Measuring Voltage Output in Soil-Based Microbial Fuel Cells and Yeast Fuel Cells

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Abstract: Amidst the booming population growth of the twenty-first century, the demand for energy is exponentially increasing. No longer can we depend upon conventional energy sources to provide power; thus, increased research into alternative forms of energy is critical. The goal of this project was to build a microbial fuel cell (MFC) that would generate electricity from benthic mud samples collected from a second-order stream, while producing pure, drinkable water as a byproduct. The MFC consisted of two acrylic boxes serving as the anode and cathode, connected by an agar-filled compression fitting that acted as the salt bridge. The anode contained benthic mud, which held various prokaryotic bacteria and organic matter required for oxidation to occur; the cathode was aerated for the reduction of oxygen to water, thus completing an electric circuit. Further trials conducted with a yeast-based cell and an open-air cathode soil MFC served as a benchmarks for comparison. An open-air cathode soil MFC yielded the highest voltage, maintaining between 450 and 500 mV per 50 cm^2 of electrode surface area for a period of 96 hours with no fuel addition. However, this MFC is less powerful than commercial batteries; an AAA battery can output 1.2 volts. In the future, MFCs could expand in scale, by connecting multiple cells in series, to convert sewage treatment plants, stream beds, and even gardens, into electrical power plants, while generating a clean source of water for consumption.

Keywords: microbial fuel cells, voltage, oxidation, electrode, renewable energy, organic matter

1 INTRODUCTION

A Microbial Fuel Cell (MFC) is an apparatus that is capable of utilizing the specific interactions of bacteria to produce energy through oxidation-reduction reactions and generate an electrical output. In a typical MFC, electrons are removed from organic matter (an oxidation reaction) and provided to an electron acceptor, such as oxygen or nitrates (a reduction reaction) thus creating an oxidation-reduction reaction. However, when bacteria are faced with anoxic conditions, where an electron acceptor is not readily available, it is possible to use a carbon electrode as a substitute. Bacteria will "deposit" electrons onto the anode to generate an electrical current.[1] The electrons will move from the electrode located in the anode chamber to the cathode, where a solution with dissolved oxygen and nitrates will readily accept electrons, thus giving the MFC the potential to power a load. With the versatile potential of bacteria, various types of biodegradable organic matter could be used as the primary energy source in an MFC. Additionally, photosynthetic and chemosynthetic bacteria open the possibility of numerous alternative compounds that could be used as fuels.

1.1 History and Use

The potential electrical output of an MFC was first explored in 1911, when Dr. Potter of the University of Durham sought to harvest the electrical energy generated by bacterial metabolism.[2] However, it was not until the 1980's, when researchers had a stronger understanding of the mechanisms and the role that the electron-transport chain played in bacterial metabolism, that further developments into MFC's were made. Researchers at the King's College London investigated the role of mediators in shuttling electrons from the transport chain to a nearby electrode, so as to create an electrical current. They discovered that they could generate a consistent electrical output with the addition of organic material at regular materials, thus forming the basis of MFC design. (Allen et al.)

[1] Microbial Fuel Cells. (n.d.). Retrieved February 1, 2015, from http://www.research.psu.edu/capabilities/documents/MFC_QandA.pdf

[2] Illumin - Microbial Fuel Cells: Generating Power from Waste. (n.d.). Retrieved February 1, 2015, from http://illumin.usc.edu/134/microbial-fuel-cells-generating-power-from-waste/

1.2 Modern Developments

Modern attempts to develop newer and more efficient MFC's have met with relative success. Indeed, these efforts have led to the development of both two-chambered, and single chamber MFC's, along with documentation of the efficiency of various types of bacteria and substrates. It has been found that the substrates that produced the largest current densities were sodium fumarate at 2.05 mA/cm^2 and glucuronic acid at 1.18 mA/cm^2. (Pant et al.) However, commercial applicability is limited, as they need to be synthesized in a laboratory, and are not readily available in naturally abundant quantities. Thus, in our study, we seek to use a substrate that is widespread: the organic materials present in benthic mud from a stream bed. Additionally, the current densities provided for the above substrates are not completely reliable, as the data was taken across various studies in which the concentrations of substrates varied, thus providing an uncontrolled influencing factor in comparison.

The effectiveness of MFC's is also heavily influenced by the nature of the wastewater used. It has been found that chocolate industry wastewater was the most effective solution in cathode construction, as it had a current density of 0.302 mA/cm^2. (Patil et al.) Another effective solution was the waste obtained from paper recycling plants, providing a current density of 0.25 mA/cm^2. (Liu et al.) However, influencing factors affecting data include the fact that the type of MFC constructed varied depending on the type of wastewater used, and the total volume of the container differed. Additionally, such types of wastewater are highly specific.

The flow rate across the salt bridge is also significant in the fuel cell efficacy. At higher flow rates, the bacterial biofilm is placed under higher stress. This affects biofilm formation stress rates, which is a contributing factor to optimal MFC performance. (Rusconi et al.)[3] This is also thought to affect the optimal time required for an MFC to achieve its maximal voltage output, and the ability of the MFC to sustain its maximal voltage output over longer periods of time.

Many designs of MFC's exist which utilize different architectural methods to achieve electrical output. However, their chemical mechanisms remain constant throughout the different designs. Initially, most MFC's utilized a two-chamber design, with each chamber serving as either the cathode or the anode. The BEAMR (bio-electrochemically assisted microbial reactor) yields hydrogen gas as an after-product of the reaction, in which redox reactions produce a measurable voltage.[4] In recent years, single-chambered MFC's have grown popular, due to their lack of spatial constraints. Single-chambered MFC's work by containing both a cathode and anode; instead of being separated by a PEM, they are separated by a gas diffusion layer (GDL), allowing for passive oxygen transfer to the cathode, alleviating the need for oxygen pumps which currently aerate the cathodes in typical two-chambered designs.[5] Again, however, the issue is raised of the widespread applicability of these devices.

In this experiment, we seek to develop an MFC using benthic mud samples containing bacteria and organic constituents, which provide the necessary materials to produce an electrical output. If such a cell could generate a measurable and consistent voltage, then it could have potential applications in regions of the world where consistent electricity is not readily available.

1.3 Purpose

We seek to compare the voltage outputs of mediator and mediator-less microbial fuel cells. A soil-based MFC, which harvests the biochemical processes of bacteria, is classified as a mediator-less MFC because it does not require a mediator – a chemical that shuttles electrons outside of the bacteria cell – to generate a current. We suspect that bacteria do not require mediators because they are prokaryotes. Prokaryotes naturally shuttle electrons outside of the cell during electron transport chain to generate ATP during cellular respiration. On the other hand, a mediated MFC requires the use of mediators. We will construct a yeast-based MFC which requires a mediator to shuttle the electrons out of yeast cells. Yeast, which are eukaryotic organisms, have mitochondria; thus, cellular respiration takes place inside the cell. As a result, yeast naturally lacks the mechanisms required to shuttle electrons outside of the cell. Therefore, in order to generate an electron flow in a yeast MFC, we will add methylene blue, a nontoxic

[3] University, P. (2015, January 22). Microbial Fuel Cells. Retrieved February 4, 2015, from http://www.princeton.edu/grandchallenges/energy/research-highlights/microfluidics/
[4] Microbial Fuel Cells. (n.d.). Retrieved February 4, 2015, from http://www.research.psu.edu/capabilities/documents/microbialfuelcells.pdf
[5] Zielke, E. (2005, December 5). Design of a Single Chamber Microbial Fuel Cell. Retrieved February 4, 2015, from http://www.engr.psu.edu/ce/enve/logan/bioenergy/pdf/Engr_499_final_zielke.pdf

mediator. By drawing a comparison between the two types of MFC, we will be able compare the voltage outputs and determine which type of cell is more efficient.

2 DISCUSSION OF THEORY

Shown below is a diagram representing the microbial mechanisms through which an MFC works.[6]

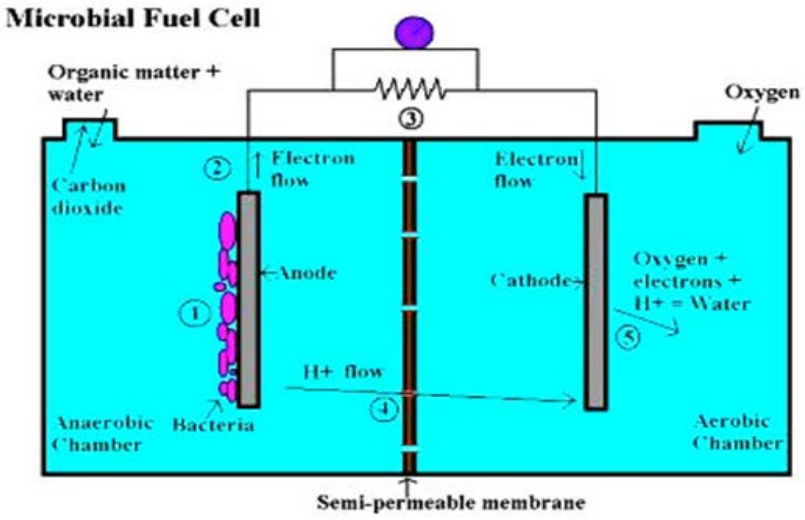


Figure I: A two-chambered MFC with an output measuring device

In the anode, the bacteria form a biofilm around the electrode. There, the bacteria oxidize the organic matter in the benthic mud, releasing electrons and protons as part of cellular respiration. The oxidation, reduction, and full balanced equation for the above described process is shown below:

Oxidation Half Reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 24H^+ + 24e^-$$

Reduction Half Reaction:

$$24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$$

Overall Redox Reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2$$

The hydrogen ions travel to the cathode through the proton exchange membrane (the salt bridge), and the electrons travel to the cathode through the electrodes. The flow of electrons generates current, and thus voltage. At the cathode, the hydrogen ions and electrons react with oxygen introduced either through solvation or through an aquarium pump, to form water. The overall reaction can be summarized as bacterial oxidation of organic constituents in benthic mud, producing carbon dioxide, hydrogen ions, and electrons; the electrons move through the electrode and the hydrogen ions move through the PEM to the cathode, where they recombine with the electrons to form water with the aid of introduced oxygen.

One of the benefits of using soil-based MFC's is the absence of a mediator to shuttle electrons outside the cell. In eukaryotic organisms such as yeast, the process of cellular respiration takes place within the interior of the cell, where electrons gradually fall down a gradient through integral membrane proteins along the interior mitochondrial membrane before finally reaching the ATP Synthase protein. As the electrons fall down the gradient, NAD+ is converted to NADH, and a stream of hydrogen ions are pumped across the inner mitochondrial membrane to the mitochondrial matrix, where a positive charge is accumulated. At the ATP synthase protein, the hydrogen ions

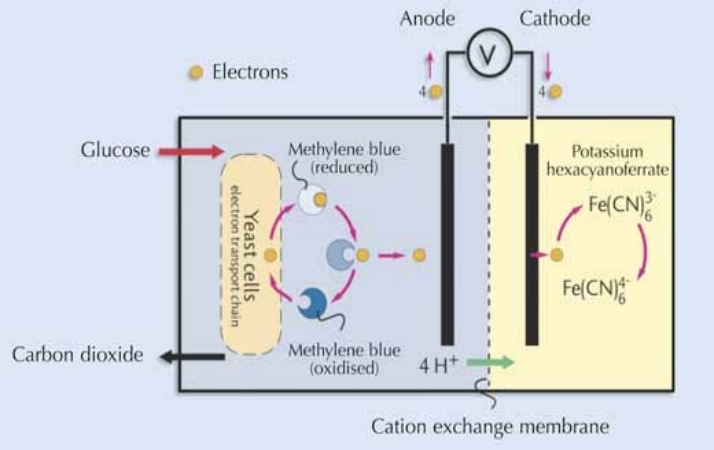
[6] Mercer. (Designer). Microbial Fuel Cell [Web Graphic]. Retrieved from http://illumin.usc.edu/assets/media/175/MFCfig2p1.jpg

move back across the gradient, facilitating the conversion of ADP into ATP. The hydrogen ions then bond with the low-energy electrons and oxygen to form water as a byproduct. While this system has its advantages, we are not capable of harvesting the electrons with high potential energy, as they are moving within the inner mitochondrial membrane, far from the cellular membrane, the gateway of exit for a cell. Thus, a mediator is needed to shuttle such electrons out of the cell for use in the MFC. Mediators are synthetically produced chemicals, and significantly add to the cost and ease of production of MFC's making commercial application difficult.

However, if prokaryotic bacteria are used in an MFC, the presence of a mediator is not required. Endosymbiotic theory postulates that eukaryotic development may have been due to digestion of prokaryotic bacteria, which would serve as chloroplasts and mitochondria. Thus, mitochondrial membranes and mechanisms for survival resemble those of prokaryotes; this is further corroborated by the fact that mitochondria contain their own unique DNA. In prokaryotes, the plasma membrane is analogous to the inner mitochondrial membrane in the mitochondria of eukaryotic cells. The electron transport chain and ATP synthase are embedded within the plasma membrane in prokaryotes. Some prokaryotic cells don't use oxygen as a final electron acceptor, instead using nitrates or sulfur, both highly electronegative compounds. When hydrogen ions move across the plasma membrane in prokaryotic cells through the movement of electrons along the cellular membrane, both are exposed to the extracellular space, where they can be readily harvested for travel through the PEM or for generating current.

In the construction of a yeast-based fuel cell, a mediator is required to shuttle the electrons to the electrodes. Shown below is a diagram detailing the cell's structure.

Figure II: A two-chambered yeast-powered microbial fuel cell[7]



Through the oxidative reactions by yeast on the glucose, electrons are shuttled down the mitochondrial membrane. Methylene blue, acting as an electron carrier, becomes reduced as it accepts the electrons, and shuttles them over to the carbon electrode. From there, as the methylene blue deposits the electrons, it becomes oxidized and becomes prepared to accept more electrons from the yeast. This is analogous to a cyclic effect, requiring minimal replenishment of methylene blue once the cell is operational. As the electrons travel through the load, they move to a final acceptor in the cathode, where a charge balance is maintained through an exchange membrane constructed from salts and agar.

In this study, we seek to develop a mediator-less soil-based MFC to determine optimal voltage output. Output will be compared to the results to a yeast-based MFC with different substrate compounds under a similar cell construction.

3 MATERIALS AND METHODS

The construction of a soil-based MFC can be divided into six primary phases.

3.1 General Design

To begin, sandpaper was rubbed against the two caps of compression fitting and its corresponding sides on acrylic containers to optimize acrylic cement sealing. Then, using a marker, circles were traced around the connection between the compression fitting and the acrylic containers. On the sides of the acrylic containers, a dot was drawn in the middle of the traced circle. Using a hand drill, a hole of diameter ¾ inch on the marked sides of the acrylic

[7] Madden, D. (2010, January 1). The Microbial Fuel Cell: Electricity from Yeast. Retrieved March 8, 2015, from http://www.scienceinschool.org/sites/default/files/teaserPdf/issue14_fuelcell.pdf

containers was made. As this hole was to serve as the connection with the PEM, the accuracy of the dimensions were re-checked. Next, a hole of diameter 2 mm at the tops of the containers was drilled for future electrode fitting. For the container made for the cathode, a similar hole was drilled for the aquarium pump tubing. Acrylic cement was then applied along the hole of ¾ inch diameter on the acrylic containers. Immediately after, acrylic cement was applied along the endcaps of the compression fitting. Then, the acrylic container and the compression fitting end caps were firmly held together for ~120 seconds. Additional tests for leakage were made.

In the construction of the electrodes, carbon cloth was cut into two equal squares of dimensions 5cm x 5cm. Then, nickel epoxy was applied to 15 cm at the end of a bare wire onto the carbon cloth. The epoxy was then left to harden over the next 24 hours to ensure a resilient connection. Before the start of the experiment, the resistance of the system was calculated. A resistance of less than 5 ohms was deemed an acceptable value.

The salt bridge, or PEM, is instrumental in the MFC to ensure H+ ion transfer from the anode to the cathode. To construct the salt bridge, a petri dish was covered with plastic wrap. Using aluminum foil reinforced with rubber bands, one end of a PVC tube was filled with agar. 300 mL of water was then poured into a beaker over a hot plate. This was combined with 30 g of agarose powder and 6 g of KCl salt. The solution was continually stirred until the agarose had dissolved, thus forming the solution of the salt bridge. The solution was then poured into the PVC tube, where it was refrigerated overnight at 4 degrees Celsius before connection to the two chambers.

3.2 Soil-Based Microbial Fuel Cell Structure

The benthic mud sample provides the organic matter and bacteria necessary in the anode portion of the MFC. Additionally, the stream water, where the mud was obtained, was used as the wastewater in the cathode for the MFC. To obtain the stream water, a 1 liter jar was used to collect the stream water from a moving section of a local second-order stream; the second-order stream used in experimentation was Wolftrap Creek, a significant stream in Difficult Run. To obtain the benthic mud sample, a spade was thrown into the stream while attached to a rope, while benthic mud was scooped from the stream bed into a bucket.

To piece together the various parts of the cells, all the components were brought to the laboratory. The salt bridge solution was taken out of the refrigerator, and carefully attached to the endcaps of both the cathode and the anode. Then, 1 liter of stream water was poured into the cathode, along with 3 tablespoons of KCl salt. The resulting solution was stirred thoroughly. One of the cathodes was then inserted into the cathode container. The aquarium pump was then inserted into the cathode container as well. In constructing the anode, 1 liter of benthic mud was poured into the anode, paying careful attention to exclude any rocks or twigs. The other electrode was then inserted, and the lid was placed on both containers. A multimeter was connected to the tips of the two electrodes to measure voltage output. Furthermore, voltage output per square centimeter was calculated based on the known proportions of the chamber. Additionally, through running the circuit in parallel under known resistance, power density per square centimeter was calculated. Finally, by adding a second multimeter to the system, current was measured through the electrodes.

3.3 Yeast-Based Fuel Cell Structure

In the construction of a yeast-based fuel cell, a similar setup with the anode and cathode chambers was observed. The cathode chamber remained unchanged from the soil-based cell. However, the anode chamber contained a glucose solution, yeast extract, and methylene blue, all prepared in a 0.1M phosphate buffer solution. All of this was prepared under appropriate quantities, 500 mL of glucose solution, 500 mL of yeast extract, and 300 mL of methylene blue, in the 1.5L cathode chamber. Data collection began immediately after the construction of the cell was completed, and an observable current could be measured from the two electrodes.

3.4 Data Collection Methods

A Fluke multimeter, connected to Flukeview® Software was used for data collection. Microsoft Excel 2013 was used for data analysis. Maximum, minimum and average values were obtained. Additionally, average error between the 45 second collection intervals were obtained for consideration in data analysis. For the yeast cell, however, due to spatial constraints, a logger pro device was used to collect the potential difference in mV over time. A significant difference between the two devices is that the logger pro software only displayed voltage up to two significant figures in millivolts, whereas the Flueview ® software displayed voltage up to 5 significant figures. Various trend

lines were constructed to determine which best suited the data, with the R-squared value used as the primary instrument of comparison. With future MFC's using different materials, ANOVA tests could be conducted for variance in duration, maximum voltage, and average voltage between the cells. Additionally, a Student's t-test could conducted between the maximum voltage output obtained from both the yeast, and soil cells, to determine statistical significance.

4 RESULTS

4.1 Closed Soil-Based Microbial Fuel Cell

Data collection began at 12:04:19 on January 26th, 2015. Data was collected over 45 second intervals. Rather than taking the exact voltage every 45 seconds, data was continually kept, and the averages were presented over a 45 second interval with the minimum and maximum voltages. Shown below is a graph of the average voltage over a period of 107 hours.

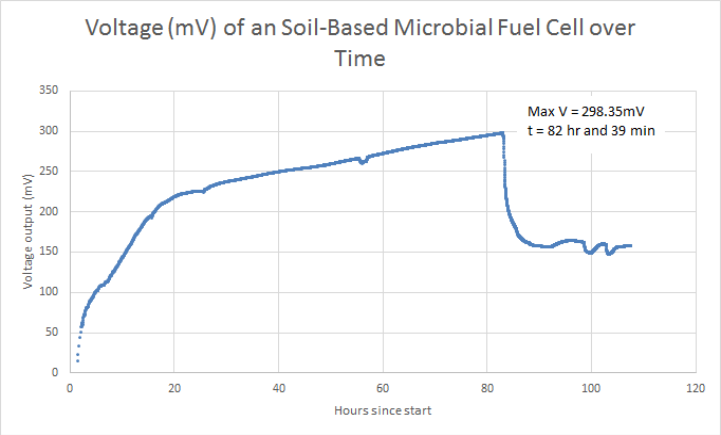


Figure III: A graph scattered with smooth lines showing the measured voltage (mV) over time (hours) since voltage production

As evidenced from the graph above, the voltage peaked at 298.35 mV at a time of 82 hours and 53 minutes after initial data collection. Following the peak, the voltage quickly dipped and re-settled at a voltage of 156-158 mV, before undergoing a slight increase over the next few hours. The graph initially displayed a rapid increase, with decreased concavity occurring at hour 20, after which the cell displayed a relatively linear increase in voltage before reaching its maximum. The mechanisms behind the sharp decrease in voltage following its peak are subject to future investigations, as similarly constructed microbial fuel cells did not display such a rapid recline before re-stabilization.

A sample of the data collected from the fluke logger is shown below:

9																
10	Reading	Sample		Start Time	Duration	Max Time	Max		Average		Min Time	Min		Descriptic	Stop Time	
11	1	0.000242	V DC	23:32.8	00:02.1	23:33.0	0.001501	V DC	0.000451	V DC	23:34.6	-1.00E-05	V DC	Unstable	23:34.9	
12	2	0.001063	V DC	23:34.9	00:00.1	23:34.9	0.001063	V DC	0.001063	V DC	23:34.9	0.001063	V DC	Interval	23:35.0	
13	3	0.001606	V DC	23:35.0	00:02.7	23:35.3	0.012602	V DC	0.00087	V DC	23:36.2	-0.00843	V DC	Unstable	23:37.7	
14	4	-0.00034	V DC	23:37.7	00:00.4	23:37.8	-0.00023	V DC	-0.00027	V DC	23:37.7	-0.00034	V DC	Interval	23:38.1	
15	5	-0.00031	V DC	23:38.1	00:02.9	23:40.9	0.003803	V DC	-0.00733	V DC	23:39.7	-0.02865	V DC	Unstable	23:41.0	
16	6	0.004986	V DC	23:41.0	00:00.1	23:41.0	0.004986	V DC	0.004986	V DC	23:41.0	0.004986	V DC	Interval	23:41.1	
17	7	-0.00124	V DC	23:41.1	00:02.8	23:43.0	0.021366	V DC	0.005363	V DC	23:41.5	-0.00694	V DC	Unstable	23:43.9	
18	8	-0.01149	V DC	23:43.9	00:00.1	23:43.9	-0.01149	V DC	-0.01149	V DC	23:43.9	-0.01149	V DC	Interval	23:44.1	
19	9	-0.00168	V DC	23:44.1	00:02.9	23:46.6	0.011099	V DC	0.004014	V DC	23:44.5	-0.00554	V DC	Unstable	23:47.0	
20	10	0.00854	V DC	23:47.0	00:00.2	23:47.0	0.00854	V DC	0.00854	V DC	23:47.0	0.00854	V DC	Interval	23:47.1	
21	11	0.005987	V DC	23:47.1	00:02.7	23:48.1	0.007497	V DC	0.001781	V DC	23:49.8	-0.0234	V DC	Unstable	23:49.9	
22	12	-0.02936	V DC	23:49.9	00:00.1	23:49.9	-0.02936	V DC	-0.02936	V DC	23:49.9	-0.02936	V DC	Interval	23:50.0	
23	13	-0.03203	V DC	23:50.0	00:02.9	23:51.8	0.001437	V DC	-0.0053	V DC	23:50.0	-0.03203	V DC	Unstable	23:52.9	
24	14	-2.00E-05	V DC	23:52.9	00:00.1	23:52.9	-2.00E-05	V DC	-2.00E-05	V DC	23:52.9	-2.00E-05	V DC	Interval	23:53.1	
25	15	-0.00015	V DC	23:53.1	00:02.8	23:55.2	0.001717	V DC	-5.54E-05	V DC	23:53.6	-0.0012	V DC	Unstable	23:55.9	
26	16	0.00043	V DC	23:55.9	00:00.2	23:55.9	0.00043	V DC	0.000392	V DC	23:56.0	0.000353	V DC	Interval	23:56.1	
27	17	-0.00038	V DC	23:56.1	00:02.7	23:57.6	0.003592	V DC	-0.00091	V DC	23:58.4	-0.0043	V DC	Unstable	23:58.8	
28	18	-0.0017	V DC	23:58.8	00:00.3	23:58.9	-0.00165	V DC	-0.00168	V DC	23:58.8	-0.0017	V DC	Interval	23:59.1	
29	19	-0.00144	V DC	23:59.1	00:02.5	24:00.8	-0.00047	V DC	-0.00267	V DC	24:00.0	-0.01026	V DC	Unstable	24:01.6	
30	20	-0.00205	V DC	24:01.6	00:00.5	24:02.0	-0.0018	V DC	-0.00194	V DC	24:01.6	-0.00205	V DC	Interval	24:02.1	
31	21	-0.00151	V DC	24:02.1	00:02.6	24:03.2	0.029297	V DC	0.00614	V DC	24:04.3	-0.00574	V DC	Unstable	24:04.7	

Figure IV: A sample of the data as collected from the Fluke Logger Program

In this sample, the start and stop times of collection, the time at which the voltage achieved a maximum value, and the time at which the voltage achieved a minimum value, are given. The average value is presented in the 9th column, which was the data primarily used in the construction of the graph. A description of the time interval during which data collection occurred is also provided. If the data did not exceed a certain delta threshold during the time period, the interval was described as being stable. However, if the delta threshold was met or exceeded, then the interval was described as unstable. The prolificacy of unstable intervals at the beginning of data collection marks the sharp growth evidenced in Figure II as the cell began operations, and also deserves further analysis as to its biological causes, and the applicability of such a system for commercial development.

Shown below is an image of the setup of the fuel cell during its operations:

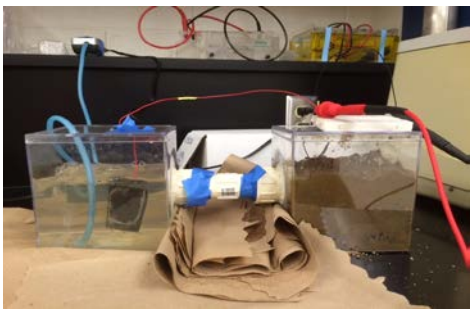


Figure V: An image of the fuel cell in operation

4.2 Open-Air Cathode Soil-Based Microbial Fuel Cell

A second trial with a modified soil-based fuel cell was also conducted. However, rather than using an oxygen pump to allow for reduction in the cathode, an open-air cathode approach was used, where air holes in the lid of the cathode were utilized to allow for aeration. Cold stream water was used in this setup, as water temperature and dissolved oxygen content have a proven negative correlation; colder water has been shown to hold greater amounts of dissolved oxygen.[8] Data from the described trial above is shown below.

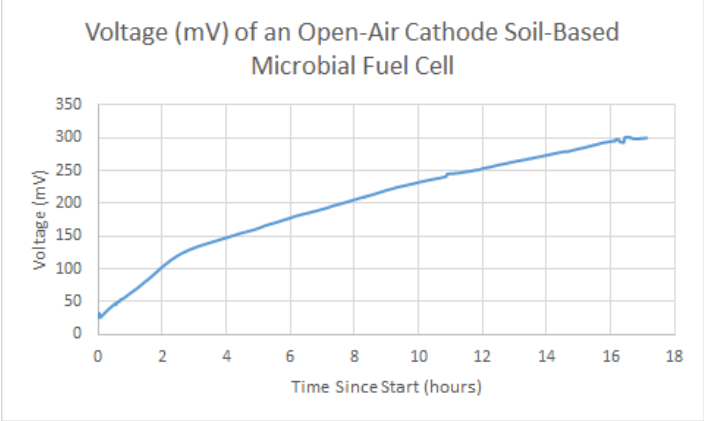


Figure VI: A graph scattered with smooth lines showing the measured voltage (mV) vs. time of an aerated cathode-designed microbial fuel cell

As seen above, the rate of growth of the open-air cathode was significantly greater than that of the closed soil-based microbial fuel cell. The voltage output quickly rose, and reached ~300mV in 16 hours. Whether the cell voltage will continue to exhibit increased growth, decrease, or stabilize is not evident from the data collected, and should be further investigated in future experiments. Indeed, this model confers a significant advantage in energy efficiency as compared to the other model, as electrical drain caused by the oxygen pump need not be accounted for in this design. Thus, a net positive electrical output, linearly increasing over time, was generated through the course of this cell's life.

[8] Water properties: Dissolved oxygen. (n.d.). Retrieved March 16, 2015, from http://water.usgs.gov/edu/dissolvedoxygen.html

4.3 Yeast Fuel Cell

Commonly researched microbial fuel cells utilize yeast as a model organism. To form a basis of comparison, data is shown below of the voltage output of a yeast-based microbial fuel cell over time.

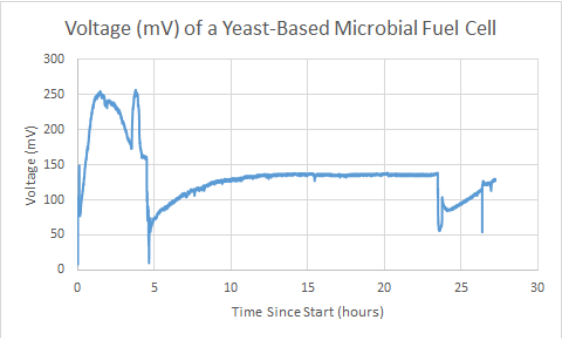


Figure VII: A graph scattered with smooth lines showing the measured voltage (mV) vs. time of a yeast-based microbial fuel cell

As seen above, the yeast-based fuel cell initially built up a large amount of voltage within the first few hours, albeit with fluctuation. However, following a plunge during the fifth hour of data collection, the yeast cell maintained a relatively constant voltage, before beginning to exhibit a more controlled growth after the twenty-fifth hour. It is important to note that no materials were added to supplement the cell upon the start of data collection. Thus, the fluctuations observed are caused by nature and not human interaction.

5 DISCUSSION AND CONCLUSION

Previous studies measuring voltage output from soil-based microbial fuel cells have shown inverse parabolic output curves as well as exponential decay curves, displaying decay over the course of 72 hours. (Kim et al.) However, the voltage output generated from our MFC trials mimics logarithmic growth prior to the maximum value, and then a decreasing logistic curve, settling around a 156-160 mV sustainable capacity over a longer period of time.

The rapid increase in voltage over the first 24 hours of activity could be attributed to the rapid formation of bacterial biofilms over the carbon cloth electrode. Biofilms are often created to form a community of various microorganisms who share a common metabolic pathway, in this case, cellular respiration. To form such biofilms, appropriate bacteria need to aggregate together. Due to the lack of a mediator, only prokaryotic bacteria would be able to partake in generating current. Thus, as the bacteria 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 was reached, the rate of growth appropriately declined until the voltage peaked. Alternatively, 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.[9] Hence, the carbon cloth, with a surface area of 50 centimeters squared, presents a limit to the amount of bacterial electron transfer through oxidative reactions.

The cause of the sharp decline after 80 hours precluded by growth 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 biofilm layer, 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 prokaryotic bacteria were to die while still physically attached to the carbon cloth, then a sharp decline in voltage could be expected. However, the dormant bacteria would eventually be replaced with new, healthy bacteria that would continue performing cellular respiration, re-stabilizing the voltage. However, due to the rate of bacterial death and slow replacement rates, the voltage would stabilize at a significantly lower output. Eventually, the bacteria will die due to a lack of organic matter present in the benthic mud, and then, the voltage would most likely exhibit a similar sharp decline. Replacement of the organic matter found in the benthic mud would most likely allow for a sustained voltage.

[9] Microbial Fuel Cell Biofilms. (n.d.). Retrieved February 4, 2015, from https://microbewiki.kenyon.edu/index.php/Microbial_Fuel_Cell_Biofilms

The second trial of a soil-based microbial fuel cell with an open-air cathode yielded encouraging results. At the start of experimentation, a sharp growth was exhibited, followed by a hypothesized plateau. If additional raw materials were to be added, a theoretical continuous reaction might be sustained, thus allowing this to serve as a perpetual source of energy from readily available materials. The product of the redox reaction, water, allows for greater dilution of the stream water present in the original sample. With the creation of drinkable water as a by-product of the reaction, the applicability of the soil-based microbial fuel cell as an energy and water source is greatly enhanced. However, a method to filter the pure water from the stream water is needed. Additionally, further research determining the composition of the newly formed product is needed to determine its suitability for human consumption.

The yeast fuel cell, which required mediating reagents to shuttle electrons outside the yeast cells, was compared to the mediator-less soil MFC. 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 a mediator-less MFC is more efficient. This could possibly be due to unfavorable conditions in the anode for the yeast, which should be a subject for future investigation. As a variety of bacteria was present in the soil-based fuel cell, unfavorable conditions for one species might have been interpreted as favorable for another. However, due to the homogeneity of yeast, a single unfavorable condition might have halted voltage output greatly. Alternatively, the effectiveness of the methylene blue in solution could have affected the voltage observed. If the methylene blue had an interaction, or was slowed down by the phosphate buffer, the rate of oxidation might have decreased, lowering the voltage.

Several limitations present in experimentation might account for minute inconsistencies between our obtained data and data from previous studies. In the construction of the salt bridge, slight leakage might have occurred, which might have disrupted hydrogen ion flow to the cathode, thus altering the efficiency and voltage output of the cell. Additionally, the environment during which the benthic mud was procured presents an uncontrollable factor in experimentation. Certain bacteria might be more prolific under certain weather conditions, thus providing more or less prokaryotic bacteria available to perform cellular respiration. Finally, the stream at which the benthic mud was procured varies between the data collected from various MFC's. Different streams might have differential bacterial populations, and might also contain altered composition of wastewater for use in the cathode. These factors provide variations in the effectiveness of MFC's across the world, and might cause different voltages under different collection conditions.

Future research considerations include continued trials comparing the duration and voltage produced by a similar soil-based MFC. Additionally, comparisons should be made between the efficiency, as measured through current or voltage density, of specifically-procured prokaryotic bacterial-based MFC's. Various commercially available wastewater treatments and substrates should be used in such experiments under constant density and volume, to determine optimal electricity production. Experiments could be conducted with eukaryotic MFC's with the use of a mediator to compare voltage output densities and duration with prokaryotic-based MFC's. Cost and accessibility should be considered in the design and comparison of such cells, as the use of synthetic compounds decreases cell viability. Alternative mechanisms which might have caused the observed sudden decline in voltage output after attaining the maximum voltage should also be studied in the future, as possible preventative measures could be taken to maintain the high voltage output duration. The applicability of MFC's in societies where conventional energy sources are either not cost-effective or available should be an issue of future study. With the MFC's capacity to produce pure water as a byproduct of electricity production, easily constructed MFC's could be implemented. Methods for the purification of the water produced are needed, as a cost-effective way to harness that water could yield monumental results. A combination of various factors in the MFC will allow for optimal performance and consumer use in the future.

The compounds used as mediators and electron acceptors in the design of a fuel cell also play a critical role in the voltage and current densities. Through experimentation with various compounds including potassium hexacyanoferrate, fumarate, or dimethyl sulfoxide, an optimal mediator may be found.

Microbial fuel cells represent a promising energy source. Specifically, mediator-less soil-based MFC's have the potential to provide a durable source of energy if developed in large quantities. Through experimentation, it

was shown that a two-chambered soil-based MFC was capable of sustaining a voltage of above 100 mV for a duration of 100 hours. Additionally, the soil MFC yielded a significantly higher sustained voltage with more infrequent fluctuations observed than that produced by the yeast fuel cell. This model could be refined through future research to develop MFC's that are capable of producing up to 1 volt for a sustained period of time.

6 APPLICATION AND FUTURE STUDIES

At the turn of the 21st century, the Millennium project set forth fifteen goals that it sought to accomplish throughout the century.[10] Accessibility to clean water and the availability of cheap energy were among these goals. The use of soil-based MFCs in generating a sustained electrical output represents a stride toward the advancement and achievement of these goals. The ease of construction of a soil-based MFC is a critical element in its versatility and applicability. Despite the use of agarose in this experiment to construct a salt bridge, many other readily accessible elements could be used. Filter paper and a saline solution could substitute for a salt bridge, thus allowing for the creation of a semi-permeable membrane. The byproduct of this reaction, water, presents another possible benefit for the use of microbial fuel cells. Although there is currently no energy-efficient way to filter the water from the system, the added water from the reaction could be filtered out through the voltage produced. If the voltage from the cell could be used to filter the water with either a net energy gain, or no net energy change, then the cell could be used as a filtration device. Corporations have utilized the microbes found in water treatment plants to generate an electrical output capable of covering the energy expenditure of distillation. However, rather than achieving a net electrical output, their sole goal was to cover the costs of the purification and filtration of water.[11] A soil-based MFC presents an innovative solution to the energy and health problems faced today.

The voltage capacity of an MFC is compatible with that of the newly synthesized blue LED created by Akasaki and his research team. The research team found that they could create an LED with an output of 300 lumens per watt.[12] Even with the reduced capacity of this MFC to approximately 100 milliwatts given the resistance of the circuit, it still meets the threshold to power a blue LED. Alternatively, low voltage infrared LED's, operating with a voltage drop of less than 1.5 V could be linked in a parallel circuit to a soil-based MFC with multiple electrodes in the substrate. With the increasing popularity of LED's today, an MFC could very well find a place in the home, as a way to power light displays at minimal cost and minimal resources used.

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