# Graphene Oxide/Polytetrafluoroethylene Composite Anode and *Chaetoceros* pre-Treated Anodic Inoculum Enhancing Performance of Microbial Fuel Cell

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Abstract-Electricity generation from microbial fuel cell (MFC) can be enhanced by proper manifestation of electrogenic bacterial growth on anode surface. Effect of graphene oxide (GO)/ Polytetrafluoroethylene (PTFE) composite bio-anode and Chaetoceros pre-treated anodic inoculum on electricity generation in MFC was investigated in the present work. MFC using GO/PTFE composite bio-anode demonstrated a maximum power density of 20.52 W/m<sup>3</sup>; whereas, MFC using bare carbon felt anode without modification produced a maximum power density of 10.25 W/m<sup>3</sup> and this was 3.43 W/m<sup>3</sup> for MFC using carbon felt anode inoculated with mixed anaerobic sludge without pre-treatment. Coulombic efficiency (CE) of 41.82 % was obtained in MFC with modified bio-anode using Chaetoceros algae pre-treated mixed anaerobic sludge as anodic inoculum. This CE obtained is far superior than the values reported earlier using mixed anaerobic sludge as inoculum. Increased catalytic current and lower charge transfer resistance were observed during linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS) for MFC with GO/PTFE modified anode as compared to MFC using unmodified anode. Thus, GO/PTFE modified carbon felt anode with Chaetoceros pre-treated mixed anaerobic sludge as inoculum could be used in MFC to enhance the power harvested by this device while simultaneously offering effective treatment to wastewater.

*Index Terms—Chaetoceros*, coulombic efficiency, graphene oxide, microbial fuel cell, suppressing methanogenesis.

### I. INTRODUCTION

Microbial fuel cells (MFCs) are novel electrochemical devices that directly convert microbial metabolic energy into electricity. However the low power density obtained and poor energy conversion efficiency of MFCs, due to the inefficient extracellular electron transfer between the microbes and electrode, limits their practical applications [1]. The specific surface area available for growth of bacteria and the interaction of the electrode material with microbes determine the extent of electrogenesis and hence power recovery in MFCs [2]. Redundant metabolic electron losses due to methanogenesis and fermentation performed by diverse group of microorganisms present in the anodic chamber, while treating actual wastewaters, have a major effect on the coulombic efficiency (CE) of MFCs [3]. Use of better

bio-compatible anode material to enhance the growth of electrogens and proper inoculum pre-treatment to reduce the substrate loss to methanogenesis can significantly enhance performance of the MFCs in terms of current harvested.

Several anode materials have been developed to increase the surface area for the effective growth of microorganisms, including graphite felt, graphite granules, activated carbon and graphite grains [4]. A bioanode should facilitate rapid heterogeneous electron transfer between bacteria and electron shuttles [5]. Graphene has been of significant interest for the fabrication of electrode. High bio-compatibility of graphene promotes bacterial growth on the electrode surface with more direct electron transfer activation centers. Liu et al. (2012) [1] used graphene modified carbon cloth anode while using Pseudomonas aeruginosa as the anodic inoculum. Graphene oxide (GO) based bioanodes have better biocompatibility and high specific surface area for accommodating large number of microbes. GO can act as the final electron acceptor for the respiratory extracellular electron transfer (EET) in MFCs. Shewanella species are reported to reduce graphene oxide to electrically conductive graphene [6]. Graphene/ Polytetrafluoroethylene (PTFE) composite electrode was used to improve the power density of an Escherichia coli catalyzed MFC [7]. Carbon felt, which has been used as anodes in MFCs due to its high conductivity and high stability, can be used as a platform for binding of the graphene oxide/PTFE composite for improving affinity and attachment of electrogenic consortium.

Coulombic loss in MFCs is attributed to the substrate loss for methanogenesis that occurs in the anodic chamber of MFCs. Methanogenesis is prevalent in MFCs while using mixed anaerobic sludge as anodic inoculum. Methanogens compete electrogens for substrate utilization and subsequently reduce the CE of MFCs [8]. Methanogenic electron loss in MFCs can be controlled by providing various pre-treatments to the mixed anaerobic sludge, generally used as inoculum for suppressing growth of methanogens [8]-[10]. The maximum power density reported by previous studies using graphene as anode material without any inoculum pre-treatment was found to be relatively less. Chaetoceros pre-treatment of anodic inoculum is found to be an efficient method for controlling methanogenesis in MFCs [11]. Hexadecatrienoic acid present in the marine algae Chaetoceros was found to inhibit the growth of Gram-positive and methanogenic archaea via adsorption and disruption of cell membranes. The present study was aimed to evaluate combined effect of use of Chaetoceros algal pre-treatment to the anodic inoculum, for suppression of methanogens in the mixed anaerobic sludge used as inoculum,

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and use of GO/PTFE composite coating on carbon felt anode to further increase biocompatibility of anode material to enhance the bio-electrogenesis in MFC.

### II. MATERIALS AND METHODS

#### A. MFC Construction and Operation

Graphene oxide was synthesized using modified Hummer's method [12]. GO/PTFE composite modified carbon felt was fabricated in laboratory for making anode of MFC. Typically, required amount of GO was dispersed in water containing 5 % PTFE solution (Sigma Aldrich, USA) to make graphene oxide concentration of 1 mg/ml and sonicated for 3 h at 150 kHz ultrasound frequency using bath sonicator. The above composite solution was then soaked on a piece of carbon felt having dry weight of 9 g and projected surface area of 192 cm<sup>2</sup> in a flat container for 24 h in electric oven at 60 °C overnight. This modified electrode was dried in electric oven for 48 h at 60 °C. After modification, the final weight of carbon felt was taken to estimate graphene deposition on the surface. GO was found to be deposited on modified carbon felt with a loading of 0.46 mg/cm<sup>2</sup>.

Four identical dual chamber aqueous cathode MFCs with an anodic liquid volume of 250 ml were fabricated. The volume of the cathode chamber of aqueous cathode MFCs was 2 L. Baked clayware cylinders served as the anodic chamber of these MFCs and the 8 mm thick wall material of the cylinder acted as a separator between anodic and cathodic chambers as well as the cation exchange membrane. MFC with unmodified carbon felt anode and GO/PTFE modified carbon felt anode in the presence of Chaetoceros inoculum pre-treatment were named as MFC-1 and MFC-2, respectively. Whereas MFC with unmodified carbon felt anode and GO/PTFE modified carbon felt anode receiving mixed anaerobic sludge inoculum without any pre-treatment were named as MFC-3 and MFC-4, respectively. The anode and cathode electrodes of all the MFCs were made up of carbon felt with a projected surface area of  $192 \text{ cm}^2$  and 260 cm<sup>2</sup>, respectively. Both electrodes were connected through concealed copper wire across a 100  $\Omega$  external resistance. Mixed anaerobic sludge of 50 ml volume collected from the bottom of a septic tank was used as the inoculum in the anodic chamber. The marine algae Chaetoceros was procured from a commercial shrimp hatchery and sundried to provide as a ground preparation. Anaerobic sludge was pretreated with 10 mg/mL of Chaetoceros powder and incubated at 39 °C for 24 h in order to suppress the growth of methanogens before inoculating in MFC-1 and MFC-2 [11]. Mixed anaerobic sludge of 50 ml volume was added in MFC-3 and MFC-4 without any inoculum pre-treatment. Synthetic wastewater containing sodium acetate as the carbon source having chemical oxygen demand (COD) of about 3000 mg/L was used as feed in all the MFCs [13]. MFCs were operated in batch mode with a feeding interval of 4 days, making retention time in each cycle of 4 days, under ambient temperature varying from 28 to 30  $^{\circ}$ C.

#### B. Analysis and Calculations

Performance of MFC was evaluated in terms of voltage

(U/V) and current (I/A) measured using a digital multimeter with a data acquisition unit (Agilent Technologies, Malaysia) and converted to power according to  $P = U^*I$ , where P =power (W). The electrode potential of both anode and cathode were measured with an Ag/AgCl reference electrode (+197 mV vs. SHE, Bioanalytical Systems Inc., USA). Open circuit voltage (OCV) was measured under no current flow condition of the circuit. Power density per unit surface area and per unit volume was calculated by normalizing power to the anode surface area and net liquid volume of anodic chamber, respectively. Polarization studies were carried out after attaining a stabilized voltage in these MFCs by varying the external resistances in steps from 20,000 to 5  $\Omega$  using the resistance box (GEC 05 R Decade Resistance Box, India). The internal resistance of the MFCs was estimated from the slope of line of voltage versus current plot [14].

Electrochemical tests were performed using a three electrode system, in which Platinum rod and saturated Ag/AgCl electrode were used as counter and reference electrode, respectively, and anode was used as working electrode. Linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS) were carried out using Autolab PGSTAT 302N potentiostat (Metrohm, The Netherland). LSV was performed in voltage ranging from -0.9 to +0.9 V at scan rate of 10 mV/s. EIS was recorded by applying alternating current of frequency range of 100 kHz to 100 mHz with voltage amplitude of 10 mV.

Influent and effluent COD concentrations were measured according to APHA standard methods [15]. The Coulombic efficiency (CE) was calculated as the fraction of total coulombs actually transferred to the anode against that theoretically present in substrate for current generation over the time period [16]. SEM image of the anode material was analyzed by cutting 1 cm<sup>2</sup> of the working electrode of MFC-1 and MFC-2. Prior to the SEM analysis the attached biofilm was fixed with glutaraldehyde and serially dried with 30 % alcohol [17].

#### III. RESULTS AND DISCUSSION

#### A. Organic Matter Removal and Coulombic Efficiency

Average COD removal efficiency in MFC-1, MFC-2, MFC-3 and MFC-4 was 66.88 ±1.32%, 69.59 ±2.59%, 76.58  $\pm 1.89\%$  and 78.34  $\pm 2.18\%$ , respectively, during 32 days of operation (Fig. 1A). Lower organic matter removal observed in MFC-1 and MFC-2 as compared to other MFCs using acetate as substrate [18] shows that substrate loss to acetoclastic methanogens was significantly reduced while using Chaetoceros algae pre-treated anodic inoculum. Average acetate removal increased from 2006 mg COD/L in MFC-1 to 2088 mg COD/L in MFC-2. Use of GO/PTFE composite as electrode material in MFC-2 and MFC-4 enhanced the hydrophilicity of anode which makes it more biocompatible for improved growth of electrogenic bacterial community and promoted slightly higher COD removal as compared to MFC-1 and MFC-3, respectively. From the stoichiometric equation it has been calculated that 99.94 ml and 90.52 ml of methane gas was produced in MFC-1 and MFC-2 while 144.04 ml and 140.61 ml of methane gas was produced in MFC-3 and MFC-4 in the 7<sup>th</sup> cycle of operation, which shows the decreased production of methane gas in MFCs in which *Chaetoceros* inoculum pre-treatment was given.

Higher current generation was noted in MFC-2 using GO/PTFE composite anode (5.75  $\pm 0.08$  mA) as compared to MFC-1 (4.75  $\pm$  0.05 mA). A maximum CE of 41.82 % and 33.65 % was noted in MFC-2 and MFC-1, respectively (Figure 1A), while the maximum CE was limited to 17.32 and 20.35 in MFC-3 and MFC-4. Higher CE was observed in MFC-2 and MFC-1 as compared to MFC-3 and MFC-4 due to the effective suppression of methanogens owing to the inoculum pre-treatment with marine algae Chaetoceros [11]. This way increased electron recovery from acetate by electrogens could be attained in the MFCs, which enhances the CE significantly. Marine algae pre-treatment of anodic inoculum prevented the substrate loss to acetoclastic methanogens and improved the CE in both the MFCs. Enhanced bio-catalytic activity in MFC-2 and MFC-4 using GO/PTFE composite bio-anode aided an increased charge recovery from acetate and resulted in further improvement in CE. Biologically reduced GO increases the electron transfer rate in the bio-anode due to an increase in the population of exoelectrogens in the MFC [19].



Fig. 1. (A) COD removal and CE in MFCs, and Fig. 1(B) Average operating voltage generation in MFCs during each cycle of operation.

#### **B.** Power Production

The electricity generation in all the MFCs reached to a stable state after 4 cycles of operation. MFC-1 delivered a maximum operating voltage of 485 mV during the 6<sup>th</sup> cycle of operation; whereas MFC-2 produced a maximum operating voltage of 597 mV in the 7<sup>th</sup> cycle of operation (Fig. 1B). During stable operation MFC-1 and MFC-2 produced average working voltage of 475  $\pm$  10 mV and 576  $\pm$  12 mV, respectively. Enhanced voltage produced by MFC-2 can be

attributed to increased bio-catalytic activity due to the increased microbial growth on GO/PTFE modified anode. Maximum voltage obtained in MFC-2 was higher than voltage of 478 mV reported in previous study using GO anode material in MFC [19]. An open circuit voltage of 794  $\pm$ 9 mV and 845  $\pm$  9 mV was produced by MFC-1 and MFC-2, respectively. The maximum operating voltage obtained in MFC-3 (296 mV) and MFC-4 (338 mV) in which no inoculum pre-treatment was given was found to be less as compared to MFC-1 and MFC-2.

Polarization study was carried out to determine the effect of external resistance on power production. Polarization curves give an idea about maximum power density that could be obtained from the MFC at an optimum resistance and also illustrates variation in power density with variation in current density. The voltage and current density curve gives different overpotential losses occurring in MFCs such as activation loss, ohmic loss and concentration loss. In addition slope of the linear part of the voltage versus current density curve represents ohmic loss which can further be used to estimate internal resistance of the system. Polarization curve illustrates that MFC-2 delivered a maximum power density of 216  $mW/m^2$  (20.52 W/m<sup>3</sup>), which was two times higher than MFC-1 having unmodified carbon felt as anode (Fig. 2A). The maximum power density obtained in this study was found to be higher than that reported using graphene/carbon cloth anode [1]. MFC-3 and MFC-4 in which no inoculum pre-treatment was given showed a maximum power density of  $34.21 \text{ mW/m}^2$  (3.43 W/m<sup>3</sup>) and 87.92 mW/m<sup>2</sup> (8.35 W/m<sup>3</sup>), respectively. This reduction in power density in MFC-3 and MFC-4 as compared to MFC-1 and MFC-2 shows that inoculum pre-treatment in MFCs using Chaetoceros has enhanced the electrochemical activity on the anode surface of both the MFCs. A maximum current density (CD) of 936  $mA/m^2$  could be obtained from MFC-2. The internal resistance of the MFC-2 (48  $\Omega$ ) was also significantly lesser than MFC-1 (82  $\Omega$ ). Lower electron transport resistance at the bacteria/electrode interface for the GO/PTFE modified anode material contributed to the higher energy conversion efficiency and higher power density in MFC-2.

The electrode potential drives electron from negative terminal of the electrode to the positive terminal of the electrode. Higher the potential difference between the two electrodes, higher current density can be observed. The anode potential (vs Ag/AgCl electrode) across 20 k $\Omega$  resistance was found to be - 465 mV for MFC-2; whereas for MFC-1 at the same external resistance it was - 417 mV. The relative increase in anode potential relative to current density was found to be in the order of MFC-3 > MFC-4 > MFC-1 > MFC-2 (Fig. 2B). The higher anode potential in MFC-3 and MFC-4 suggests the decreased electrogenic activity in the absence of inoculum pre-treatment; while the lesser anode potential in MFC-2 as compared to MFC-1 shows the enhanced anodic reaction kinetics on the GO modified surface of the carbon felt anode. At a current density of 407 mA/m<sup>2</sup>, MFC-2 noted an anode potential of - 326 mV; whereas the anode potential of MFC-1 was only - 221 mV at the same current density. These results demonstrate an enhanced electrogenic activity in the GO/PTFE bio-anode due to increased microbial growth on the reduced GO surface of the

anode.



Fig. 2. (A) Polarization curve of MFCs, and Fig. 2(B) Electrode potentials of MFCs.

## C. Electrochemical Analysis

Bio-catalytic activity of anodes in MFC-1 and MFC-2 were inspected using electrochemical tests. Linear sweep voltammetry (LSV) is a powerful tool to investigate the electrode kinetics in MFCs. Compared to the unmodified carbon felt anode, the GO/PTFE modified carbon felt anode exhibited higher current density response over an applied potential range during LSV (Fig. 3A). Oxidative current densities were found to be lower in MFC-1 (37 mA/m<sup>2</sup>) as compared to MFC-2 (95 mA/m<sup>2</sup>) using unmodified and GO/PTFE modified anode, respectively. This higher current density in MFC-2 suggests that the GO/PTFE modified anode facilitated higher specific surface area to adhere bacteria on the anode surface. In addition, the unique thin sheet like structure of GO provided large surface area for bacterial attachment and increased reaction sites for acetate oxidation, which eventually enhanced the current density by 2.56 fold in MFC-2 as compared to the MFC-1.



Fig. 3. (A) LSV analysis of anode of MFCs.

In addition, the EIS results showed significant difference in individual components of internal resistance viz. charge transfer resistance ( $R_{ct}$ ) and solution resistance ( $R_s$ ), when these values are compared for both the anodes. Anode with GO/PTFE composite had lower  $R_{ct}$  of 1.2  $\Omega$  as compared to unmodified anode having  $R_{ct}$  of 2.8  $\Omega$  (Fig. 3B). These results indicate that the GO present on anode surface acted as terminal electron acceptor for extracellular electron transfer and reduced to graphene, which substantially increased the conductivity of anode.  $R_s$  value represents the resistance offered by the ionic solution (electrolyte), which contributes to the overall impedance of the cell [20].



Significant difference in  $R_s$  values were estimated in MFC using unmodified anode (MFC-1) and GO/PTFE modified anode (MFC-2) with a value of 18.7  $\Omega$  and 3.2  $\Omega$ , respectively, probably due to higher overpotential loss in MFC-1 as compared to MFC-2. At higher over potential, solution resistance dominates and decreases the performance of MFC [21]. Enhanced bio-catalytic property of anode using GO/PTFE modified carbon felt was observed due to synergetic effect of graphene oxide and PTFE. Moreover, high conductivity of GO/PTFE composite posited lower hindrance in charge transfer thereby increased the oxidative current.

### D. Microscopic Analysis

SEM analysis of the biofilms grown on the anode electrode materials have been taken to study the qualitative examination of the presence of electrogenic microbes.SEM image of the plain graphite felt is shown in the Fig. 4A which had no coating of GO/PTFE composite, as clearly visible. GO/PTFE coated anode (Fig. 4B) shows the morphology of graphene sheets entangled with the carbon felt fiber matrix. Image of the biofilm grown on the bare carbon felt fibers shows that the electrogenic microbial biomass were not uniformly distributed in each of the single carbon fiber, might be due to the lesser affinity of the bacteria to the carbon fiber surface (Fig. 4C); whereas the biofilm formed on the surface of GO/PTFE composite anode was found to be covered with an extensive layer of bacteria (Fig. 4D). GO/PTFE composite anode did not show any detrimental effect for the growth of electrogenic bacterial consortium. Extensive growth of biofilm on the surface of GO/PTFE bio-anode leads to an increased bio-electrocatalytic activity in MFC-2, which lead to an increase in electric current and power density.



Fig. 4. SEM image of (A) Plain carbon felt, (B) PTFE/GO modified carbon felt, (C) Biofilm growth on plain carbon felt and (D) Biofilm growth on PTFE/GO modified carbon felt.

## IV. CONCLUSION

Increased power generation was observed in MFC using Graphene oxide/PTFE modified anode, while marine algae pre-treatment of anodic inoculum reduced the substrate loss to methanogens and enhanced the growth of electrogenic microbes. As a result, coulombic efficiency of the MFC using modified anode in the presence of Chaetoceros inoculum pre-treatment was found to be 1.24 times higher than the MFC using carbon felt anode without any modification. Linear analysis noted voltammetry an increased sweep bio-electrocatalytic activity on the surface of GO/PTFE composite anode and a substantial reduction in charge transfer resistance was observed. Electrochemical impedance spectroscopy analysis showed a decrease in charge transfer resistance in the MFC using GO/PTFE modified anode as compared to MFC using unmodified anode. The use of GO/PTFE carbon felt composite anode in MFC significantly enhanced power production of MFC, hence it is recommended for practical application. Future research is required for scaling up of the system for practical application.

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