# Creating Sustainable Energy from Microbial Interactions in Various Media

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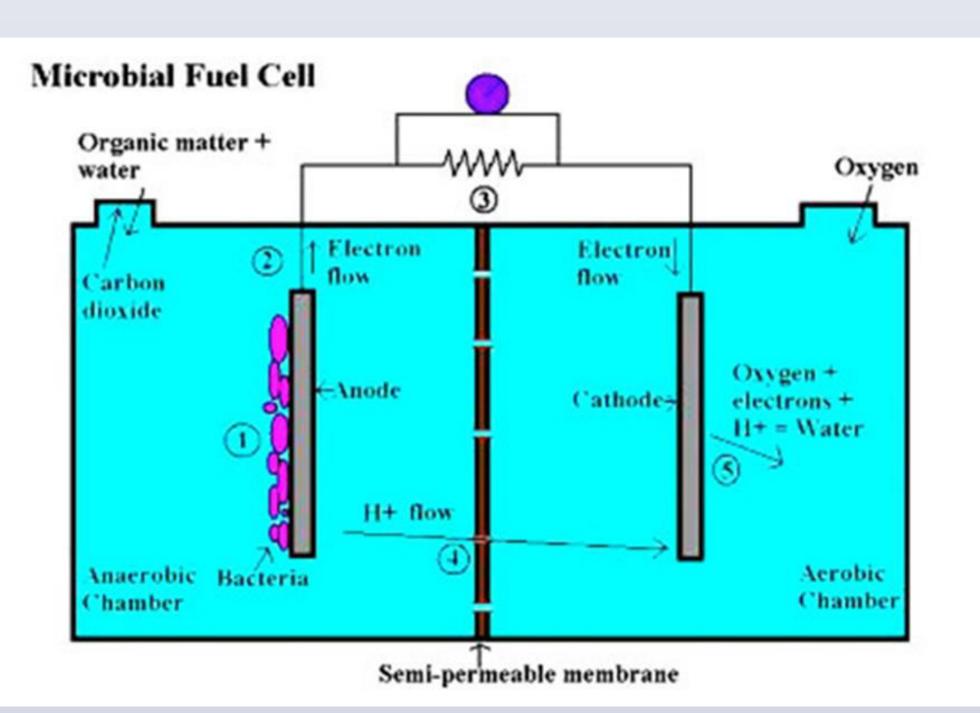
## Introduction

In order to cope with the growing energy needs of the 21st century, alternative energy sources that are quickly constructed, easily implemented, and provide long-term sustenance, are greatly needed. Microbial fuel cells (MFCs) provide a solution to this problem, as they generate an electrical output through the oxidation-reduction reactions that microbes carry out on a daily basis in order to survive. However, existing MFCs use specific microbes, only available in a laboratory setting, and hazardous materials which serve as electron mediators. A soil-based MFC, however, does not have any constraints regarding the materials involved in its construction. Soil taken from a riverbed is teeming with bacteria that can form a biofilm, an aggregate of various bacterial colonies that share resources. Through our experiment, we have seen that the soil-based MFC produced a voltage significantly higher than that of the yeast MFC, which was a model of an MFC usually constructed in laboratories. Soil based cells are not only much more energy and cost efficient, but also more readily available.

## **Engineering Goal**

The viability of the output of a soil-based microbial fuel cell has yet to be tested against other, more commonly used microbial fuel cells. Thus, the engineering goal of this project was to build a twochambered microbial fuel cell that generates a sustainable, measurable electrical output in mV from benthic mud collected at a local second order stream. In order to compare voltage outputs, we also engineered a yeast cell that was similar to conventional MFCs constructed in research labs.

## **Discussion of Theory**



Mercer. (Designer). Microbial Fuel Cell [Web Graphic]. Retrieved from http://illumin.usc.edu/assets/media/175/MFCfig2p1.jpg

In the construction of an MFC, microbes oxidize organic matter in the cathode. The electrons lost travel to the cathode, where they bond with dissolved oxygen in the water of the cathode, and the hydrogen ions in solution to form pure water. To maintain the charge balance between the anode and cathode, a proton exchange membrane (PEM) allows for the flow of H+ ions to the cathode as necessary. Carbon dioxide released by the bacteria through their metabolic processes is excreted as a waste product, along with water. The survival of the microbes present in the anode is critical to the electrical output of the cell, as their cellular machinery allow for the redox reaction to occur. The reactions that occur in both the cathode and anode are shown below: Anode Reaction:

 $D - [C_6H_{12}O_6] + 2ADP + 2PO_4^{3-} \rightarrow 2C_3H_3O_3^- + 2ATP + 6e^- + 6H^+$ Cathode Reaction:

 $4H^+ + O_2 \rightarrow 2H_2O$ 

## Procedure

Benthic mud samples taken from a local second order stream and collected stream water serve as the materials placed in the anode and cathode respectively. For the yeast cell, a solution containing 500 mL of 100 g / L yeast extract, 250 mL of 10 mM methylene blue solution, and 500 mL of 3M Dextrose monohydrate solution, all prepared in a 0.1 M phosphate buffer, were added to the anode.



Image taken by the student researchers

controls ion flow.

A salt bridge, containing a solution of 30 g agarose powder, 6 g potassium chloride, and 300 mL of water was refrigerated overnight, and fitted between the two chambers of the cell.

Two electrodes of surface area 50 square centimeters were constructed by attaching a copper wire to

carbon cloth with nickel epoxy. Another piece of carbon cloth was then placed over the wire to maximize surface area

Two holes of 3/4 inch diameter were drilled on the sides of acrylic containers. The two containers will serve as the two chambers of the MFC, connected by a salt bridge that

Figure I: The voltage shown over time for a soil-

underwent a several decline before re-stabilizing

based MFC with an oxygen pump to ensure

dissolved oxygen. After hour 83, the voltage

at approximately 170 mV.



Image taken by the student researchers

In the construction of the first soil MFC, an aquarium pump was placed in the cathode, and wires attached to a multimeter were attached to the ends of the electrodes. Towels were placed by the salt bridge to prevent



A Fluke ® multimeter was

attached to a laptop.

recorded at 45 second

intervals. For the yeast

based fuel cell, a GoLink

® probe was connected

collection and analysis.

where voltage was

to the LoggerPro

software for data



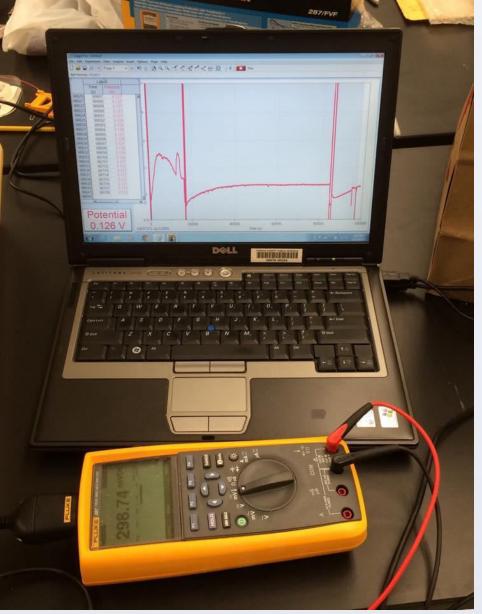


Image taken by the student researchers

## Results

Image taken by the student researchers

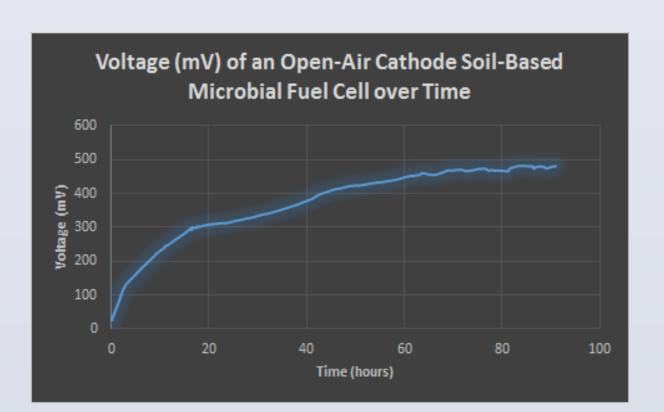


Figure II: The voltage shown over time for a yeast-based microbial fuel cell. During the first 40 hours of experimentation, rapid fluctuations were observed in the voltage before finally settling at 150 mV.

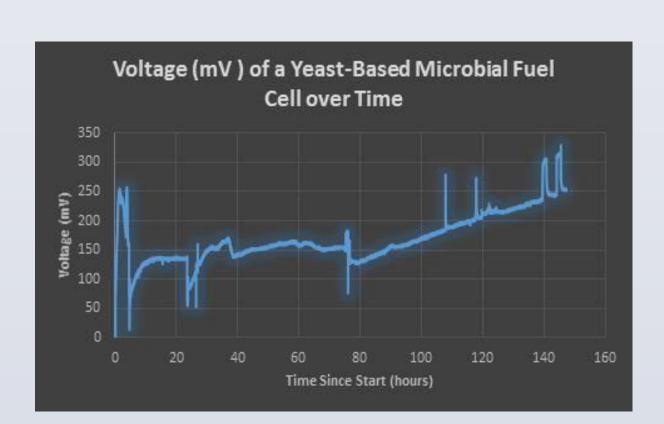


Figure III: The voltage shown over time for a soil-based MFC with an open-air cathode. The voltage exhibited a steady rise, with a relative leveling out at 450 mV at 80 hours. There were no sudden observed drops in voltage throughout the data collection period.

## Analysis

The results obtained from the logarithmic growth exhibited by the open-air cathode cell can be modeled by the following equation, which displayed an R<sup>2</sup> value of 0.98, lending accuracy to the curve modeled. V(t) = 99.903 ln(x) + 22.951

From the closed cathode MFC, the prior to the decline in voltage, the growth also exhibited a logarithmic patter, modeled by the following regression at an R<sup>2</sup> value of 0.95. V(t) = 65.835 ln(x) + 5.903 As both trials were conducted using a similar volume of substrate in the cathode and anode, a comparison of the average voltage generated over the course of 80 hours, would yield an accurate comparison of the effectiveness of both cells. To obtain the average voltage over 80 hours, an integral over the time period is required to be taken.

Such an analysis yielded an average voltage of 366 mV for an open-air cathode MFC. When compared to the average voltage of 225 mV for a closed cathode soil MFC, an open-air cathode yields a comparatively higher voltage. In the calculation of the voltage density of benthic mud samples, the average voltage produced was divided by the volume of cathode space used, yielding a voltage density of 0.41 mV/ cm<sup>3</sup> for the open-air cathode cell. At the new perceived equilibrium point of 480 mV however, a 0.53 mV/cm<sup>3</sup> voltage density is obtained. Based upon an analysis of the ratios of the coefficients for the modeled voltage for both types of cells, the open-air cathode soil-based MFC is proven to be ~1.5 times more effective than the mechanically oxygenated soil-based MFC.

Although the resistance of the circuit varies as the reaction proceeds, at equilibrium, a measured resistance of  $5\Omega$  was obtained. Using Ohm's Law, 96 mA of current is generated through the circuit, yielding a power 46 mW.





Figure IV and V: Photos of the yeast-based MFC (left) and soilbased MFC (right) during operation. The oxygen pump was only utilized for the yeast-based MFC. Image taken by student researchers.

### Discussion

In all three data collections, the initial rapid increase in voltage could be attributed to the rapid formation of microbial biofilms over the carbon cloth electrode. To form such biofilms, appropriate bacteria or yeast needs to aggregate together. Then, as the microbes began to aggregate together to form biofilms at an increasing rate, the voltage generated displayed similar growth. However, there is a limit to the amount of bacteria present in the benthic mud sample, and as that limit reached, the rate of growth appropriately declined until the voltage peaked. Similarly, there was a limit to the amount of yeast. Additionally, the proportion of electrical current that could be generated from the MFC has been shown to be proportional to the surface area of the electrode. Hence, the carbon cloth has a surface area of 50 centimeters squared, presenting a limit to the amount of bacterial electron transfer through oxidative reactions.

In the first soil MFC, the cause of the sharp decline after 80 hours has not appeared in similar experiments using an MFC. However, analysis of biofilm formation has shown that after a surface has been coated with a layer of biofilm, the process of replacement after bacterial death is slow to occur, but can stabilize over time. (O'Toole et al.) This provides a possible explanation for the sharp decline in bacterial growth following the peak voltage. If large numbers of the prokaryotic bacteria were to die while still physically connected to the carbon cloth, then a sharp decline in voltage could be expected. However, the dormant bacteria would eventually be replaced with new, health bacteria, who would continue performing cellular respiration, re-stabilizing the voltage. However, due to the rate of bacterial death, and the slower onset of replacement, the voltage would continue at a significantly lower output range. However, replacement of the organic matter found in the benthic mud would most likely allow for a sustained voltage.

The second trial of a soil-based microbial fuel cell with an open-air cathode yielded more encouraging results, yielding a stabilizing voltage output of approximately 480mV. Furthermore, we did not use an oxygen pump to dissolve in oxygen in the cathode; this was done to determine if the air pump is vital in the construction of MFC.

The yeast microbial fuel cell, which requires mediating reagents to shuttle electrons outside the yeast cells, was compared to the soil MFC, which does not require mediators, as bacteria naturally have the mechanism to shuttle the electrons out of their cell. From the data, the yeast MFC settled around 150mV, which was comparatively lower than 480mV, the voltage output yielded by the soil MFC in the second trial. This seems to suggest that mediator-less MFC is more efficient, as they do not require additional reagents to shuttle the electrons from inside of the cell body to the electrode.

#### Conclusion

- The open-air cathode soil-based MFC yielded a significantly higher average voltage than either of the two other MFCs (Yeast MFC & Closed-air cathode soil MFC).
- Both soil-based MFCs exhibited less fluctuation in their voltage as compared to the
- The construction of the soil-based MFC is easily reproducible, as it only requires a source of benthic mud and stream water.
- The open-air cathode soil-based MFC had a positive net electrical output, as an oxygen pump was not required in the engineering design.
- Water was produced in the cathode during the reduction reaction.
- A soil-based MFC presents a promising novel potential source of clean energy.

#### **Application**

Availability of energy and accessibility to clean water are two of the fifteen Millennium Project goals. Soil-based microbial fuel cells represent a stride forward in the availability of clean energy. Due to the fact that their fuel source is benthic mud, and the cathode is simply composed of cold stream water, the materials necessary for the construction of a soil MFC are easily obtained. Additionally, soil MFC's have a shown durability, as they were able to exhibit sustained growth over a period of ninety hours with no fuel addition.

The production of pure water at the cathode of the soil-based MFC presents another application that should be pursued to the fullest. Although a mechanism to harness the pure water and separate it from the stream water already in the cathode does not yet exist, a method to filter these newly created water molecules presents a promising potential to generate not only electricity but also clean water.

The maximum voltage observed throughout the operation of the open-air cathode soil-based MFC was ~490 mV. Although the resistance in the circuit was not continuously measured, a measurement taken during the peak interval revealed a resistance of ~5 ohms, amounting to ~50 milliwatts of power, which is enough, if hooked in series, to power low voltage infrared LED. Multiple electrodes allow for increased biofilm surface area, also increasing output. Soil-based MFC's could utilize various wastewater treatments instead of using stream water,

while using the very same wastewater in the cathode. Because the presence of bacteria and organic material are the only two prerequisites to the generation of electricity from an MFC, many MFC's could move into various sectors in industry, utilizing the waste to produce an electrical output.

#### References

Deng, H., Wu, Y. C., Zhang, F., Huang, Z. C., Chen, Z., Xu, H. J. and Zhao, F. 2014. Factors affecting the performance of single-chamber soil microbial

Huang, D., Zhou, S., Chen, Q., Zhao, B., Yuan, Y., & Zhuang, L. (2011). Enhanced anaerobic degradation of organic pollutants in a soil microbial fuel cell. Chemical Engineering Journal, 172(2-3), 647-653

Kim, H., Park, H., Hyun, M., Chang, I., Kim, M., & Kim, B. (2002.). A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme and Microbial Technology, 145-152. Logrono, W., Recalde, C., & Echeverria, M. (2014). Influence of cell size on performance of microbial fuel cells of single chamber using organic solid

waste with Amazonian and High Andeans soils from Ecuador. New Biotechnology, 31 (Supplement), S213. O'Toole, G. (2000). Biofilm formation as microbial development. Annu Rev Microbiol., 54, 49-79. Pant, D., Bogaert, G., Diels, L., & Vanbroekhoven, K. (2010). A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy

Rahimnejad, M., Najafpour, G., Ghoreyshi, A., Shakeri, M., & Zare, H. (2011). Methylene blue as electron promoters in microbial fuel cell. International

Raj, S. B., Solomon, J. R., Prathipa, R., & Kumar, A. M. (2013). Production of Electricity from Agricultural Soil and Dye Industrial Effluent Soil Using Microbial Fuel Cell. International Journal of Research in Engineering and Technology, 2(10), 140-148 Sayed, E., Saito, Y., Tsujiguchi, T., & Nakagawa, N. (2012.). Catalytic activity of yeast extract in biofuel cell. Journal of Bioscience and Bioengineering,

Zhang, X., Shi, J., Liang, P., Wei, J., Huang, X., Zhang, C., & Logan, B. (2013). Catalytic activity of yeast extract in biofuel cell. Bioresource Technology,