SUBSTRATE AND MICROBIAL CONSORTIUM IN A MFC FOR GENERATION OF BIOELECTRICITY

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ABSTRACT
As carbon based natural energy supplies are dwindling, other sources of energy are being explored. The need for alternate eco-friendly fuel is growing rapidly with depletion of non-renewable energy resources. Microbial Fuel Cells (MFCs) represent a new form of renewable energy which converts the organic matter into electricity (direct current) by the action of bacteria present in wastewater, while simultaneously treating the waste water. Waste management is one of the major areas of concern in today’s environment. In this research both the human needs - treatment of waste water and production of electricity are addressed. The present study was aimed at designing a MFC for generation of direct current from the wastewater of Treatment Plant in SRM University and at optimizing the substrate and microbial consortium in the MFC. The substrates used include that of samples obtained from different stages of wastewater treatment plant. The substrate obtained from lamellar separator gave good results with a maximum of 175 µA; comparatively the paddy straw gave a maximum of 150 µA and the synthetic media with a maximum of 100 µA for 2 liter of the substrate used. Microbial consortium varied with aerobic inhibitors, which were used as oxygen scavengers, to facilitate the growth of facultative microorganisms present in the wastewater. The results emphasize that - with optimal carbohydrate concentration, the sample gave better results, while the consortium was devoid of aerobes and methanogens.

Key Words : Microbial fuel cell, Mediator less MFC, Substrate, Microbial consortium, Bioelectricity.

INTRODUCTION
Increase in fuel consumption, owing to burgeoning of world population, has made it vital to find alternative methods for energy generation and organic waste utilization that are sustainable in future. Energy consumption has increased dramatically over the decade and an unbalanced energy management exists today. While there are no signs indicative of an abate in this growing demand (particularly amongst the developing nations), however, over the there is a steady increase in awareness in the transience of non-renewable resources and the irreversible damage caused to the environment. Bio-fuel cells potentially offer solutions to all these problems, by taking nature’s solutions to generate energy and utilizing them to our own needs. They take the readily available substrates from renewable sources and convert them into benign by-products with the generation of electricity. Since they use concentrated sources of chemical energy, they can be small and light.
Today electricity production from renewable resources without a net carbon dioxide emission is much desired. The collection, transportation, processing and recycling or disposal of waste materials is a major threat that the developed and developing nations are facing. The term “Waste”, in this context, relates to materials produced by human activity. Waste management is basically carried out to recover resources from it and is undertaken to reduce their effect on health, environment or aesthetics. Millions of gallons of waste water is produced from industries, households etc., worldwide. 2 billion people in the world lack adequate sanitation and the economic means to afford it\(^2\). Hence, the treatment of wastewater is highly essential for maintaining a clean pollution free environment\(^1\). In this research, both the human needs are addressed.

MFCs represent a completely new method of renewable energy recovery: direct conversion of organic matter to electricity (direct current) using bacteria. In the absence of oxygen, Bacteria transfers the electrons obtained from the metabolism it undergoes to the next electronegative component, i.e., anode. In MFC, these electrons therefore are transferred to the anode, while the counter electrode (cathode) is exposed to oxygen (terminal electron acceptor). At cathode the electrons, oxygen and protons combine to form water. MFCs can be used to generate electricity from various forms of biodegradable organic matter like domestic, agricultural and industrial wastewaters. Waste water is used as a substrate in this experiment since it is ubiquitous and cost effective\(^3\).

MFCs are classified into a mediator based and a mediator less MFC. The electron transfer from microbial cells to the electrode is facilitated by mediators such as Thionine, Methyl Violegen, Methyl Blue, Humic acid, Neutral red\(^4\). Most of the mediators are expensive and toxic. Bacteria in mediator-less MFCs, typically use electrochemically-active redox enzymes such as cytobchromes on their outer membrane that can transfer electrons to external materials\(^5\). Such MFCs were initially engineered at the Korea Institute of Science and Technology\(^6\). The present model constructed is a mediator less MFC considering the economic aspects of the reactor.

**Generation of Bioelectricity**

Reduction of sugars in an aerobic chamber by bacteria leads to the production of CO\(_2\) and H\(_2\)O. While, in an anaerobic chamber it leads to the production of electrons\(^7\). Trapping these electrons, leads to the generation of current.

\[
\text{C}_{12}\text{H}_{22}\text{O}_{11} + 13\text{H}_2\text{O} \rightarrow 12\text{CO}_2 + 48\text{H}^+ + 48\text{e}^-
\]

Some of the bacteria present possess electrochemically active redox enzymes which help this reaction to take place\(^8\). And these enzymes act as inorganic mediators to trap the electron from the electron transport chain of bacterial cells\(^8\). Trapping these electrons is performed strictly under anaerobic condition\(^9\). Hence experiments were conducted to optimize the reactor design in an attempt to maintain the anaerobic chamber.

**Consortium of Microorganisms**

The Microbial Consortium that exists in the substrate upon analysis reveals a great diversity in composition. Some of these species have also been used as pure culture to generate electricity in MFCs. However, the results disclose that they have relatively low energy transfer efficiency compared to
mixed microbial consortium that exists in wastewater, marine sediments, and livestock manures. MFCs that make use of mixed bacterial cultures have some important advantages over MFCs driven by pure cultures: higher resistance to process disturbances, higher substrate consumption rates, lower substrate specificity, and higher power output. These microbial consortium allow the electrochemically active bacteria to take advantage of the hydrolysis, fermentation, and anaerobic oxidation performed by other species to provide readily degradable substrates, making the general food chain in MFCs similar to methanogenesis in all but the final step. The combined activity of fermentative microorganisms coupled with the oxidation of fermentation products by Geobacteraceae appears to be a more competitive process. Highly effective microbial consortium can be obtained by repeatedly harvesting the bacteria from the anode, leading to a consortium with coulombic efficiency of 80%.

We, in SRM University, started with a platinum/ carbon based two chamber MFC, converted to a plastic H shaped model, further a single chamber air cathode which is termed as a Salt Bridge-Immersed-Air Cathode (SBIAC) was designed with carbon electrode to make it economical. While we worked on various model designing aspects, we also worked on the physical parameters like salt bridge length, ionic concentration and the agar concentrations, where in we tried to optimize such parameters for better current production. While, the power generated increased with increase in the head space and regular mixing of the substrate. The stacking of MFCs in a series contributed to increased current production.

MATERIAL AND METHODS

MFC Components

Microbial Fuel Cell majorly constitutes electrodes, anodic and cathodic chamber and a salt bridge. The Anodic chamber is anaerobic, holds the substrate and microorganisms as biocatalyst. The cathodic chamber was maintained aerobic with catholyte (air-oxygen rich) in it. The salt bridge that forms a porous barrier between cathodic and anodic chamber, facilitates the transfer of ions (protons). Carbon electrodes (85 cm²) were used as both anode and cathode.

Construction of MFC

Salt Bridge-Immersed-Air Cathode MFC (SBIAC-MFC) consists of a plastic container of capacity 2 liters served as the anodic chamber. The anodic compartment contained the substrate and the carbon electrode (anode ~85cm²). A similar carbon electrode which was used as anode served as cathode. The salt bridge served as an electrolyte in transfer of protons. The cathode was immersed in the salt bridge when it was in molten stage to ensure complete surface contact. The 50% cathode surface was exposed to atmospheric air. Salt bridge employed here was made with 5M NaCl and 10% Agar. The salt bridge was cast in a PVC pipe (12 cm X 2cm). Proper precautions were taken to ensure complete sealing of anodic chamber by means of applying epoxy and wax to ensure anaerobic conditions. The assembly was put together to create a single chamber cell. The external circuit was completed by connecting a resistor (270 Ω) between the two leads of the electrodes. The setup is depicted in the schematic representation of the design is given in Fig. 1.
MFC Operation

Substrate was added in the anodic chamber and was completely sealed to maintain anaerobic condition. The reactor was spurge with CO\(_2\) before sealing completely to ensure complete removal of oxygen. A batch configuration was employed and readings were taken for a period of 21 days\(^2\). The rise and decline in readings was noted for this period and readings were recorded. A graph was generated (Current Vs Time) to visually represent the comparison at the end of the experiment.

Substrate

Waste water which served as the substrate of the MFC was collected from SRM University- Sewage Treatment Plant (100000 cubic meter capacity). The source of the plant includes wastes from laboratories, toiletries etc. The inlet of the lamellar filter, effluent of Carbon Filter and effluent of Sand Filter, Agriculture waste- Paddy Straw, Synthetic Media (Table 1) served as substrates. Synthetic Media A,B and C corresponds to Lovely, Logan and IICT ‘s formulated media. These phases were selected in order to avoid the coarse and large particles found in raw waste water. The natural consortium present in the waste water was majorly used in all studies (unless mentioned).
Table 1: Composition of Synthetic wastewater used in MFC

<table>
<thead>
<tr>
<th>Medium A</th>
<th>Medium B</th>
<th>Medium C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Components (pH 7)</strong></td>
<td><strong>Quantity</strong></td>
<td><strong>Components</strong></td>
</tr>
<tr>
<td>NaCl</td>
<td>8880 g/L</td>
<td>KCl</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>3000 g/L</td>
<td>NaH₂PO₄</td>
</tr>
<tr>
<td>MgCl₂·7H₂O</td>
<td>330 g/L</td>
<td>NaCl</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>275 g/L</td>
<td>NaHCO₃</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>14 mg/L</td>
<td>KCl</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>21 mg/L</td>
<td>NaH₂PO₄</td>
</tr>
<tr>
<td>Na₂HPO₄·7H₂O</td>
<td>56 mg/L</td>
<td>CuCl₂</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>10 mg/L</td>
<td>CaCl₂</td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>5 mg/L</td>
<td>MnCl₂</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>3.1 mg/L</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>2 mg/L</td>
<td></td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>1 mg/L</td>
<td></td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>1 mg/L</td>
<td></td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.1 mg/L</td>
<td></td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.1 mg/L</td>
<td></td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>0.25 mg/L</td>
<td></td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>0.24 mg/L</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mg/L</td>
<td></td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>385 g/L</td>
<td></td>
</tr>
</tbody>
</table>

Microbial Consortium

Heat treatment

The wastewater collected from treatment plant was heat treated at a temperature 90°C to kill the non-spore forming aerobes and methanogens. The heat treated wastewater was cooled down to room temperature and then used as in MFC (SBIAC-MFC). The unaltered wastewater was used as substrate in control MFC (SBIAC-MFC).

Aerobic Inhibitors

The wastewater collected from treatment plant and 2% cystiene HCl and sodium azide which served as the oxygen scavenger were added in the setup. The unaltered wastewater was used as a substrate in control SBIAC-MFC. The reactors were run for 21 days and readings were noted at regular intervals.
RESULTS AND DISCUSSION

Substrate Analysis

Identifying a potential renewable substrate that is enormously available at low cost, and yields high energy for alternate energy production is highly critical. Wastes with high organic content are a good candidature of choice in MFC as a substrate. Wastes from domestic and agriculture were studied. The experiment in which the wastewater collected from the SRM Wastewater Treatment Plant from various stages namely lamellar separator, sand filter and carbon resulted with a maximum current generation of 175 µA, 130 µA and 120 µA respectively between 13th to 18th days. This result also suggests that the integration of Microbial Fuel Cell system at the lamellar stage shall be beneficial. The results suggest that the influent of lamellar separator is an ideal choice. Highly homogenized substrate and availability for microbial consortium can be attributed to the maximum current obtained.

The experiments conducted with synthetic waste as substrate in anodic chamber suggests that the carbohydrate breakdown metabolism shows increased output. This is evident, in the SBIAC-MFC with medium C, Glucose composition was 3% (by volume) yielded a maximum current of 160 µA.

The onset of the peak in the reading is attributed to the acclimatization of the microbes to the new anaerobic atmosphere and the time taken for the microbes to proliferate on the anode surface for efficient electron transfer (Anode acclimatization process).

Table 2: Maximum current generated from various substrate samples used in MFC

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Maximum Current (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamellar Separator</td>
<td>175</td>
</tr>
<tr>
<td>Sand Filter</td>
<td>130</td>
</tr>
<tr>
<td>Carbon Filter</td>
<td>120</td>
</tr>
<tr>
<td>Paddy Straw</td>
<td>150</td>
</tr>
<tr>
<td>Medium A</td>
<td>100</td>
</tr>
<tr>
<td>Medium B</td>
<td>87</td>
</tr>
<tr>
<td>Medium C</td>
<td>160</td>
</tr>
</tbody>
</table>

Fig. 2: Comparison of substrates used.
Microbial Consortium

Adding of aerobic inhibitors like Cysteine HCl and Sodium azide inhibits aerobic organisms in the anodic chamber, increasing the MFC efficiency from 25 to 60%. In the absence of the inhibitor, there were chances for aerobic organisms to thrive in the medium and hence the current generation was less.

Heat treatment decreased the non-spore forming methanogens in the anaerobic system. It was observed that methane accumulation was lower in the system. Alternatively, the faster growth of the spore-forming methanogens may have proliferated and resulted in methane production. This is a more likely explanation as the gas head space removal increased the output.

Table 3: Maximum current generated from MFC after adopting various strategies for Microbial Consortium.

<table>
<thead>
<tr>
<th>Microbial Consortium</th>
<th>Maximum Current (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Treatment</td>
<td>220</td>
</tr>
<tr>
<td>Aerobic Inhibitors</td>
<td>270</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>236</td>
</tr>
<tr>
<td>Standard MFC (Control)</td>
<td>175</td>
</tr>
</tbody>
</table>

Fig. 3: Microbial consortium
Other influencing parameters

Ionic strength of the salt bridge, conductivity of the substrate, pH of the medium, electrode materials, role of inhibiting microorganisms are various other parameters that influence the current generation in MFC. The optimization of every parameter is very critical which is also dependent on each other for efficient results.

There are challenges in using oxygen as a cathode in MFC.

CONCLUSION

MFCs represent a promising technology for renewable energy production; their most likely near-term applications are as a method of simultaneous wastewater treatment and electricity production. They will be useful in other specialized applications as well for example, as power sources for environmental sensors and environmental bioremediation. With modifications, MFC technology could find applications ranging from H₂ production to renewable energy production from agricultural biomass. The ability of a diverse range of bacteria to function and persist in an MFC is a truly fascinating occurrence, and understanding why high bacterial diversion appears to exist in such communities will enhance our knowledge of the microbial ecology of bio-films and bacteria. MFCs are rapidly evolving technologies that will fascinate scientists and engineers who are challenged with disposal and utilization of waste and energy production in the coming decades.

REFERENCES

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