Low Level Electricity Production and COD Removal in Wastewater Using a Dual Chamber Microbial Fuel Cell with Pseudomonas Fluorescens as Biocatalyst

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Abstract. Urbanization intensifies global energy crisis and contributes largely to environmental pollution. The use of microbial fuel cell (MFC) helps in addressing both problems. This study aims to construct a low-cost MFC which simultaneously produce low level electricity and has the capacity to lower chemical oxygen demand in wastewater. The MFC used various carbon sources and copper wire as electrodes. Variation in voltages were observed using various carbon sources in solution. The microbial fuel cell utilizing P. fluorescens was able to produce voltage of more than 800 mV in fermented rice and more than 1000 mV for both sewage sludge and kitchen sink wastewater substrates. It was also observed that the COD reduction was seen in different types of wastewater.

1. Introduction
Today, the main source of energy used around the globe is fossil fuel. However, this energy source is non-renewable and will deplete over time. Also, production and use of fossil fuels cause ecological imbalance [1] as well as environmental hazards [2].

In the Philippines, wastewater treatment facilities are rare with only 5% of the total population connected to a sewer network. Most of domestic wastewater is discharge in major rivers and bodies of water leading to its pollution. Also, new reliable ways of generating electricity is one of the pressing concerns of the county. With that in mind, a method to generate electricity from wastewater is a potential solution to both concerns of the Philippines.

A microbial fuel cell (MFC) is an alternative source of energy that directly converts chemical energy to electrical energy [3] without generating toxic by- product [1]. Aside from electricity production, MFC maybe a potential treatment to wastewater [4] that contains carbohydrate residues.

MFC is an electrochemical cell which is thermodynamically driven by redox processes that occurs in solution with microbes mediating the process. The efficiency of MFC depends on the components such as the type of electrodes (anode and cathode) used, proton exchange membrane (PEM) and microorganism used in the culture. Reactants of MFC are utilized by the microorganisms in the bioreactor and electron transfer leads to the generation of electrical current. Continuous flow can be achieved as long as electrodes and PEM are stable and intact [5].

Microbial fuel cells make use of various microbes as catalysts to carry out oxidation of organic substrate to generate electrical current. An MFC’s efficiency is dependent on the type of substrate used for the consumption of the microbes present in the anode. Using wastewater as a substrate can generate electricity as well as treat the wastewater inexpensively. From agro-wastes, high amounts of...
wastewater rich in starch is disposed without treatment. Particularly, cassava starch production produces starch-rich wastewater that have a high chemical oxygen demand (COD), biochemical oxygen demand, and total solids [1]. In this study, we have constructed a low-cost microbial fuel cell which generates low voltage electricity and simultaneously treat domestic wastewater which is a common scenario in the remote provincial islands in the Philippines. Moreover, the effects of using different substrate components and addition of *Pseudomonas fluorescens* culture as biocatalyst were taken into account in this study.

2. **Methodology**

2.1. **Bacterial culture**

*Pseudomonas fluorescens* was obtained from Philippine National Collection of Microorganisms (PNCM), UP Los Baños (Los Baños, Philippines). The strain of bacteria was grown and amplified using Tryptic Soy Broth at 32°C with pH=5. Broth was prepared according to company’s instructions. Swab from primary culture was transferred to TSB for secondary culture. The secondary culture undergone three consecutive days of subculture for optimization before the culture was amplified. Bacterial growth was observed by indirect method using spectrophotometer. At 600 nm wavelength, optical density of 0.5 was measured before the culture was transferred to chamber for MFC run.

2.2. **Proton exchange membrane**

Gelatinized cassava starch and agar as PEM were subjected to preliminary testing. PEM out of cassava starch was constructed according to Obasi and colleagues’ methods [6]. Cassava starch weighing 85g was dissolved in 75mL water and heated at 80 to 100°C. NaCl (5g) was gently added to the solution when the starch was completely dissolved. The solution was stirred every 5 minutes. After 30 minutes, the solution was allowed to cool down and solidify. On the other hand, 5g of nutrient agar and 5g NaCl was dissolved in 75mL water at 80 to 100°C with continuous stirring. The solution was then solidified at room temperature. The two salt bridges were submerged in water for 24hours. Results showed that PEM made up of agar has firmer, and stable structure as compared to gelatinized cassava starch which partially disintegrates in water at the said time. For this study, salt bridge made up of agar was utilized.

2.3. **Electrode**

Three electrodes made up of different materials (aluminum mesh wire, graphite rod, copper wire) were tested. Each electrode was used for a test run and voltages were compared afterwards. Preliminary data showed that the use of copper wire is best among the three and thus preferred in this study.

2.4. **MFC assembly**

A low-budget MFC setup was assembled. In the design as seen in Figure 1, plastic containers with volume of 750mL were used as cathode and anode chambers. Organic components were placed at the anode chamber where reactions happened in an anaerobic condition. While, an aerated cathode chamber was filled with 600mL KMnO₄ (1.5g L⁻¹). KMnO₄ was changed when it was fully oxidized. Chambers were connected by an agar salt bridge. Agar solution was placed and was allowed to solidify in a PVC pipe with dimensions: 6 inches long and 1.5 inches in diameter. The pipe was secured to chambers with glue gun to avoid any spilling. Voltage difference was measured with DMM via totally submerged copper wire electrodes connected on each chamber.
Figure 1. MFC Design.

2.5. MFC run

Six set-ups were used in the MFC runs in this study. The anodic substrates were varied as seen in Table 1. In the first run, Maltose and cassava starch (with different concentration: 0.5, 1.5, 2.0 g L$^{-1}$) was used as carbon substrates. In second run, the contents of the anode were identical to the first run with the addition of fermented rice as sludge. Cooked rice was fermented for three days before use. In the third run, the contents of the anode was identical to the second setup only with addition of $P.$ fluorescens (150 mL broth). In the fourth run, sucrose and corn starch (with different concentration: 0.5, 1.5, 2.0 g L$^{-1}$) was used as carbon substrates. In fifth run, the contents of the anode were identical to the fourth run with the addition of sewage sludge. Lastly, in the sixth setup, the contents of the anode was identical to the fifth run only with addition of $P.$ fluorescens (150 mL broth).

Table 1. Experimental Setup of MFC Assembly.

| MFC      | Anode                        | Cathode | Electrode  |
|----------|------------------------------|---------|------------|
| MFC Run 1| Maltose (5g L$^{-1}$) + Cassava Starch (0.5, 1.5, 2.0 g L$^{-1}$) | KMnO$_4$ | Copper Wire |
| MFC Run 2| Maltose (5g L$^{-1}$) + Cassava Starch (0.5, 1.5, 2.0 g L$^{-1}$) + sludge (fermented rice) | KMnO$_4$ | Copper Wire |
| MFC Run 3| Maltose (5g L$^{-1}$) + Cassava Starch (0.5, 1.5, 2.0 g L$^{-1}$) + sludge (fermented rice) + $P.$ fluorescens | KMnO$_4$ | Copper Wire |
| MFC Run 4| Sucrose (5g L$^{-1}$) + Corn Starch (0.5, 1.5, 2.0 g L$^{-1}$) | KMnO$_4$ | Copper Wire |
| MFC Run 5| Sucrose (5g L$^{-1}$) + Corn Starch (0.5, 1.5, 2.0 g L$^{-1}$) + sewage sludge | KMnO$_4$ | Copper Wire |
| MFC Run 6| Sucrose (5g L$^{-1}$) + Corn Starch (0.5, 1.5, 2.0 g L$^{-1}$) + sewage sludge + $P.$ fluorescens | KMnO$_4$ | Copper Wire |

2.6. Wastewater treatment

Rather than voltage production, the ability of MFC to treat wastewater was also tested. Using the MFC design setup seen in Figure 1, Kitchen sink wastewater was collected from Sun Residences (Manila, Philippines). $P.$ fluorescens was added to 600 mL wastewater and served as anode substrates. In another run, $P.$ fluorescens was added to sewage sludge and served as anode substrates. The effectiveness of the constructed MFC in treating kitchen wastewater and sewage sludge was determined by measuring the Chemical Oxygen Demand of the sewage sludge before and after 24 hours of the MFC run. Samples were brought to Mach Union Laboratory (Las Piñas, Philippines) for COD tests.
3. Results and discussion

3.1. Bacterial growth
Selection of bacterial biocatalyst is critical in voltage production. Metabolism of bacteria and its electrogenic properties will determine the conversion of substrate and transmission of electrons. In this study, *Pseudomonas fluorescens* was used. *P. fluorescens* is a Gram-negative, motile rod-shaped bacteria. It is a chemoorganotroph that can grow in an aerobic or anaerobic environment. This is due to its metabolism variability [7]. It is closely related and mostly have same properties as *P. aeruginosa* which is a successful electrogenic bacteria that produces metabolites that serve as electron shuttles.

Bacteria was first optimized to minimize bacterial mutations and to make sure that only young bacterial cells were isolated and amplified. Rate of growth of organism was observed using spectrophotometer. It was made sure that the bacteria to be used is under its exponential stage. This is verified by having 0.5 optical density under 600nm wavelength.

As seen in Figure 2, the data shows that as time increase the optical density increases. This indicates that as time increases, the bacterial population increases as well. However, during the 215th minute run, the optical density starts to become relatively constant. This indicates that the bacteria enter its stationary phase of its growth phase. This may due to an essential nutrient depleting or a formation of an inhibitory product.

![Figure 2. Bacterial Growth of Pseudomonas fluorescens.](image)

In this study, the *P. fluorescens* culture is added to the anode when the culture is during its exponential phase.

3.2. Voltage production
The effects of different anodic substrate used were tested in this study. Substrates were selected in consideration to the microorganism that will be utilized. The team used maltose as the main carbon source since *P. fluorescens* was unable to ferment glucose [8]. Since *P. fluorescens* is abundant in leguminous roots, cassava starch was used and added.

In the MFC 1 run, maltose and different cassava starch concentrations (0.5, 1.5, 2.0g L⁻¹) were used as anodic substrates. Based on the result graphed at Figure 3, the anode chamber containing the lowest concentration of cassava starch generated the highest voltage for hours. While the anode chamber containing the highest concentration produced the lowest voltage and decreased drastically starting its 8th hour run.
Compared to the MFC 4 run, the anodic substrate used were sucrose and different corn starch concentrations (0.5, 1.5, 2.0 g L\(^{-1}\)). As seen in Figure 4, the anode substrate with the highest corn starch concentration (2.0 g L\(^{-1}\)) generated the highest voltage throughout the entire run.

In the MFC 2 run, fermented rice was added to the anode containing maltose and cassava starch. The presence of sludge allows the growth of mixed culture of external microorganisms. Several studies stated that mixed culture inoculum produces higher voltage measurement. As seen in Figure 5, the addition of sludge (fermented rice) to the setup gave lower initial voltage than the first run. It is also observed that highest concentration of cassava starch also generated the highest voltage. Voltages at all concentration have stable measurement at range of 2 to 8 hours and begin to ascend at 9-hour run.

**Figure 3.** Voltage Generated with anode having maltose:cassava starch.

**Figure 4.** Voltage Generated with anode having sucrose:corn starch.
In the MFC 5 run, sewage sludge was added to the anode containing sucrose and corn starch. This was done to investigate the effects of a mixed culture inoculum to the voltage generation. As seen in Figure 6, the addition of the sludge to the setup gave higher initial voltage readings than the setup for the anode substrate containing 0.5 and 1.5 g L\(^{-1}\) corn starch while lower initial voltage readings for the anode substrate containing 2.0 g L\(^{-1}\) corn starch. It can also be observed that the anode substrate containing 2.0 g L\(^{-1}\) corn starch generated the highest voltage. Several studies showed that a mixed culture inoculum resulted to a higher voltage generation. Upon the comparison of MFC 4 run and MFC 5 run, the run containing a mixed culture inoculum generated a much greater voltage (1255 mV) compared to the setup without it (719 mV). The voltage readings taken from all set-up had steadily increased for 13 hours and stabilized after the 13\(^{th}\) hour run.

For the MFC 3 run, *P. fluorescens* was added. Figure 7 shows that with the presence of *P. fluorescens* voltage at each concentration produce longer hours of stable voltage measurement.
Lastly, for the MFC 6 run, *P. fluorescens* was added to serve as a biocatalyst for voltage generation. As seen in Figure 8, the addition of *P. fluorescens* allowed relatively constant voltage generation for longer hours. The voltage generated for all anode substrates steadily increased for 7 hours and remained relatively constant after the 7th hour run. Like the MFC 5 run, the anode substrate containing 2.0 g L\(^{-1}\) corn starch generated the highest voltage.

*P. fluorescens* produces pyoverdine, a siderophore that contributes to production of biofilm and serves as colonization factor. As known, biofilm formation is needed to obtain desirable voltage results [9]. Pyoverdine is released when under a stressed environment, when it is on anaerobic and iron-limited conditions [10].

Based from the results of the MFC runs, it can be inferred that concentration of added cassava starch do not have any relationship with the voltage generated by the bioreactor. On the other hand, it can be inferred that concentration of corn starch added to the anode substrate has a direct relationship with the voltage generated by the microbial fuel cell.

### 3.3 Wastewater treatment

After the MFC run, kitchen wastewater was found to have reduced COD measurement. Based on the COD result, from 14,433 mg L\(^{-1}\) COD it decreased to 6,200 mg L\(^{-1}\) after 24h of MFC run. This only imply that the constructed MFC was able to significantly treat kitchen wastewater based on COD. In addition, we measured the voltage after 24h of MFC ran and found that kitchen sink wastewater...
substrate with \textit{P. fluorescens} generate more than 1200 mV. This value is higher than the other set-ups. This may be a result of presence more bacterial culture than that of fermented rice substrate. Similarly, After the microbial fuel cell run with the anode containing sewage sludge, the COD measurements of the sewage sludge decreased from 15,449 mg L\(^{-1}\) to 6,096 mg L\(^{-1}\) after 24 hours of the MFC run. This only shows that the constructed microbial fuel cell was able to treat sewage sludge based on the COD results.

4. Conclusions
In this study, the constructed low-budget MFC utilizing the mixed inoculum from the fermented rice was able to produce a voltage of 845 mV. Upon the addition of \textit{P. fluorescens} to the anode containing the substrates and fermented rice, the MFC was able to produce voltage of 830 mV. The addition of \textit{P. fluorescens} made it possible to generate a voltage of more than 800 mV for a longer period. The MFC was able to produce voltage of more than 1200 mV utilizing sewage sludge without \textit{P. fluorescens} and 1200 mV for longer periods with \textit{P. fluorescens}. Using the MFC design, the COD measurements of the sewage sludge decreased from 14,433 mg L\(^{-1}\) to 6,200 mg L\(^{-1}\) after 24 hours of the MFC run. It was also able to reduce the COD measurements of the kitchen wastewater from 15,449 mg L\(^{-1}\) to 6,096 mg L\(^{-1}\) after 24 hours of the MFC run. Though this study is considered to be a success, there are still many things to be considered and methods to be tested for MFC improvement.

5. References
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