Pursue: Undergraduate Research Journal

Volume 3 | Issue 1 Article 1

2020

Harvesting Electrical Energy Produced by Electrogenic Bacteria in Microbial Fuel Cells

Chinedu Ahuchaogu
University of Maryland Eastern Shore

Kausik Das
University of Maryland Eastern Shore, kdas@umes.edu

Harvesting Electrical Energy Produced by Electrogenic Bacteria in Microbial Fuel Cells

Cover Page Footnote

This work was partially supported by the National Science Foundation (HBCU-UP, Award #1719425) and the Department of Education (MSEIP, Award #P120A70068).

Harvesting Electrical Energy Produced by Electrogenic Bacteria in Microbial Fuel Cells

Chinedu D. Ahuchaogu and Kausik S. Das

Department of Natural Sciences, University of Maryland Eastern Shore

Corresponding Author:

Kausik S. Das

Department of Natural Sciences

University of Maryland Eastern Shore

Princess Anne, MD 21853

kdas@umes.edu

Abstract

Cellular respiration is the process by which organic matter oxidizes, and the energy stored in the chemical bonds of the food releases. Normally, cellular respiration occurs inside the mitochondria of cells; however, a unique type of bacteria releases electrons externally. These specialized organisms are called electrogenic bacteria. Our goal is to construct a microbial fuel cell (MFC) with electrogenic bacteria, harvest the external electrons created by cellular respiration, and channel them through an external circuit to generate electricity. Mud soil, which has a high number of electrogenic bacteria in the environment, was used to construct an MFC. In the presence of gram-negative bacteria, which exist in both aerobic and anaerobic conditions, the constructed MFC delivered electrical energy to an external circuit. The MFC can generate electricity, and thereby power, from biodegradable substances and organic wastes found in the environment and landfills. They can also be used to power small devices and sensors used in day-to-day activities. To determine the effect of sugar on the growth and development of bacteria present in the MFC, the quantity of sugar administered will be monitored in relation to the power generated per day.

Keywords: Power generation, Electrogenic bacteria, Bioenergy, Microbes, Microbial Fuel Cell.

Introduction

With an increase in the generation of organic waste and the constant need for electricity in day-to-day activities, the use of microbial fuel cells (MFCs) is gaining momentum. MFCs can be used to treat wastewater and generate electricity from organic waste (Mercer, 2010). Also known as the redox reaction, two key reactions occur in MFCs: 1) reduction of hydrogen and 2) oxidation (loss of electrons); they occur in separate regions of the fuel cell (Rozendal, Hamelers, Rabaey, Keller, & Buisman, 2008). Also known as biological fuel cells, MFCs can drive current using microbes/bacteria found in the environment in their natural state. In MFCs, microorganisms undergo a major bioelectrochemical reaction, which converts the mass of organisms in each area into electricity or hydrogen/chemical products (Pant et al., 2012). Ideally, an MFC consists of a cathode, where hydrogen ions generated by the microbes interact with the electrons and undergo reduction and an anode where oxidation occurs. The cathode and anode are typically separated by a proton exchange membrane, such as porous mud (Ghasemi et al., 2012). The organic electron donor is present in the anode chamber, where oxidation takes place (Liu & Logan, 2004). During the generation of electrons by the microbes, a biofilm is developed around the anode, and this is spurred by the growth of cells on the surface of the electrode (Gottenbos, Vander Mei, & Busscher, 1999). The biofilm found in MFCs accommodates microbes, which allows free electrons to be transmitted to the anode. For an MFC to function effectively, electrons generated by the bacteria in the anode chamber must flow through a wire to the cathode where it reacts with oxygen from the exposed cathode (Lohner & Rowland, 2016) for continuous current. The electron donor produces CO_2, protons, and electrons when oxidized, as seen in equation 1. The protons produced at the anode pass through the proton exchange membrane to the cathode (Rahimnejad, Najafpour & Ghoreyshi, 2011), and the reaction of protons and electrons at the cathode in the presence of oxygen results in the formation of water as seen in equation 2. Based on the level of energy generated, the MFC can be renewable sources of energy for small devices such as biosensors (Rahimnejad, Adhami, Darvari, Zirepour, & Oh, 2015).

$$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \quad (1)$$

$$2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O \quad (2)$$

The MFC has two halves: aerobic and anaerobic (Mercer, 2010). The aerobic chamber has a positively charged electrode and is oxygenated. The anaerobic chamber does not have oxygen, thereby enabling a negatively charged electrode to serve as the electron acceptor for the bacterial process.

There are two types of MFCs: mediated and mediator-free (Huang, Zeng, & Angelidaki, 2008).

- Mediated MFCs: The mediators in these MFCs spur or facilitate the movement of electrons to the electrode from the microbial cells (Delaney et al., 1984/2008). Examples of such mediators are thionine, methylene blue, and benzyl viologen (Roller et al., 1984). Mediated MFCS mainly utilize electrochemically inactive microbial cells.
- Mediator-free MFCs: In this case, electrons move to the electrodes via electrochemically active bacteria. Examples of such bacteria are Shewanella sp (Kim, Kim, Hyun, & Park, 1999a) and Geobacteraceae (Bond & Lovley, 2003).

The kind of electron transfer mediator and the bacteria used determine the efficiency of an MFC, which is often measured by the quantity of oxygen consumed (Roller et al., 1984). Thus, the MFC needs to operate at a pH close to 7 and a temperature between 68°F and 104°F (20°C and 40°C) (Bullen, Arnot, Lakeman, & Walsh, 2006).

Shewanella sp. is gram-negative bacteria that can respire in both aerobic and anaerobic environments (Nordberg et al., 2014). They possess thread-like structures known as flagella that enable motility and aid in generating and passing of electrons. Shewanella appears rod-like, as seen in Figure 1A. Shewanella form a biofilm on the anode in which they stick together (Hall- Stoodley, Costerton, & Stoodley, 2004) and produce protons. This biofilm helps decompose acetate to generate electricity (Reguera et al., 2006).

Geobacter sp. live in anaerobic conditions, which has made them relevant in the bioremediation of organic compounds (Childers, 2002) and the production of electricity. They are gram-negative bacteria that generate electricity by oxidizing compounds and reducing the anode where they are attached. Geobacter have long nanowires known as pili--extracellular tubules believed to conduct the flow of electrons. The high level of electron transfer via the pili encourages the collation of Geobacter at the anode, the formation of a thick biofilm, and the generation of current

(Reguera et al., 2006). The pili can grow up to 20 micrometers (Strycharz-Glaven, Snider, Guiseppi-Eliec, & Tender, 2011). Figure 1B shows the nature of a Geobacter.

Figure 1A: Shewanella sp. all connected with the flagella

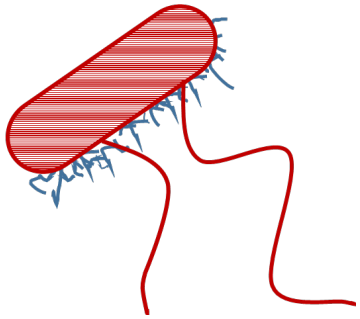


Figure 1B: Geobacter sp. with the pili

Due to *Shewanella sp.*'s respiration mode, there can be a correlation between the electricity generation and the growth of the bacteria on the electrodes. In our experiments, we have tried to quantify that effect. It is important to note that both bacteria possess flagella/pili, which aid in the generation and transmission of electrons. Mud soil may naturally have a high number of microbes in the environment; therefore, mud soil has the potential for high energy generation. With this in view, we used mud soil collected from a field beside a poultry farm to create a model MFC system and test the hypothesis that energy generation will increase when table sugar is present with the MFC's microbes.

Materials and Method

Various structures can improve the performance of MFCs (Du, Li, & Gu, 2007). The optimal design is necessary for maximum efficiency and power generation. Two identical MFCs were assembled, one to serve as a control, which has no treatment after setting up, and the other was supplied with sugar as a food source. The number of bacteria were measured at the inception of the project and when sugar was added to one MFC, and the power output was compared with the control.

MFC Preparation

A Sieve #18 with a 1.00 mm opening and 0.0394 inches was used to strain the soil. Small, hard particles such as rocks and pebbles were removed. These particles are

removed to reduce the chances of aeration within the soil, particularly for bacteria which operate in anaerobic condition. Two teaspoons full of the soil were scooped into a beaker and set aside after the soil was prepared. This beaker of soil is used to measure the initial quantity of bacteria in the soil (See Bacteria Count Section). After preparing the soil, the MFCs were assembled and labeled as "control" or "sugar." The MFC units are filled with soil up to the point marking 1 centimeter on the unit and patted to give the soil a smooth surface. It is important to make a smooth surface to avoid any form or aeration which might affect the bacteria within the anode compartment. The anode was placed on and pressed against the soil to remove air bubbles. A wire was connected to the anode and stretched on the side of the unit. The MFC is then filled with more soil up to the 5-centimeter mark and patted to get a smooth surface. The cathode, which is a thicker, black graphite foam with another wire connected to it, was placed on the soil. It is important to avoid any form of liquid or soil to cover the top of the cathode as it might affect the conversion of oxygen to water. The cathode wire was placed into the "+" port of the hacker board (MudWatt Inc.), and the anode wire was placed into the "-" port of the same. A 10μF 50 V capacitor and a LED were then plugged into the ports of the hacker board. It is important that the capacitor and LED are inserted the right way to avoid any form of interference.

Bacteria Count

Comparing the total number of cells present in the MFCs at the inception of the project to the number present at the time sugar is added gives a better understanding of how the cell count affects the level of power generated by the MFCs. After setting up the MFCs, the beaker of soil that was collected was used for the initial bacteria count. Three beakers are prepared and labeled A, B, and C for a serial dilution factor of 10^{-2}, 10^{-4} and, 10^{-6}, respectively. A balance (Ohaus Adventurer AR3130) was used to measure 0.5 g of soil that was placed in Beaker A; each beaker was then filled with 49.5 mL of distilled water. Beaker A was stirred thoroughly with a sterilized spoon. Afterward, 0.5 mL of the soil-water mixture from Beaker A was aspirated with a pipette and added to Beaker B, which was then mixed with a sterilized spoon. Beaker C received 0.5 mL of the solution from Beaker B and was stirred. A sample (0.5 mL) was drawn from each beaker and dropped on the surface of a labeled nutrient agar plate and spread over the surface of the agar until the surface appears dry. The lid is closed, and the plates are then inverted and incubated at 85°F (29.44°C) for 24 hours in an incubator (Quincy Lab Inc., Model 10-140). Sugar was added to one MFC (See Power Generation Section), and another bacteria count was done, following the same process.

However, a more diluted sample was needed because of the increased number of bacteria. Dilutions of 10^{-6}, and 10^{-8} were prepared for these experiments.

Electricity Generation in MFCs

The LED connected to the external surface of the MFC starts to blink once the MFC generates electrical energy. The rate of blinking served as a measurement of power generated. The time difference between each blink was tracked with a stopwatch and recorded.

Measuring Power Generated

The voltage generated by the MFCs was measured daily to check the power generated. Measuring voltage generated from a microbial fuel cell requires a connection to the hacker board and special configuration. The LED and capacitor were removed from the board, and a WPA N73 Resistance Box Voltage Divider was plugged into the circuit to measure the potential drop across the external resistance. For this experiment, seven resistors were used: 4700 Ω, 2200 Ω, 1000 Ω, 470 Ω, 220 Ω, 100 Ω, and 50 Ω. When plugged, the resistor was left on for at least five minutes before the voltage generated is measured by a multimeter (Keithley 2450 SourceMeter, Tektronix, Inc, Beaverton, Oregon). The voltage was checked for each of the resistors on both MFCs. After the voltage was measured, the LED and 10μF 50 V capacitor were placed back into the hacker board. Figure 2 shows the schematic of the whole MFC set up.

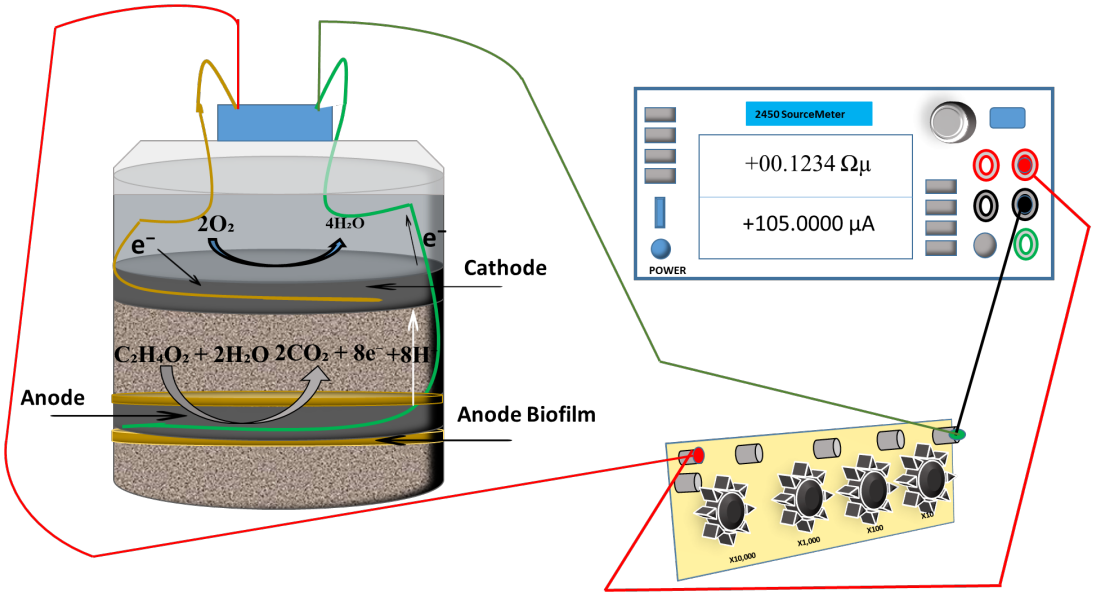


Figure 2: Schematic of the microbial fuel cell

Power Generation

As the level of power generation from both MFCs stabilized, table sugar was added to the MFC labeled "Sugar." The addition of sugar to the fuel cell was completed as follows. Table sugar (0.5 g) was added to a beaker of 10 mL of distilled water. All the cables were disconnected from the hacker board, and the lid was opened. A teaspoon scooped ~1 cm of soil into the unit. A transfer pipette was used to spread all of the sugar solution (10mL) on top of and mixed into the soil. After ~5 minutes, the MFC was reassembled. At the same time a soil sample is taken for a bacterial count using the same methods as stated above. After five days, another 0.5 g of table sugar is added to the MFC. From the decline in the number of blinks observed on the LED and the voltage measured, another 0.5 g of sugar would be added to the MFC after seven days.

Results

Cell Count for MFC

To determine the number of cells present, we counted the number of clusters present on the nutrient agar plates after incubation for 24 hours. The control plate with the 10^{-4} dilution had 147 cell clusters (Figure 3A), and the control plate with the 10^{-6}

dilution had 128 cell clusters (Figure 3B). The second round of cell counting was completed after the addition of distilled water and/or table sugar and is detailed in Table 1. The lower solution shows a significant reduction in the number of clusters, this is a result of the dilution from preceding plates as seen in Figure 4C and 4D.

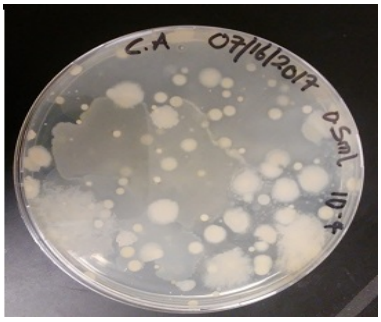


Figure 3A: Nutrient agar with the 10^{-4} dilution of the initial soil

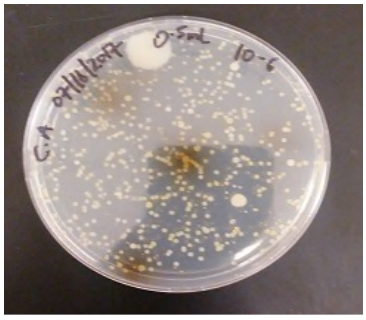


Figure 3B: Nutrient agar with the 10^{-6} dilution of the initial soil

Table 1: Cell counts based on the dilution factor for each MFC.		
Dilution	Number of Cell Clusters for MFC (Control)	Number of Cell Clusters for MFC (Sugar)
10^{-6}	49	30
10^{-8}	25	27

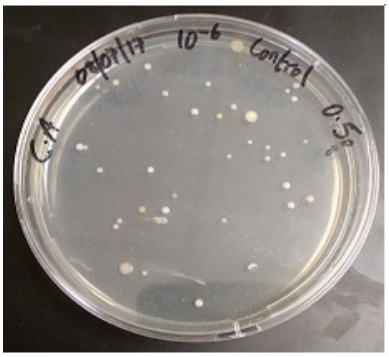


Figure 4A: Nutrient agar with the 10^{-6} dilution for control MFC

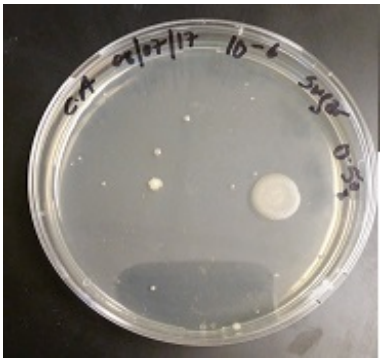


Figure 4B: Nutrient agar with the 10^{-6} dilution for sugar MFC

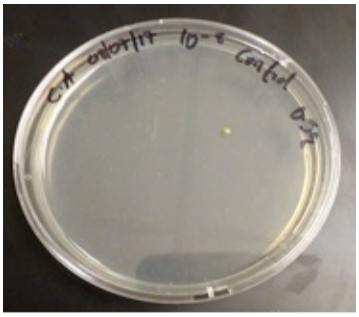


Figure 4C: Nutrient agar with the 10^{-8} dilution for control MFC

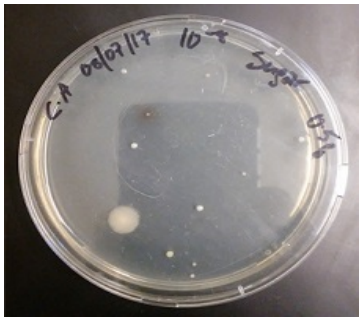


Figure 4D: Nutrient agar with the 10^{-8} dilution for sugar MFC

Power Output from MFCs

The voltage measured across each resistor was tabulated for each day as shown in Table 2. The power generated was then calculated from Ohm's law, as shown in equation 3.

$$P = V^2/R \qquad (3)$$

Where P is power in Watt (W)
V is the voltage in volts (V)
R is resistance in ohms (Ω)

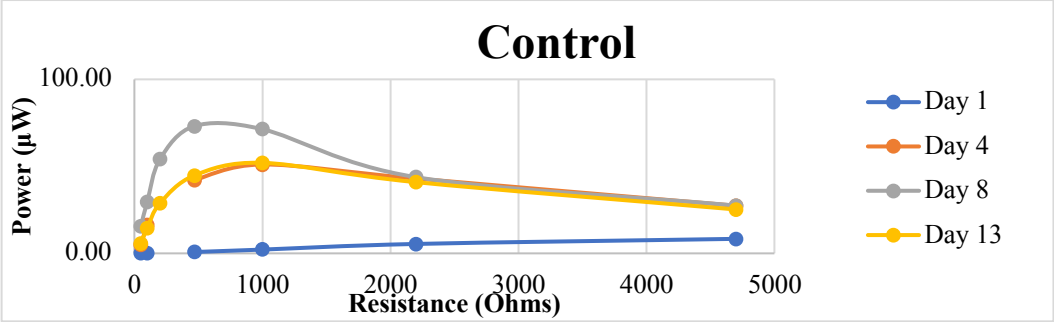
With the use of several resistors, a curve is generated, which shows the relationship between the power and the resistance for each MFC as seen in Figure 5. This also exhibits the level of power generated by the microbes in each MFC.

Table 2: Maximum voltage measurement for each microbial fuel cell daily.

	Control		Sugar	
Resistance (Ω)	Voltage (mV)	Power (W)	Voltage (mV)	Power (W)
4700	278.700	16.53	134.567	3.85
2200	256.900	30.00	119.947	6.54
1000	206.400	42.60	91.567	8.39
470	134.625	38.56	52.213	5.80
220	47.510	13.93	28.751	3.76
100	37.325	10.26	13.217	1.75
50	18.127	6.57	6.513	0.85

By the end of the thirteenth day of voltage measurement, the power-resistance curve showed some stability in the power generated. The peak power was not more or less than 10% different on days 11, 12 and 13 (Figure 5A and 5B). With this observation, 10 mL of 0.5 g diluted table sugar was introduced to the MFC labeled "Sugar," and 10 mL of distilled water was introduced to the MFC labeled "Control." Figure 6 shows the power-resistance curves after sugar was added. There was an increase in the number of volts generated for two consecutive days in each MFC after the addition of water/sugar. However, by Day 16, the number of volts generated by the MFC with sugar reduced gradually. By Day 18, the maximum power generated by the sugar MFC was 50% less than that the power generated on Day 15.

A.



B.

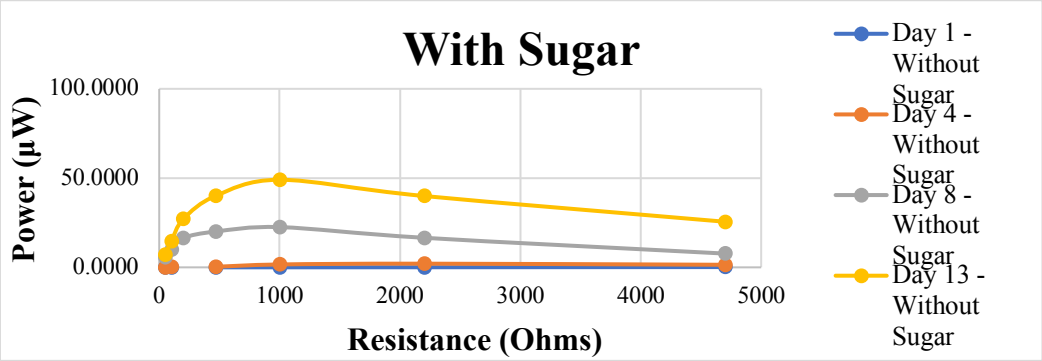


Figure 5: Curves showing the relationship between power and resistance for each microbial fuel cell from Day 1 to Day 13.

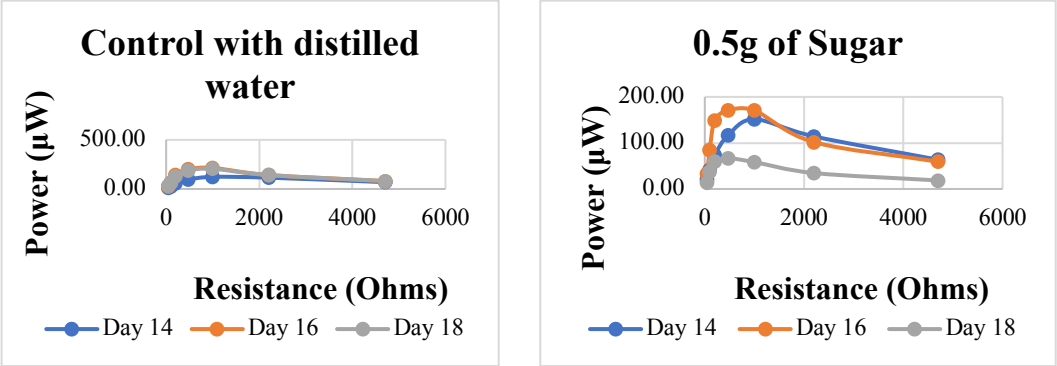


Figure 6: Curves showing the relationship between power and resistance for each microbial fuel cell after 10 mL of distilled water was added to control MFC and 10 mL of 0.5 g table sugar was added to the sugar MFC.

Investigators wanted to determine how fast the quantity of sugar introduced is consumed and metabolized (Figure 7). On Day 19, 10 mL of 0.5 g of table sugar or distilled water were added to appropriate MFC. Once again, an increase in the maximum power generated was observed. The Control MFC generated only slighted higher power, but the Sugar MFC had a 73% increase in the maximum power generated. By Day 22, the maximum power generated by the Sugar MFC began to subside. Figure 8 compares the maximum power generated per day for each MFC.

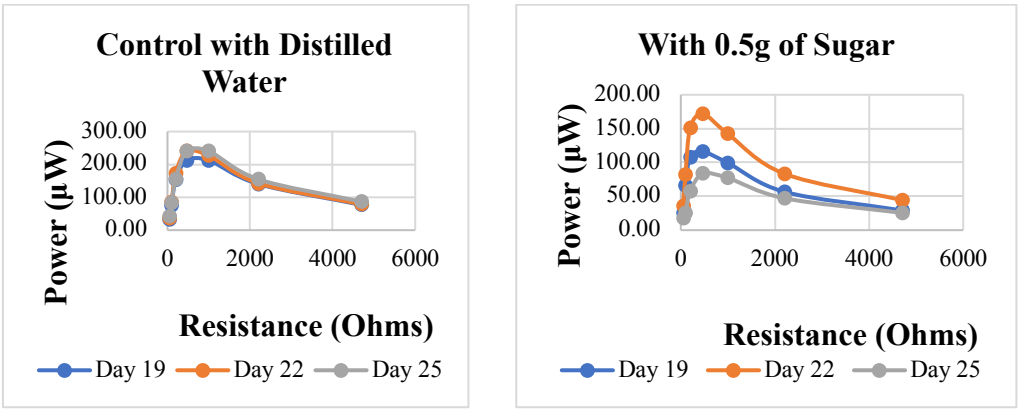


Figure 7: Curves showing the relationship between power and resistance for each microbial fuel cell after 10 mL of distilled water was added to the control MFC and 10 mL of 0.5 g table sugar was added to the sugar MFC.

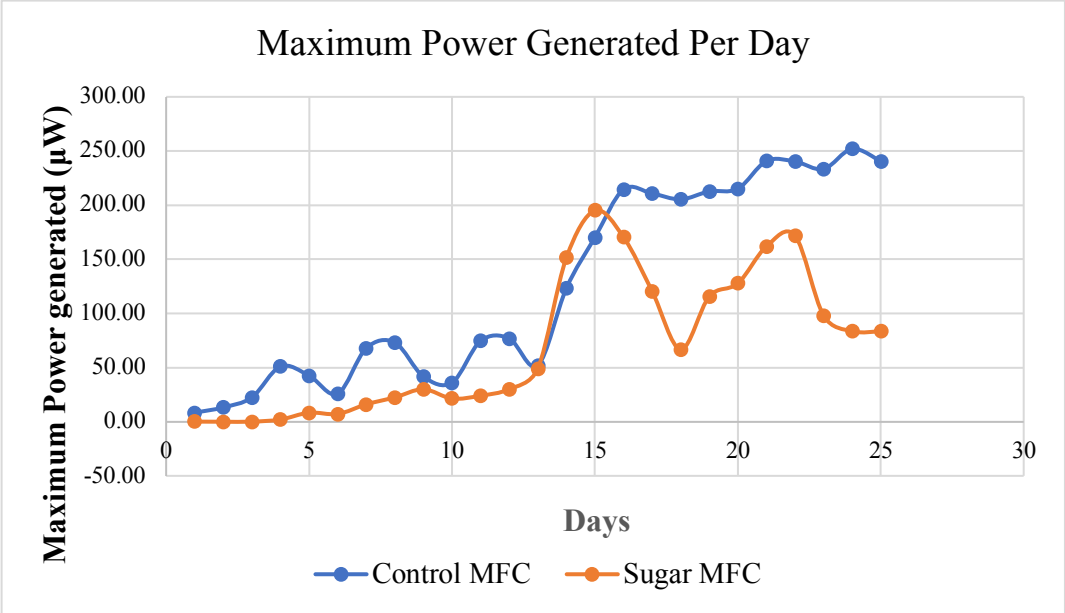


Figure 8: Maximum power generated per day for each MFC.

Discussion and Conclusion

From the results obtained, the power generated increases soon after the introduction of table sugar (glucose). However, the power lasts for only a few days. The inhibition effect occurs because of voltammetry. Glucose was fed directly into the soil, and the time of degradation of the sugar increased in each case as a result of the reduction in the amount of oxygen present within the MFC. This is most likely due to the bacteria directly consuming oxygen, which flows to the anode as an electron acceptor. Ideally, the anode bacteria use oxygen around the anode to generate electrons which are passed from the anode chamber to the cathode chamber via the connecting wire. However, with the sugar introduced into the MFC, the protons are passed freely to the cathode through the soil separating both chambers along with the protons passed through the wire. The low energy produced might be a result of the fermentation of glucose, which cannot produce electricity. Methanogenesis occurs during the anaerobic respiration of *Geobacter sp,* which hampers the growth of the bacteria. Rabaey, Lissens, Siciliano and Verstraete (2003) built an MFC which worked on generating more power with glucose, but that was after making it anoxic before administration. Sugar should be used to generate more power in MFCs and if a Geobacter is used, attention should be paid to making the MFC an anoxic environment to reduce the toxic effect on the *Geobacter species.*

Acknowledgment

This work was partially supported by the National Science Foundation (HBCU-UP, Award #1719425) and the Department of Education (MSEIP, Award #P120A70068).

References

Bond, D. R., & Lovley, D. R. (2003). Electricity Production by Geobacter sulfurreducens Attached to Electrodes. *Appl. Environ. Microbiol.* vol. 69 no. 3 1548-1555.

Bullen, R. A., Arnot, T. C., Lakeman, J. B., & Walsh, F. C. (2006). Biofuel cells and their development. *Biosensors & Bioelectronics.* 21 (11): 2015–45.

Childers, S. (2002). *Geobacter metallireducens* accesses insoluble Fe (III) oxide by chemotaxis. *Nature*. 416: 767–769.

Delaney, G. M., Bennetto, H. P., Mason, J. R., Roller, S. D., Stirling, J. L., Thurston, C. F. (2008). Electron-transfer coupling in microbial fuel cells: 2. Performance of fuel cells containing selected microorganism-mediator-substrate combinations. *Journal of Chemical Technology and Biotechnology. Biotechnology.* 34: (pp. 13–27). (Original work published 1984)

Du, Z., Li, H., & Gu, T., (2007). A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotech. Adv.* 25, 464–482.

Ghasemi, M., Wan, W.R., Ismail, W.R., Rahimnejad, M., Ismail, A.F., Leong, J.X., Ben Liew, K. (2012) Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance. Int. *J. Hydrogen Energy,* 38, (pp. 5480-5484).

Gottenbos, B., van der Mei, H.C., & Busscher, H.J. (1999). Models for studying initial adhesion and surface growth in biofilm formation on surfaces. *Methods in Enzymology.*

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology.* 2 (2): 95–108.

Huang, L., Zeng R. J., & Angelidaki, I. (2008). Electricity production from xylose using a mediator-less microbial fuel cell. *Bioresource Technol.* 99, 4178–4184.

Kim, B.H., Kim, H.J., Hyun, M.S., & Park, D.H. (1999a). Direct electrode reaction of Fe (III) reducing bacterium, *Shewanella putrefacience. J Microbiol. Biotechnol.* 9: 127–131.

Liu, H., & Logan, B. E. (2004). Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane. *Environ. Sci. Technol.,* 38 (14), (pp. 4040–4046).

Lohner, S., & Rowland, T. (2016). How Do Bacteria Produce Power in a Microbial Fuel Cell? *Science Buddies.* Science Buddies, 28 Nov.

Mercer, J. (2010). Microbial Fuel Cells: Generating Power from Waste. *Illumini.* Volume XII Issue II.

Nordberg, H., Cantor, M., Dusheyko, S., Hua, S., Poliakov, A., Shabalov, I., … Dubchak, I. (2014). The genome portal of the Department of Energy Joint Genome Institute: 2014 updates. *Nucleic Acids Res.* 42(1): D26-31.

Pant, D., Singh, A., Van Bogaert, G., Olsen, S. I., Nigam, P. S., Diels, L., & Vanbroekhoven, K. (2012). Bioelectrochemical systems (BES) for sustainable energy production and product recovery from organic wastes and industrial wastewaters" *RSC Adv.,* 2, 1248-1263.

Rabaey, K., Lissens, G., Siciliano, S. D., & Verstraete, W. (2003). A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol.* Lett. 25, 1531–1535.

Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A., & Oh, S. (2015). Microbial fuel cell as new technology for bioelectricity generation: A review. *Alexandria Engineering Journal* Volume 54, Issue 3, (pp. 745-756).

Rahimnejad, M., Najafpour, G., & Ghoreyshi, A.A. (2011). Effect of mass transfer on performance of microbial fuel cell, *Intech* 5, 233–250.

Reguera, G., Nevin, K. P., Nicoll, J. S., Covalla, S. F., Woodard, T. L., & Lovely, D. R. (2006). Biofilm and nanowire production leads to increased current in Geobacter sulfurreducens fuel cells. *Appl Environ Microbiol.* Nov; 72(11):7345-8.

Roller, S. D., Bennetto, H. P., Delaney, G. M., Mason, J. R., Stirling, J. L., & Thurston, C. F. (1984). Electron transfer Coupling in Microbial Fuel Cells: 1.

Comparison of Redox Mediator Reduction Rates and Respiratory Rates of Bacteria. *Journal of Chemical Technology and Biotechnology* 34B: (pp. 3-12).

Rozendal, R. V., Hamelers, H. V. M., Rabaey, K., Keller, J., & Buisman, C. J. N. (2008). Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnology,* vol. 26, no. 8, (pp. 450–459).

Strycharz-Glaven, S. M., Snider, R. M., Guiseppi-Eliec, A., & Tender, L. M. (2011). On the electrical conductivity of microbial nanowires and biofilms. *Energy Environ. Sci.,* 4, 4366 - 4379.