Fabrication and characterization of high power dual chamber 
\textit{E. coli} microbial fuel cell

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Abstract. This work reports the fabrication of a dual chamber microbial fuel cell with \textit{E. coli} modified graphite as the anode and lead dioxide cathode. At the optimized operating conditions, the cell provided 778 mV open circuit potential, 3.47 mA m$^{-2}$ of current density and 1660 mW m$^{-2}$ power density. Morphology of the \textit{E. coli} biofilm on the electrode was analysed using AFM and the electrochemical characterization of the fuel cell was carried out using electrochemical impedance spectroscopy (EIS) and polarization curves. The composition of the anode and the time duration for \textit{E. coli} biofilm formation were varied to obtain maximum power density. The MFC fabricated in this study was found to have improved power density in comparison with other reported fuel cells.

Keywords— \textit{E. coli} fuel cell, lead dioxide cathode, graphite electrode, microbial fuel cell electrodes.

1. Introduction

Microbial fuel cell (MFC) is a sustainable device that employs anaerobic oxidation of organic matter by microorganisms to generate electrical energy. Typically, an MFC consists of an anode and a cathode in two chambers separated by a proton exchange membrane (PEM). When bacteria immobilized electrode is introduced into the de-aerated anodic chamber, bacteria oxidize the organic compounds (e.g., organic substances in wastewater) to form protons and electrons. The protons pass through the PEM to the cathode, while the electrons are transferred through an external circuit from the anode to the cathode. The electronic flow drives an external load and reduces the electron acceptor (e.g., oxygen) at the cathode producing water. Thus, MFC is a renewable energy sources as it can produce electricity from wastewater using microorganisms. MFCs are safe to operate and find its application in wastewater treatment \cite{1, 2}, bioremediation \cite{3} and toxic recovery \cite{4} to name a few.
The power density of MFC is dependent on the design of the fuel cell, the electrodes used, the type of fuel cell (mediator/mediatorless), surface area of the electrode, internal resistance and over potential of electrodes [5]. Since there is high movement of electrons in an MFC, the electrodes used should be highly conductive and the cathode compartment should be filled with materials that are good electron acceptors. The choice of materials used for electrode construction plays an important role in influencing the performance of the MFC. In the past decade, a large number of electrodes have been well studied and employed to improve current and power density output from MFC [6]. Carbon electrodes in the form of graphite are the most commonly used for both as anode and cathode construction. Even though platinum modified electrodes are commonly used as cathode, efficient oxygen reduction reaction catalysts such as PbO\(_2\) and MnO\(_2\) have also been reported for the construction of the cathode [7].

Another important factor that influences the power density of MFC is the extracellular electron transfer between the bacterial cell and the anode. Many microorganisms like G. sulfurreducens [8], C. butyricum [9], Lactobacillus [10] and E. coli [11] are well known for their extracellular electron transfer. Out of these, E. coli is the most prevalent species in wastewater [12] and can be easily cultured [13].

The present work details the construction and electrochemical characterization of a microbial fuel cell containing using E. coli modified graphite electrodes as the anode and PbO\(_2\) as cathode with improved power density.

2. Experimental

2.1. Materials and reagents

Graphite powder (282863, 99.99% purity) was purchased from Sigma - Aldrich. Carbon ink was obtained from Anabond Constance, Chennai. Analytical grade potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)), disodium hydrogen phosphate (Na\(_2\)HPO\(_4\)), potassium chloride (KCl), sodium chloride (NaCl), lead dioxide (PbO\(_2\)) and potassium ferricyanide were purchased from Finar chemicals, India and used as such without further purification. Agar, peptone, beef extract, yeast extract and sodium chloride used for the preparation of microbial culture media were procured from HiMedia Laboratories.

2.2. Sub culturing of E. coli

For sub culturing of the E. coli, a small amount of culture was inoculated into a nutrient broth medium from agar plates using a sterile loop. For preparation of nutrient broth (NB) medium of pH 7.0 containing 5 g L\(^{-1}\) of peptone, 3 g L\(^{-1}\) of beef extract, 2 g L\(^{-1}\) of yeast extract, 5 g L\(^{-1}\) of sodium chloride were thoroughly mixed in 1 L of water and sterilized and cooled before use. After inoculation, the nutrient broth was incubated at 37 \(^{\circ}\)C under constant stirring.

2.3. Fabrication of MFC electrodes

For the fabrication of the anode, 0.75 g of graphite powder was thoroughly mixed with 5 g of carbon ink to form a thick paste. This paste was uniformly applied onto a cotton cloth of dimension 2 cm X 2 cm and dried at 60 \(^{\circ}\)C for 10 hours. The anode was then immersed in nutrient broth inoculated with E. coli to obtain a microbial biofilm on the surface. The time duration for incubation was varied and the fuel cell characteristics were studied to obtain high efficiency. A cotton cloth (2 cm X 2 cm) coated with a conducting paste consisting of 0.5 g PbO\(_2\), 0.25 g of graphite powder and 5 g of carbon ink was used as the cathode.
2.4. Design and fabrication of MFC

A two compartment MFC was built using acrylic sheet of 2 mm thickness. The design consisted of an anodic and cathodic chamber of dimension 27.5 mm X 40 mm X 60 mm (LXWXH). The two chambers are separated by a 5 mm thick agar- KCl mixture acting as the proton exchange membrane (PEM). The two walls of the PEM have a slit of dimension 30 mm X 50 mm to allow transport of protons from anode to cathode. The schematics and the photograph of the MFC are shown in Figure 1 A and B respectively. The anolyte and catholyte were NB medium and phosphate buffer saline-ferricyanide mixture respectively. The anolyte was deoxygenated using nitrogen while oxygen was supplied to the catholyte.

![Figure 1. Schematics (A) and Photograph (B) of dual chamber E. coli microbial fuel cell](image.png)

2.5. Characterization and testing

MASTECH M92A high precision multimeter was used for current and potential measurement. All electrochemical measurements were performed using CHI660C electrochemical workstation (CH instruments, TX, USA). Morphology of the electrodes was analysed using atomic force microscope (AFM)- XE70 (Park systems, Korea).

3. Results and Discussion

3.1. Morphology of biofilm on graphite electrode

Figure 2 depicts the 2D and 3D AFM images of the graphite and E. coli modified graphite electrodes. From Figure 1 it is observed that the graphitic anode surface is highly uniform and nonporous (A & B) while the E. coli biofilm formation was found to be highly porous and uniform (C&D).
3.2. **Optimization of electrode fabrication process of MFC**

To maximize the efficiency of the MFC, conductivity experiments were conducted by varying the composition of the carbon ink and graphite on the anode. From the results obtained it was observed that 0.75 g of graphite powder in 5.0 g of carbon ink was best suited for the construction of the anode. The power density from the MFC constructed with *E. coli* biofilm formation at 24 hrs was greater than those incubated in culture medium for 48 and 72 hrs. Hence for all further experiments, the anode used consisted of biofilm formation at 24 hours. In order to form the salt bridge between the anodic and cathodic compartments, agar with supporting electrolyte KCl was used for reducing internal resistance.

3.3. **Effect of mediators on cathodic reaction**

Figure 3 depicts the Nyquist plots recorded for the cathode with and without ferricyanide mediator. Electrochemical equivalent circuit (inset of figure 3) was designed for the plots and the values for the equivalent electrical components were derived and are shown in Table 1. The components $R_s$, $R_{ct}$, $C_{dl}$, $Q$ and $W$ of the circuit represent the, solution resistance, charge transfer resistance, double layer capacitance, constant phase element (CPE) and the mass transfer impedance (Warburg impedance) respectively. In an ideal condition, the impedance spectrum for the circuit should be a semicircle, but the plot obtained does not show complete semicircles at the given frequency range. This frequency dispersion is due to complex double layer capacitance and is depicted by CPE, which is the measure of capacitive dispersion arising from the surface heterogeneities. CPE is mathematically represented as $Z = |Q(j\omega)^\alpha|^{-1}$, where $Y_0$ is a constant, $\omega$ is the angular frequency, $\alpha$ is the measure of arc depression. When the ‘$\alpha$’ value becomes unity the CPE behaves like a capacitor with a value of $Q$. 

![Figure 2. Noncontact 2D and 3D AFM images of graphite electrode (A and B) and *E. coli* biofilm modified electrode (C and D).](image)
Electrochemical impedance plots for the PbO\textsubscript{2} cathode with and without the presence of ferricyanide mediator. Inset shows the best fit equivalent circuit for the EIS plots.

Table 1. The best fit parameters for PbO\textsubscript{2} modified cathode with and without the presence of ferricyanide mediator in catholyte

| Electrode                  | R\textsubscript{s} (Ω) | R\textsubscript{ct} (Ω) | W (S\textsuperscript{0.5}) | Q or CPE (S\textsuperscript{n}) |
|----------------------------|----------------------|------------------------|-----------------------------|---------------------------------|
| Without ferricyanide       | 23.33                | 841.7                  | 0.0231                      | 0.0032                          |
|                           |                      |                        |                             | \(\alpha = 0.5642\)             |
| With ferricyanide          | 25.16                | 417.7                  | 0.0376                      | 0.004                           |
|                           |                      |                        |                             | \(\alpha = 0.5246\)             |

From Table 1 it is observed that in the presence of ferricyanide, the charge transfer coefficient \(R_{ct}\) drops to 417.7 Ω in comparison to the 841.7 Ω observed without ferricyanide. This implies that the electron transfer resistance for oxygen reduction on the PbO\textsubscript{2} electrode decreased after the introduction of ferricyanide ions. Hence it can be inferred that ferricyanide effectively mediates the transfer of electron to the cathode.

3.4. MFC operation and results

The MFC was operated in batch mode at 30 °C. Freshly fabricated MFC showed an open circuit voltage (OCV) of 590 mV. The anode compartment was deoxygenated using nitrogen and the cell was left undisturbed for 3 days. During this time the OCV increased to 778 mV and was also found to be stable. By using varying load values (10 Ω to 10000 Ω), the current and potential were measured. Power density and Polarization curve of MFC with variation in the load is shown in Figure 4. The voltage of the cell was found to vary based on the load that was connected. The current (\(I\), µA) measured was also theoretically calculated using Ohm's law (equation 1)
where, $V =$ Voltage measured across the load
\[ R_{\text{ext}} = \text{External resistance connected to the circuit} \]

The performance of MFC is generally expressed in terms of power density with respect to the anode surface area as the anode is exposed to the biological reactions. The power density ($P$, W m$^{-2}$) is calculated from equation (2).

\[ P = \frac{V^2}{R_{\text{ext}}A} \]  

where, $A =$ area of the anode taking part in the reaction (10.5 cm$^2$).

The current density ($CD$, µA cm$^{-2}$) is calculated from equation (3).

\[ CD = \frac{V}{R_{\text{ext}}A} \]

A maximum power density of 1660 mW m$^{-2}$ was obtained from the MFC within the operating range of 10 $\Omega$ to 10000 $\Omega$.

![Figure 4. Power density curve and Polarization curve of MFC with variation in the load](image)

3.5 Working mechanism of MFC:

During aerobic cycle, *E. coli* breaks down the nutrient in the growth medium to produce carbon dioxide and water. However, when oxygen, the anaerobic cycle yields carbon dioxide, protons, and electrons. The movement of these electrons released electrons from the anode to the cathode results in the generation of electricity. On the other hand, the protons will pass through the proton exchange membrane and mix with oxygen in the cathode side to form water. The higher composition of KCl helps in the effective transfer of H$^+$ ions to the cathode compartment [14]. The electrode reactions are represented in Figure 5.
3.5. Comparison of power densities of different E. coli based systems

Comparison of the power density of the MFC fabricated in this work with those is shown in Table 2. From the comparison it is clear that the fabricated dual chamber MFC produces much superior power density.

Table 2. Power density of the present MFC compared with recently represented MFC’s

| Electrodes used                                | Power density (mW m⁻²) | Reference |
|------------------------------------------------|------------------------|-----------|
| PTFE coated graphite anode, air cathode        | 760                    | [15]      |
| Carbon cloth electrodes                        | 640                    | [16]      |
| CNC electrodes                                 | 1304                   |           |
| Graphite anode and cathode                     | 0.30                   |           |
| Graphite anode, Fe³⁺-graphite cathode          | 0.44                   |           |
| NR- graphite anode, Fe³⁺-graphite cathode      | 1.20                   | [17]      |
| Mn⁴⁺-graphite anode, Fe⁵⁺-graphite cathode     | 91                     |           |
Graphite felt anode, Graphite felt cathode | 285 | [18]
Graphite anode, PbO$_2$ cathode | 1660 | This work

4. Conclusion
In summary, we have successfully demonstrated the fabrication and characterization of a dual chamber microbial fuel cell using *E. coli*. The use of PbO$_2$ as cathode and ferricyanide as mediator increased the rate of oxygen reduction reaction on cathode and improved the cell performance. The use of agar in place of commercially available proton exchange membranes could improve the cost effectiveness of the fuel cell. With excellent voltage and power density being obtained from the fabricated fuel cell, future work can be directed towards the use of wastewater where *E. coli* are abundant.

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