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Article

The effect of food type on mediator-less microbial fuel cell electricity output

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SUMMARY

Clean power production is a heavily investigated topic in science today. The journey to find a clean, sustainable power source capable of producing enough energy to power an entire city is still in progress. Among the developments of different methods, the microbial fuel cell (MFC) is a promising idea, harnessing microbes to generate power through consumption of organic substances. In this investigation, we compared the different power outputs from using various foods as fuel sources, including sugar, cheese, and grapes. We hypothesized that different types of food, when placed inside the MFC, will produce different amounts of power. From the experiment, we observed a higher energy output for the sugar solution which suggests the potential energy output generated by the MFC of *Shewanella putrefaciens* is indeed affected by the fuel source used. From this study we have shown that the type of fuel used to power an MFC plays an instrumental role in the efficiency of the MFC and should be of the utmost importance when developing MFCs in the future.

INTRODUCTION

Even as scientists and engineers develop new ways to obtain natural resources to replace fossil fuels, the power outputs of the alternatives don't always match the oil and gas used as power sources today (1). A promising development in alternative electricity sources, however, is the microbial fuel cell (MFC), which employs microorganisms to consume organic substances, producing energy (2). In the past, MFC were difficult to make because of the expensive and often toxic chemical mediators required to take electrons from the microbes to be used as power (3). When Kim et al. discovered the ability for some bacteria to transfer electrons extracellularly, the mediators were no longer required, which opened the door to modern mediator-less MFC designs (4).

Mediator-less MFCs employ electroactive bacteria and their ability to transfer electrons extracellularly through one of three ways: direct contact, electron transferring shuttles, or cellular extensions (5). In this study, we focused on MFCs that use electron transfer via cellular extensions. By expanding, branching, and retracting membrane vesicles, bacteria such as *Shewanella* spp. give electrons to extracellular electron acceptors (5). These vesicles, which contain cytochromes filled with electrons, allow the bacteria to release electrons in an anaerobic environment (5). Mediator-less MFCs employ this concept, creating an anaerobic environment for bacteria to decompose organic matter, before providing an extracellular electron acceptor to accept the extra electrons (6). The vesicles send the electrons through a circuit, before electrons and stray protons decomposed by the bacteria combine at the cathode to create water, completing the circuit (6).

Many studies regarding MFCs are directed towards finding ways to improve the efficiency of the fuel cell using chemistry or microbiology means, by experimenting with different anodes, cathodes, microbes, or electron transport systems (7,8). However, few studies have explored the importance of different fuel sources in MFC power production (9). Developing MFCs with a focus on different fuel sources allows scientists to use the fuel source they have efficiently (10).

As described by Mercer, MFCs also have the ability to treat waste, consuming organic waste products to produce energy (11). In this experiment, we tested distinct types of food waste as fuel, and used *Shewanella putrefaciens* in a double chamber MFC to produce electricity. As a dissimilatory metal-reducing bacteria, *S. putrefaciens* depends on organic electron donors, such as food waste, to reduce metals, transferring energy between chemical bonds, into electrical energy (9). We identified cheese and grapes as promising forms of fuels in a previous experiment. We hypothesized that cheese would produce the most power because there is more energy stored in the form of lipids, which means there is more energy for the microbes to convert to power (12). Grapes, which contain high levels of potassium and dietary fiber, consist of less calories, which means there will be less energy for microbes to transform to power (13). However, from our experiments, the cheese produced only a fraction of the power that the grape was able to generate, indicating that the amount of energy contained in a food does not necessarily correlate with the amount of power MFCs can produce.

From further research, we theorized that ingredients in the cheese could have disrupted the electron flow from the bacteria to the electrodes, or that *S. putrefaciens* wasn't able to consume cheese as efficiently, resulting in the reduced power output compared to the grape or sugar. These results indicate that unforeseen factors relating to the fuel source used can drastically affect the efficiency of MFCs.

RESULTS

For the purpose of this experiment, a double chamber MFC was used because it is a commonly used and reliable fuel cell design. *S. putrefaciens* was the microbe of choice because of its accessibility.

After constructing the double chamber MFC, food waste was added to the anode chamber of each MFC. There were four test groups: a positive control (sugar), the cheese, the grape, and a negative control containing no food waste, each of which had three trials, resulting in a total of 12 MFC runs. Each of the MFCs were wired to a voltage splitter, with one side running to the cathode and one side running to an Arduino circuit board. The Arduino board then collected the voltage data once every five seconds for a total of 16 hours. The data have specific values and intervals because the Arduino circuit board can only read voltages on a discrete scale.

The MFCs using grape as fuel performed almost as well as the positive control, with the positive control MFC reaching a maximum of 0.37 V and the grape MFC reaching 0.35 V at around 6 hours (**Figure 1**). The cheese MFC performed considerably worse, reaching only up to 0.03 V at most (**Figure 1**). The negative control produced no voltage (**Figure 1**).

DISCUSSION

From our results, contrary to our hypothesis, using cheese in the MFC did not produce the most power. Instead, the positive control produced the most power, followed by the grape, cheese, and finally the negative control with no food waste provided. Because the positive control and the grape produced more power than the other groups in an MFC, it can be concluded that sugar, specifically glucose and sucrose because they are the predominant sugars in grapes, is consumed efficiently by *S. putrefaciens* for the production of electricity (14).

We concluded that the cheese was not able to produce as much power. This is likely because the fat content in cheese can reach up to 33% of the mass of the cheese, causing a lot of the fat to be released when the cheese is set in the water (15). One possible implication of this is that the fat mixes into the water, preventing the electrons from being able to reach the anode. According to Rouabeh et al., at approximately 30ºC, some oils reach a resistivity of up to 6TΩ•cm, which could explain the lower power output (16). But given that from our experiment the MFC with cheese still produced voltage, it is still possible that the *S. putrefaciens* was able to consume part of the cheese and produce a slight bit of power with it.

Another possible reason why the cheese MFC failed to produce much power is because the bacteria could not reduce the complex structure of the cheese. Cheese is made up primarily of casein protein and fat, with trace amounts of sugar (17). The coagulation of cheese removed most of the sugar, which *S. putrefaciens* reduces preferentially, leaving the proteins and the fat (17). This means the bacteria may only have been able to reduce the small amounts of sugar, resulting in less power being generated.

Instead of the cheese producing more power, the grape was the food product that produced the most power. As carbohydrates make up about 17% of a grape's mass, the amount of sugar in this chamber exceeded any of the others by far, apart from the chamber with just sugar (18). This sugar content allows the *S. putrefaciens* to produce energy. Grapes also have a very low level of fat, preventing grapes from generating the same issue possibly caused by the cheese (18).

One observation to be made is the oscillation of the input voltages. Throughout the experiment, the voltage did not remain constant but fluctuated between a range of values (**Figure 1**). It can also be noted that the range of values moved up in discrete intervals as opposed to a gradual increase of voltages. This may be the result of limitations with the voltage input of the Arduino microcontroller but should not affect the other conclusions made.

The limitations in our experiment included performing the experiment at different time periods and using an Arduino circuit board to record the data. Because only six MFCs were available, the entire experiment had to be divided into two

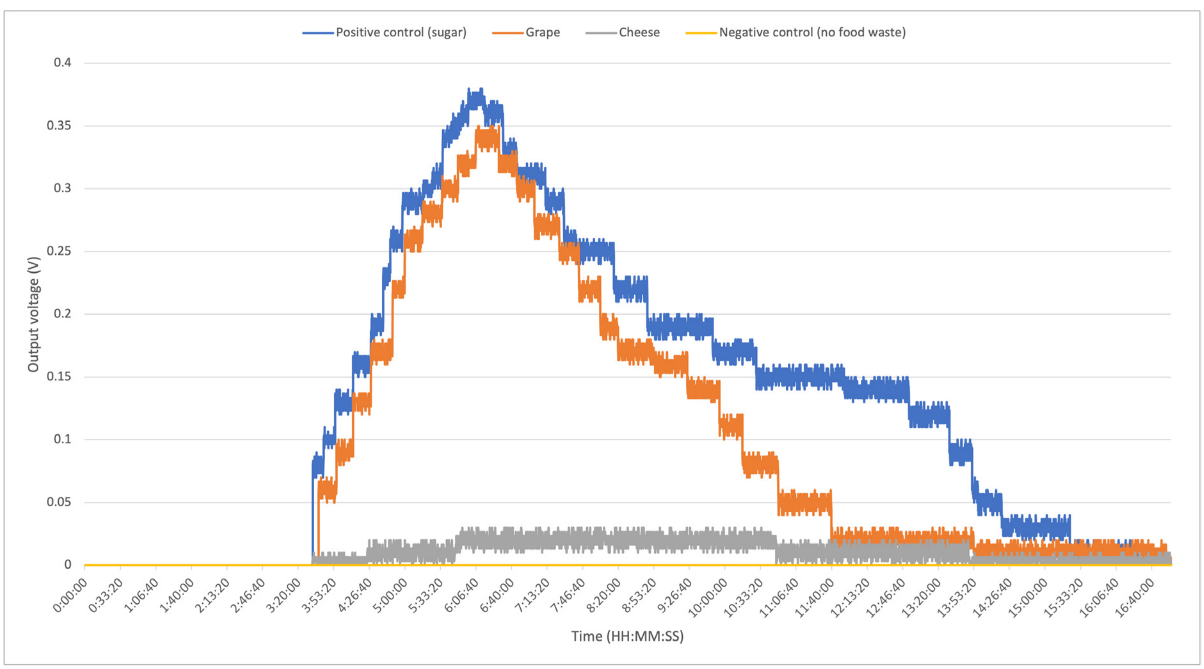

Figure 1. Difference in voltage output of the MFCs showing the positive control and the grape sample producing more power. Voltages produced by the fuel cells over a time span of 17 hours (n=3). 15 g of the three types of food waste, sugar (blue), grape (orange), and cheese (grey), were placed in a double chambered MFC, using an agar salt bridge and *S. putrefaciens* as the microbe.

separate time periods, during which environmental factors may have affected the results, even though the temperature was kept at a consistent 30ºC. Also, because we used an Arduino circuit board to record data, the voltages recorded showed up as discrete values, which could make the results less accurate. Because the circuit board reads input voltage at intervals of approximately 0.04V, at such low input voltages the circuit board becomes slightly less precise (19).

To eliminate errors and inaccuracies in future experiments, a more accurate voltmeter at low voltages can be used, with each MFC having its own voltmeter. The biggest advantage of using the Arduino circuit board to read voltages is that it can read multiple voltages simultaneously, but a more ideal experiment would have voltmeters that are more accurate with low voltages. Then, if each MFC had its own voltmeter, there would not be any problems with the voltage intervals read affecting the results. Additional MFCs could also have been constructed to minimize inaccuracies caused by environmental factors.

Future experiments could be conducted regarding different types of bacteria and what types of food waste produce the most power with each. From our research and experiment, *S. putrefaciens* consumes different types of sugar more efficiently than others (20). However, not all bacteria may behave that way, and experimenting with different types of bacteria would allow us to find bacteria that may consume other foods, such as dairy, better, allowing MFCs targeting different types of food waste to be constructed.

Another possible experiment is experimenting with more types of foods. In this experiment, one type of dairy and one type of fruit was used, but each type of food will have its own compounds and may produce different amounts of power. Inedible organic substances can also be experimented on to find new purposes for waste such as fish scales or bones.

In conclusion, out of all of the test groups, the positive control (sugar) MFCs performed the best, followed by grape, cheese, and finally the negative control, indicating that *S. putrefaciens* prefers sugars as a food source in MFCs. Currently, the majority of MFC research is focused on different MFC structures, but in this experiment, we have shown that different microbes with different food sources can vastly impact the power output, meaning that this is a field worth further research in the future.

MATERIALS AND METHODS

S. putrefaciens Growth and Salt-bridge Preparation

A freeze-dried sample of *S. putrefaciens* was obtained from the Bioresource Collection and Research Center from Hsinchu, Taiwan. After the sample of bacteria was thawed rapidly in a water bath, 225 mL of tryptic soy broth was used to culture the bacteria for two days. Next, the salt bridges were constructed. To create the salt bridges, a solution of 250 mL 0.15 M NaCl was mixed with 7.5 g of agar, heated, stirred. Then poured into six plastic test tubes cut off at each end to form a cylinder. The solution was then set to cool inside the plastic cylinders until they were solid, forming the salt bridge.

MFC Construction

Each of the chambers consisted of a T25 cell culture flask, with a hole drilled in the side of each flask. Two chambers were placed opening-to-opening, with the salt bridges connecting the two flasks, and the chambers were sealed together using Parafilm, ensuring the setup was free of water leaks. Cylindrical graphite electrodes were inserted into the hole in the side of the flasks, attached by a wire to a circuit board (**Figure 2**). The circuit board consisted of a current divider circuit that allowed an Arduino microcontroller to measure the voltage coming out of the electrodes. The program used to measure the voltage is found at the following website: gist.github.com/i3ta/b9618e918b259148963ea241ecbc6d04.

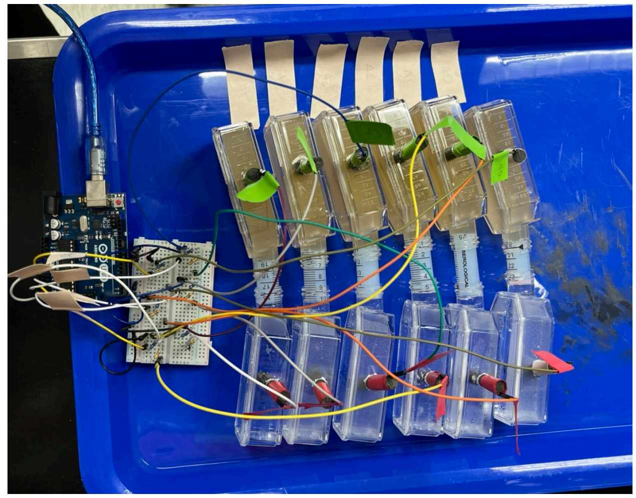

Figure 2. Experimental setup with six double-chambered MFCs. Anodes are labeled with green tape and cathodes labeled with red tape, wired up to voltage splitters (breadboard), connected to the Arduino circuit board (left). The test groups in this image are the sugar positive control (right three) and the grape (left three)."

Experimental Procedure

15 g of each type of food waste was ground down to smaller pieces and inserted into the anode chambers. The bacteria was then injected into the anode chambers with a pipette. The cathode chambers were filled with water. Finally, the two electrodes were placed into their respective holes in the chamber, one in the anode and one in the cathode chambers. The MFCs were placed into a temperature-controlled chamber, and an Arduino microcontroller collected the voltage from the MFC once per 10 seconds. The MFCs stopped producing power after 16 hours; the data was collected continuously during this time period. This process was repeated three times for each test group, for a total of four test groups (two experimental groups and two control groups). Because we only had six MFCs, we tested the two test groups first, before cleaning them out and repeating with the remaining test groups.

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