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Article

From Waste to Wealth: Making Millivolts from Microbes!

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SUMMARY

Biofuels can reduce our reliance on fossil energy sources while also protecting our environment. A Microbial Fuel Cell (MFC) is a system in which microorganisms produce electricity while performing their normal metabolism. The purpose of this project was to engineer a series of MFCs and manipulate circuit components to optimize voltage production. Each two-chamber MFC was created using creek mud, presumably rich in anaerobic exoelectrogenic bacteria, in the anode chamber and water in the cathode chamber. Four types of MFCs (three in each group) were constructed: 1) Mud in anode and water in cathode chambers; 2) Mud and sucrose in anode and water in cathode chambers; 3) Mud in anode and water and iron filings in cathode chambers; 4) Mud and sucrose in anode and water and iron filings in cathode chambers. Voltage output was recoded over twelve days. MFCs with iron filings (alone or with sucrose) consistently produced more voltage than the MFC with mud alone. Adding iron filings to the cathode chamber increased voltage output by 49%. While sugar alone in the anode chamber did not increase voltage output, the combination of sugar in the anode and iron filings in the cathode chambers did increase voltage output by 69% when compared to the MFC with mud alone. Our experiment demonstrated that MCFs can be optimized by manipulating bacterial substrate and using metal electron acceptors. We must invest in our planet's future by supporting large scale research on MFCs that can produce clean electric energy and purify environmental waste.

INTRODUCTION

Worldwide, human energy use requires over 11 billion tons of non-renewable fuels every year. This rapid consumption of fossil fuels, such as coal, oil, and natural gas, is depleting these non-renewable energy sources and polluting the earth. While there are several greenhouse gases responsible for global warming, carbon dioxide (CO2) is the major contributor, and it is produced by the combustion of fossil fuels in cars, factories, and electricity production facilities (1). Research shows that the earth's population is growing at a fast rate and is anticipated to reach 9.7 billion by 2050 (2), which means the world's energy needs will continue to increase. Renewable clean energy sources are one solution to the current global problem, allowing energy production while respecting the environment and reducing contamination in the atmosphere.

Electricity is a part of nature and one of the most widely used forms of energy. Conductors are materials or elements in which electrons flow easily between atoms (e.g. copper, silver, aluminum). It is this flow of electrons that constitutes an electric current (measured in Amperes), and any opposition to this flow is called the resistance (measured in Ohms). Thus, an electric circuit is a system of conductors and components forming a complete path for current to travel. In a battery, for example, the electrons flow out of the negative side (anode) of the battery, through the circuit, and back to the positive side (cathode) of the battery. The force or pressure that causes the current to flow in a circuit is called the voltage (measured in Volts). In summary, all one needs to generate electricity is a source of electrons and a voltage that causes the electrons to move through a circuit (3).

In the world today, we produce electricity mostly by using primary energy sources, such as coal and natural gas, which are non-renewable and pollute the environment. Solar, wind, and water are examples of clean energy sources that are already in use, while there are others sources that are in various stages of development. One such fascinating possibility is the idea of a microbial fuel cell (MFC) in which microorganisms produce an electric current while performing their metabolic processes. The basic principle of a MFC is that the chemical energy stored in organic matter is converted to electrical energy when the microorganisms metabolize the organic matter (4). All living organisms, including bacteria, undergo cellular respiration, a series of metabolic processes by which the chemical energy in organic matter/food (such as glucose) is broken down to produce energy for the living cells (5). The energy of the substrate is stored in the chemical bonds, or shared electrons, of the organic molecules. As the bonds are broken, and electrons flow through various metabolic reactions, energy is transformed into usable forms by the cells and living organisms. Eventually, the electrons are transferred to an electron acceptor, and when that final acceptor is oxygen, the process is called aerobic respiration. Unlike most living creatures, some bacteria do not require oxygen to survive, and they conduct their cellular respiration without oxygen. Some of these anaerobic bacteria can transfer the electrons obtained during cellular metabolism to a final electron acceptor in the outside environment (6). It is these types of exoelectrogenic bacteria that can be used to create MCFs.

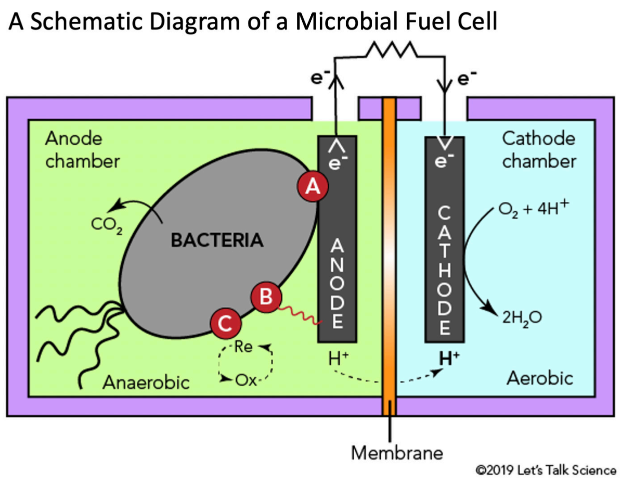

Figure 1: A microbial fuel cell is composed of four basic parts: an anode, a cathode, electrodes and an external load. Electrons can be carried by protein carriers (A), nanowires (B) or mediators (C) (© 2019 used with permission from Let's Talk Science [linkprotect.cudasvc.com]).

A microbial fuel cell is composed of four basic parts: an anode, a cathode, electrodes, and an external load (**Figure 1**) (7). The anode is where the organic matter is present and where the microorganisms grow. It has electron-rich substrate but contains no oxygen or final electron acceptor, hence it is an anaerobic environment. In contrast, the cathode is rich in oxygen, usually contains water, and sometimes has other electron acceptors, such as metals.

To connect the anode and cathode, electrodes and an external load are used. These components make up an electric circuit in which electrons given off by the bacteria travel from the anode compartment to the cathode compartment as an electric current, which can be harnessed or measured through the external load. Lastly, the fuel cell includes a semipermeable connection between the two chambers that allows protons to flow from the anode to the cathode, where they can recombine with electrons and oxygen to form water. This membrane does not allow the passage of electron acceptors from the cathode into the anode, thereby forcing the electrons to travel the circuit and generate current. A salt bridge is commonly used for the semi-permeable membrane connection between the two compartments. The flow of electrons through the circuit creates electricity by producing a potential difference across the two electrodes, and this is the ultimate voltage output of the fuel cell, which can be measured using a voltmeter. Voltage output is directly related to microbial growth and will decrease as the bacteria start to die.

As Rabaey and colleagues have described, MFCs present an exciting opportunity to create clean, renewable energy and have many advantages. First, microorganisms can be found in all environments and easily accessed in soil and wastewater, for example. They are also easy to grow and replenish. Second, by providing microorganisms with organic matter, we can extend the lifespan of these cultures, making this technology sustainable. Third, since oxygen is the final electron acceptor, and water is the byproduct, no harmful chemical waste is produced. Finally, besides producing electricity, MFCs can be used in wastewater treatment for food processing industries and sewage, since this water is rich in organic material. Microorganisms simultaneously break down this organic matter and clean up the water (8). Despite all of these advantages, MFCs are not widely used as they have limited voltage output (9). Much current research focuses on how best to improve efficiency by decreasing loss of energy, boosting the substrate, and decreasing the resistance.

Parameter	Voltage in mV			
	Mud	Mud + Sucrose	Mud + Iron	Mud + Sucrose + Iron
Mean	156.8	163.5	227.9	280.7
Standard Deviation	15.8	17.7	27.9	37.1
Standard Error	4.6	5.1	8.0	10.7
Maximum Voltage	190	188	282	351
Minimum Voltage	121	122	180	205

Table 1: Comparison between the density, faradic capacity, and theoretical voltage of magnesium, aluminum, and zinc (5).

We have therefore designed and constructed a series of fuel cells to determine whether we can improve the performance of the MFC by enhancing the food supply of the bacterial colonies, optimizing the electrical circuit with the addition of iron as an electron acceptor, and combining substrate augmentation and using iron as an electron acceptor. We hypothesized that the addition of sucrose to the anode chamber, iron to the cathode chamber, and the combination of these factors would lead to an increase in voltage output by the MFC. Our results showed that the addition of iron filings in the cathode chamber, with or without sucrose, increased voltage output by 69% or 49% respectively, while sucrose alone did not increase voltage output.

RESULTS

We used a total of 12 MFCs, with 3 MFCs for each group: Mud only, Mud + Sucrose, Mud + Iron, and Mud + Sucrose +

Experimental Setup of Microbial Fuel Cells (used in This Project)

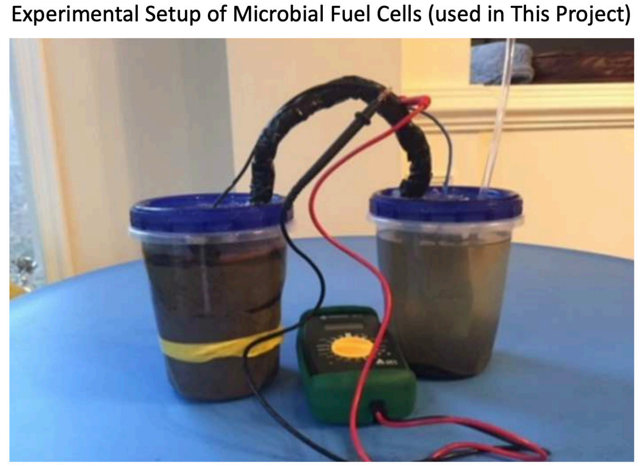

Figure 2: A photograph of one of the MFCs constructed

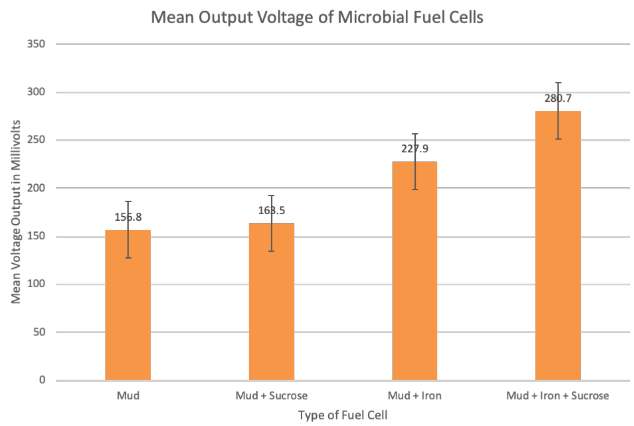

Figure 3: A total of 12 Microbial Fuel Cells were used, with 3 MFCs for each: Mud only, Mud + Sucrose, Mud + Iron and Mud + Sucrose + Iron. The mean voltage output and standard errors for Mud only, Mud + Sucrose, Mud + Iron and Mud + Sucrose + Iron were 121 mV (SE 4.6), 122 mV (SE 5.1), 180 mV (SE 8.0) and 205 mV (SE 10.7) respectively.

Iron. The mean voltage output and standard errors for Mud only, Mud + Sucrose, Mud + Iron, and Mud + Sucrose + Iron were 121 ± 4.6 mV, 122 ± 5.1 mV, 180 ± 8.0 mV, and 205 ± 10.7 mV, respectively (**Table 1** and **Figure 2**). **Figure 3** shows the voltage generated by each type of MFC over a period of 14 days. Maximum voltage was attained with Mud + Iron, and minimum voltage was with Mud alone. There was no significant difference in voltage output with sugar added to the anode chamber compared to mud alone. However, there was a significant increase of 49% in voltage output by adding iron filings to the cathode chamber. While the addition of sugar to the anode chamber did not significantly improve the voltage output, the sugar in combination with the iron filings did cause an increase in voltage output of 69% when compared to the MFC with mud alone. We observed that adding sucrose as a substrate for the bacteria seemed to slightly decrease the rate at which the voltage decreased (**Figure 4**).

We found no significant difference in voltage output with sugar added to the MFC (p = 0.900); however, there was a significant increase in voltage output by adding iron filings (p<0.001) when compared to the MFC with mud alone. While adding sugar to the anode chamber did not significantly improve the voltage output, the sugar in combination with iron filings did cause an increase in voltage output when compared to mud alone (p<0.001) and when compared to the MFC with mud and iron filings (p<0.001) as well.

DISCUSSION

Electricity production and maintaining a clean environment play a vital role in human life; the MFC is a technology that performs both functions by employing microbes in a controlled environment. In our project, we successfully engineered multiple types of MCFs to determine whether we can improve the performance of the MFC by manipulating bacterial substrate and adding a metal electron acceptor. We found that the addition of sucrose did not significantly improve

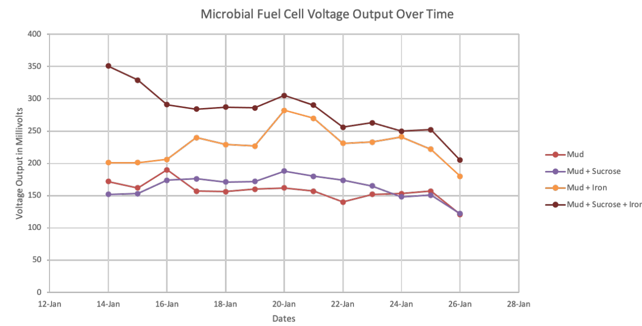

Figure 4: The voltage output of MFC over a period of fourteen days. The MFC with iron and sucrose consistently performed better than the other fuel cells.

the performance of the fuel cell. However, our data showed that the addition of iron filings did significantly enhance the voltage output of the fuel cell. The combination fuel cell with sucrose in the anode and iron in the cathode did much better than the fuel cell with mud alone.

In MFCs, the substrate is regarded as one of the most important factors affecting electricity generation as it can affect the composition and density of the microbial community, which is the electrical charge generator (11). We chose to add sucrose (a polymer of glucose and fructose) to our mud anode chamber as prior research has shown bacteria found within mud can metabolize glucose to successfully produce electricity (9). Unlike Rabaey and colleagues, we did not see any significant improvement in voltage output with this addition (9). It is possible that the specific bacterial composition found in our creek mud differed from that of others. Also, perhaps using the simplest sugar forms, such as glucose or fructose, might have yielded better higher voltage output had the more complex form, sucrose. It is possible that the bacteria lacked the enzymatic reactions necessary to break down this complex sugar.

Electron acceptors receive electrons from the cathode and make a significant contribution to the performance of the MFC. Different electron acceptors exhibit physically and chemically different properties (e.g., oxidation potential) and therefore affect the efficiency of electricity production. Ferric iron can be reduced to ferrous iron in the cathode chamber by accepting electrons. This reversible electron transfer reaction provides several advantages, such as fast reaction, high standard potentials, and biological degradability (12). Our work confirmed prior research (8) that iron can be successfully used as electron acceptors and thus reduced to the less toxic ferrous form.

While the addition of sugar to the anode chamber did not significantly improve the voltage output, the sugar in combination with the iron filings did cause an increase in voltage output when compared to the MFC with iron filings alone, and this closely approached statistical significance (p-value = $1.1102e^{-16}$). To our knowledge, prior research has not looked at substrate manipulation in combination with the addition of electron acceptors. It is possible that in the MFC with sucrose added, the bacterial colonies may have been able to grow and multiply more robustly, but the electrons produced could not be delivered with any more efficiency to the cathode. Future experiments may be able to evaluate this by measuring bacterial growth in conjunction with voltage output. This indicates that perhaps adding metal conductors to the cathode chamber can have a much larger impact on fuel cell efficiency than simply providing a greater substrate concentration to the bacteria in any given MFC. Future studies should expand the types of substrate used, as well as electron acceptors, to find the combination that yields maximal energy output.

There are many aspects to our experiment that we could not entirely predict. For example, towards the end of two weeks we may have had bacteria growing in the cathode area or even other small organisms, such as fungi. Also, the bacteria may not be able to grow and reproduce under our experimental conditions as well as they would in their natural habitat and associated parameters of light, temperature and other microorganisms.

We successfully constructed our own MFCs from individual components with minimal outside assistance. Our findings with regards to combining substrate and electron acceptor manipulations suggest a potential new strategy to maximize microbial fuel cell efficiency. The size of the fuel cells used and type of electrode components were limited by space and budget limitations. As with prior studies, our MFCs were hindered by relatively low voltage output, which can be attributed to a number of factors, such as high internal resistance, nature of the electrodes, and substrate.

MFC technology must overcome many hurdles before it can be implemented as a form of bioelectricity and wastewater treatment. As with other renewable energy sources it must face challenges of having an extensive infrastructure, including space and installation technologies, to support large scale production. However, our experiment has provided major insight into the creation and potential optimization of MFCs. We must invest in our planet's future by supporting further studies on MFCs, conducted on a larger scale, to produce clean electric energy and purify environmental waste.

MATERIALS AND METHODS

Based on the literature, we engineered a microbial fuel cell and used existing research to optimize the production of electric current. Voltage output is directly related to microbial growth rate, which in turn depends on a supply of food substrate. We included sucrose as a food substrate, to our anode chamber, because sucrose can be broken down to the simple sugars - glucose and fructose. We expected this to increase electricity production since carbohydrates are by far the most abundant group used in prior studies such as the one conducted by Rabaey and colleagues where investigated the power output of an MFC in relation to glucose dosage containing a mixed bacterial culture utilizing glucose as a substrate. They found that electron recovery, in terms of bioelectricity, of up to 89% occurred for glucose as a substrate in the anodic chamber (9).

We decided to use iron as an electron mediator to enhance the performance in the cathode compartment. Ferric iron can be reduced to ferrous iron in the cathode chamber according to equation, $Fe^{3+} + e^- \rightarrow Fe^{2+}$ (10). In terms of electron acceptors used in the cathode compartment, oxygen is the most widely used, due to its high oxidation potential and the fact that it yields a clean product (water) after reduction. However, research suggests that the use of alternative electron acceptors may not only increase the power generation and reduce the operating costs but also expand the potential applications of MFCs (10). Prior work in this area demonstrates that metals such as iron and mercury (8), can also be used as electron acceptors and are reduced to less toxic forms. Thus, electricity generation and wastewater treatment take place simultaneously.

Each two-chamber MFC was created using mud in the anode chamber and water in the cathode chamber (see **Figure 4**). mud was obtained from the bottom of a creek in Laurenwood Estates in Manassas, VA with the assumption that this aquatic source would contain sufficient quantities of anaerobic bacteria, such as *Shewanella oneidensis*. The mud was thoroughly mixed so that each sample contained approximately equal bacterial species. The chambers were constructed from plastic Ziploc containers. On the container lids, we drilled one hole for copper wire, an additional hole for the air pump, and one hole into each lid for the salt bridge. Electrodes made from aluminum mesh were fixed to both chambers and connected via a copper wire to the external resistor or voltmeter. A salt bridge (using rope saturated with salt water) was also constructed, connecting the chambers. All experiments were conducted under room temperature and normal ambient light conditions. We used 750 mL of mud, 1 tablespoon of sucrose, and 1 tablespoon of iron filings per chamber (x6).

A total of 12 MFCs were engineered, with 3 replicates designed to test each of 4 experimental conditions: 1) mud in anode and water in cathode chambers; 2) mud and sucrose in anode and water in cathode chambers; 3) mud in anode and water and iron filings in cathode chambers; and 4) mud and sucrose in anode and water and iron filings in cathode chambers. Voltage output was recoded using a voltmeter twice daily for 12 days. A picture of one of the MFCs constructed shown in **Figure 4**.

From voltage measurements, the mean and standard deviation was computed using Excel. To determine statistical significance, we ran a one-way ANOVA test for our four independent experimental groups and derived an F-statistic of 65.5243 and a p-value of $1.1102e^{-16}$. Next, we applied the Tukey HSD post-hoc test to identify which pairs of groups were significantly different from each other.

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