF-3P were only able to sustain peak plateau voltage for 4 and 2 days correspondingly. At higher loading, the dense presence of PEDOT blocked the pores of the felt configuration; interfering with the diffusion of substrate into the central section of the felt. This condition could trigger microbial fouling in the anode as nutrient deprived microbes faced an endogenous condition and consequently reached the death phase. The dead biomass then accumulates inside the felt fibers and leads to fouling[31–33]. This circumstances obstruct the available open structure of the graphite felt thereby considerably decrease the active surface area. Hence, the anodes with high PEDOT loading (GF-2P and GF-3P) are less stable compared to GF-P or even GF.

Internal resistance of the MFC systems
The components of the internal resistance of the MFC system are computed through EIS analysis. The Bode plots and its corresponding fitting were presented in Fig. 4 and tabulated in Table 1. Generally, PEDOT loading brought about significant reduction in the overall $R_{\text{int}}$ as seen from the ~50% reduction exhibited by the modified anodes. Additionally, all the modified anodes yielded almost similar $R_a$ ranging from ~14 to 18 $\Omega$, which explained the similar voltage and maximum power density achieved. The $R_a$ of GF, GF-2P and GF-3P were in the range of 9 Ω–13 Ω. However, the decline of membrane quality due to prolonged operation increased the membrane...
resistance for GF-P system, causing the exceptionally high $R_U$. The $R_c$ of the systems ranged from 10 to 59 $\Omega$. All felt anodes exhibited $R_d$ although it is not the dominant resistance in this study. The presence of $R_d$ is consistent with other 3D anodes as reported by literatures [24,34].

COD and CE performance of the anodes

The COD removal and CE performances of the studied anodes are presented in Table 2. The COD removals of the anodes ranged from 65.6% to 86.3% while the CE from 26.3% to 51.0%. The GF-P emerged as most efficient candidate among the four with highest COD removal and CE. The ephemeral nature of the voltage cycle exhibited by GF-2P and GF-3P leads to the low CE although they exhibited high current and power density. This further ascertains that high loading of PEDOT on felt anodes are disadvantageous to the efficiency of the MFC performance in terms of treatment and electricity generation.

POME wastewater as carbon source for MFC

POME, a wastewater with high organic content was used as carbon source in the MFC system to validate the functionality of the modified anode in removing complex pollutants. GF-P anode was chosen as the sample anode as it was observed as the optimum anode. The voltage generation and polarization curve for all three ratio of POME was demonstrated in Figs. 5 and 6 respectively. In the inception, 50% POME was added to 50% acetate (1 g/L) to acclimatize the anode. The

![Fig. 4 – Bode impedance modulus plot and its corresponding phase angle under 1 kΩ for felt anodes (single column).](image)

![Table 1 – Components of internal resistance for felt anodes.](image)

<table>
<thead>
<tr>
<th>Anode</th>
<th>$R_o$</th>
<th>$R_a$</th>
<th>$R_c$</th>
<th>$R_d$</th>
<th>$R_{int}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF</td>
<td>13.2</td>
<td>27.7</td>
<td>58.6</td>
<td>27.1</td>
<td>126.6</td>
</tr>
<tr>
<td>GF-P</td>
<td>33.8</td>
<td>13.7</td>
<td>9.8</td>
<td>1.1</td>
<td>58.4</td>
</tr>
<tr>
<td>GF-2P</td>
<td>8.8</td>
<td>17.7</td>
<td>13.5</td>
<td>5.0</td>
<td>45.0</td>
</tr>
<tr>
<td>GF-3P</td>
<td>9.0</td>
<td>14.1</td>
<td>14.8</td>
<td>6.2</td>
<td>44.1</td>
</tr>
</tbody>
</table>

![Fig. 5 – Voltage generation for GF-P using different ratios of POME (single column).](image)
generated voltage was much lower than that of pure acetate substrate (573.7 ± 13.8 mV compared to 710.0 ± 2.7 mV). Concurrently, maximum current and power density (3.0 A/m²; 1.1 W/m²) also declined in similar trend. The subsequent stage of experiment extends the percentage of POME to 75% exhibits a performance equivalent to that of acetate conditions. The voltage generation, maximum current and power density were found to be 707.5 ± 7.7 mV, 3.9 A/m² and 1.23 W/m² respectively. The increase in overall performance of the system at higher percentage of POME was due to the acclimatization of the biofilm towards the substrate. However, in the final stage when the anolyte was replaced with 100% POME, the electricity generation of the reactor declined marginally. The reactor delivered a peak voltage of 685.5 ± 3.5 mV, current and power density of 3.2 A/m² and 1.1 W/m² respectively.

The overall results demonstrate that the functionality of the anode was not compromised by complex substrate. GF-P was able to achieve comparable voltage generation when utilizing POME substrate, especially with minimal addition of simple carbon source. On the other hand, the COD removal and current efficiency of all the wastewater experiments (Table 3) were inferior compared to synthetic conditions owing to the complex nature of the pollutant.

Distribution of exoelectrogen in MFC system

The contribution of anodic biofilm and planktonic cells to voltage generation were investigated to reveal the distribution of exoelectrogen in the MFC system. In this part of the study, GF and GF-P were employed to represent the control and PEDOT modified anode respectively. Both the biofilm and planktonic cell experiments for GF recorded almost similar peak voltage after 48 h, as clearly depicted in Fig. 7(a).

However, peak voltage was attained in 3 h by anodic biofilm whereas the latter took ~24 h to achieve the peak voltage. The obtained result verifies that anodic biofilm dictates the electricity generation of the GF system [28]. Conversely, the contribution from planktonic cells cannot be neglected as it is able to achieve 93% of the peak voltage attained by anodic biofilm.

The voltage generation in the GF-P system was solely contributed by anodic biofilm. This is clearly observed from Fig. 7(b) as the anodic biofilm achieved peak voltage in ~1 h whereas there was no significant voltage measured during the planktonic cells study. Therefore, this circumstance suggests that the electricity generation in the present GF-P system is dominantly attributed by the anodic biofilm.

Biocide was injected into the anode compartment at the end of each experiment to establish that live cells are crucial for electron transfer as well as to determine the fraction of voltage generation from abiotic factors. In the space of 1 h after biocide was injected into the chamber containing GF anode, the voltage plunged to 10% of its peak voltage. The steep fall in the peak voltage ascertained the notable contribution of the live microbes for electron transfer and eliminated the contribution by the abiotic factors. A slightly longer time was required for GF-P to achieve similar reduction (~2 h) that was indicative of the capacitive effect of the PEDOT [35].

<table>
<thead>
<tr>
<th>% POME</th>
<th>CE (%)</th>
<th>COD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>24.3</td>
<td>76.3</td>
</tr>
<tr>
<td>75</td>
<td>34.9</td>
<td>68.8</td>
</tr>
<tr>
<td>100</td>
<td>34.2</td>
<td>59.7</td>
</tr>
</tbody>
</table>

Biofilm characterization

Comprehensive characterization of the biofilm attached onto anodes could provide information on the interaction between
the microbes and the anodes. The FESEM images of biofilm formed on the GF and GF-P anodes were obtained and shown in Fig. 8. It is evident from the images that rod shaped bacteria are predominant among the consortium. These microbes were well attached to the fibers of GF and the PEDOT particles of GF-P anodes. GF-P had higher microbial density compared to GF as the addition of PEDOT particles increased the surface roughness and area, facilitating better biofilm immobilization and growth. Furthermore, the electrostatic force between negatively charged cell walls and positively charged polymer backbone of PEDOT regulated more stable biofilm formation [19,36].

Microbial diversity in the inoculum and anode biofilms was investigated with the support of Next Generation Sequencing (NGS) technique. Alpha diversity analysis of inoculum, GF and GF-P anodes is presented in Table 4. The number of sequence ranged from ~20,000 to 23,900, providing a consistent sequencing depth for all the samples. The richness of the samples are analyzed by the number of OTUs and Chao1 index. The table showed that all the anodes have decreased richness compared to the sludge used for inoculation. This was due to the selection of exoelectrogens during acclimatization phase [37]. The richness index further declined for GF-P, proving that PEDOT incorporation further developed the selection of anodic biofilm.

The comparison of microbial community representing dominant 50 genera is demonstrated in Fig. 9. The most dominant genus for the sludge community is the Trichococcus while all the anodes were dominated by Geobacter followed by Hydrogenophaga. Identified exoelectrogens such as Geobacter, Hydrogenophaga and Shewamella were present in the anodic biofilms but is below detectable level (<1%) in the inoculum as tabulated in Table 5. On the contrary, the Pseudomonas, an exoelectrogen was reduced or disappeared from the anodic biofilm population although it was detected in the sludge. This strain was less competent compared to the other genera for electron generation and was eliminated from the community [37]. The acclimatization has significantly increased the frequency of exoelectrogens in all the anodes compared to the sludge inoculum. The adopted acclimatization condition allowed the selection of biofilm with external electron transfer capabilities to achieve a steady-state cell potential [38].

The prevalence of Geobacter is consistent with most acetate-fed operation as they are able to oxidize acetate and transfer electron directly to the anode [39-41]. The second majority of exoelectrogens found on the anodic biofilms were the Hydrogenophaga. They are hydrogen-consuming exoelectrogens that utilizes the by-product of acetate metabolism by Geobacter. Hence, Hydrogenophaga established a syntrophic

| Table 4 – Summary of alpha diversity of the inoculum and anodic biofilm. |
|-----------------|-----------------|-----------------|-----------------|
| Sample          | No. of sequence | No. of OTU      | Chao1 index     |
| Sludge          | 23,899          | 2848            | 8287.58         |
| GF              | 23,504          | 2516            | 5577.07         |
| GF-P            | 20,675          | 1914            | 4169.40         |

Fig. 8 – FESEM images of biofilm attachment of (a, b) GF and (c, d) GF-P anodes under different magnification (single column).
relationship with Geobacter and usually found alongside Geobacter in MFC systems [42]. Table 5 also showed the nominal presence of Shewanella. However, their contribution to electricity generation cannot be ignored, as predominance of a strain does not necessarily relate to their current production ability [43]. Additionally, the frequency of exoelectrogens on PEDOT modified anode were higher compared to the control anode. It is evident that the presence of PEDOT supports the growth and development of these exoelectrogens. The robust electrical conductivity of the PEDOT stimulates the biofilm formation as compared to control anode [20].

Table 5 – Exoelectrogen distribution in sludge inoculum and tested anodes (>1%).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Sludge (%)</th>
<th>GF (%)</th>
<th>GF-P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geobacter</td>
<td>–</td>
<td>22.9</td>
<td>63.5</td>
</tr>
<tr>
<td>Hydrogenophaga</td>
<td>–</td>
<td>4.8</td>
<td>17.2</td>
</tr>
<tr>
<td>Shewanella</td>
<td>–</td>
<td>–</td>
<td>2.3</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>3.7</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>3.7</td>
<td>30.3</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Fig. 9 – Relative abundances of the genus-level phylogenetic groups based on 16S rDNA extracted from inoculum sludge and biofilm from GF and GF-P anodes (2-column).

The present findings revealed the robustness of PEDOT as an enhancer for MFC anode. The electrochemical and power generation potential of the anode drastically enhanced with minor addition of PEDOT. It was also evident that higher loading of PEDOT conversely lead to detrimental performance and functionality. The modified anode demonstrated its ability in removing synthetic as well as complex carbon source derived from wastewater. The incorporation of PEDOT onto the anode not only improved electrochemical characteristics but also stimulated the biocatalyst selectivity and subsequently benefits the overall productivity of the MFC operation.

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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.2016.09.059.

References


