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Article

Voltage, power, and energy production of a *Shewanella oneidensis* biofilm microbial fuel cell in microgravity

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SUMMARY

Future long-term space travel requires both efficient waste management and renewable energy production to be feasible. One such option in addressing these issues is a microbial fuel cell (MFC) that converts chemical energy in organic matter to electrical energy through biological processes of microbes. Electroactive biofilms are special colonies of microbes that utilize an extracellular matrix to increase the endurance and growth of bacterial colonies through the sharing of resources and the depositing of electrons. We studied the power production of a biofilm MFC by testing the fuel cell in microgravity over time on the International Space Station (ISS). We utilized *Shewanella oneidensis*, an established electroactive biofilm, to break down a nutrient solution and release electrons and protons, producing a voltage difference across the cell. The *S. onedensis* biofilm grew more prolifically under low-pressure conditions, making it well suited for microgravity; consequently, the consumption of sodium lactate in a larger biofilm caused an increase in anaerobic respiration of the bacteria. This increased the voltage difference recorded across the cell and the corresponding power of the MFC. Our results are consistent with our hypothesis that there would be an increase in voltage and power production over time; however, an insufficient amount of growth medium eventually led to a decrease in voltage and power production as the biofilm died out. Power output during microgravity testing increased over time, coinciding with nutrient solution pump cycles. This experiment established that an MFC is a promising avenue for the development of renewable energy in microgravity.

INTRODUCTION

Bacteria undergo surface association in natural environments to maintain a favorable environment (1). The bacteria organize themselves into a biological extracellular matrix constructed from extracellular polymeric substances and carbohydrate-binding proteins. The biofilm structure allows for cell-to-cell interaction that is crucial for electrochemical processes in electrochemically active bacteria such as *Shewanella oneidensis* (2).

The microbial fuel cell (MFC) is an area of promising research in renewable energy (3). The potential in MFCs lies within the ability to utilize unwanted biomass, which would otherwise be treated as waste, as nutrients for the bacteria to generate electricity through catalytic reactions of bacteria (3). These bacteria harness and store energy as adenosine triphosphate (ATP) by oxidizing reduced substrates like sodium lactate (SL) and transferring electrons to respiratory enzymes via NADH, creating a proton gradient across an internal membrane (4). This gradient drives ATP production through the enzyme ATPase and the release of electrons to a terminal electron acceptor, which results in current generation as electrons flow through an external circuit from the anodic chamber, where bacterial oxidation of substrates occurs, to the cathode and electron acceptor (4). This electrochemical nature of bacteria, and its usage to generate power has been observed in numerous other bacteria, such as *Klebsiella, Candida, Escherichia, Saccharomyces*, *Aeromonas*, and *Clostridium* (5).

MFCs utilize bacterial colonies or biofilms to extract nutrients from an organic source and convert the energy contained in those nutrients into electrical energy (6, 7). These fuel cells consist of two chambers: an anodic chamber and a cathodic chamber (6). The biofilm in the anodic chamber releases electrons through aerobic or anaerobic respiration, which are then deposited in the cathodic chamber by a copper wire and accepted by diluted potassium ferricyanide (6). The chambers are separated by a semipermeable membrane, allowing the flow of protons (**Figure 1**) (6).

In recent renewable energy research and in industry use, electrochemically active biofilms have become increasingly common and widespread (7, 8). The purpose of our research was to utilize an electrochemically active biofilm to generate renewable energy in microgravity. The microgravity aspect of this investigation was heavily inspired by previously demonstrated results by Kim et al. showing that spaceflight can promote the growth of biofilms (9). In such experiments, biomass and thickness of the bacteria of *Pseudomonas aeruginosa* biofilms were shown to increase significantly due to the lack of pressure in microgravity. Additionally, the biofilms formed a column-and-canopy structure not previously observed on earth (9). This structure has also been found in the biofilm colonies of *Pseudomonas* and *Staphylococcus* spp. in 2010 and 2011 on experiments aboard Space Shuttle Atlantis examining the growth of biofilms (10).

We hypothesized that the stimulated growth of biofilm in microgravity would lead to increased power and energy production of a biofilm MFC over time, primarily due to the enhanced efficiency of anaerobic respiration facilitated by the growing biofilm. We conducted the study by testing a biofilm MFC on the International Space Station (ISS) over a 30-day period. To accomplish this, we utilized *S. oneidensis*, an established electroactive biofilm, to break down a nutrient solution and generate a voltage difference across the cell by releasing electrons.

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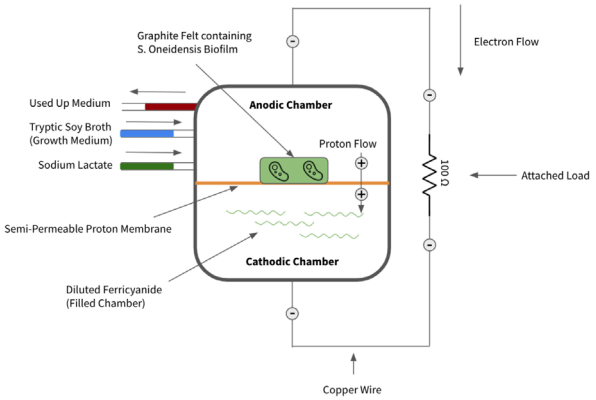

Figure 1: Microbial Fuel Cell Diagram. Displayed is a full diagram of the anodic and cathodic chambers that comprised the MFC. Proton flow was mediated by a semipermeable membrane, and electron flow was mediated by two copper wires, one placed in the ferricyanide solution of the cathodic chamber and the other threaded through the graphite felt inside of the anodic chamber.

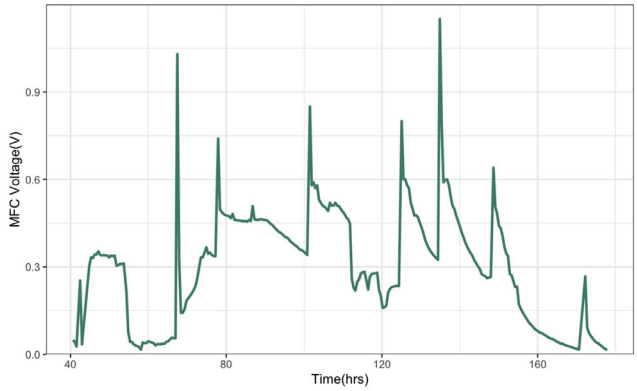

Figure 2: Measured MFC voltage in microgravity over time. Plotted in the graph are data measured from an amplification circuit, which allowed for precise voltage readings with uncertainty of approximately ±0.0024 V. Each voltage reading was measured in 10 bits or 1024 steps out of a maximum of 2.5 V. The solid green line represents the voltage readings of the MFC taken by the microcontroller.

RESULTS

To be sent to the ISS, the *S. oneidensis* needed to be lyophilized to survive the journey. Prior to lyophilizing the *S. oneidensis* biofilm for the actual chamber of the experiment, we conducted a test run of four different excipients - whey, milk powder, trehalose, and a control - to see whether the *S. oneidensis* biofilms would survive after lyophilization. We found that *S. oneidensis* biofilms lyophilized with 0.75 g of milk powder survived, which led to our usage of milk powder as the excipient for lyophilization of the biofilm in the experiment (**Table 1**).

Throughout the experiment's 30-day span, we used a microcontroller to record the net voltage difference across our experimental unit, taking readings every 30 minutes. The voltage graph reflects voltage data across the MFC throughout the duration of the experiment (**Figure 2**). The two largest voltage differences in microgravity occurred at 72 and 144 hours with values of 1.03 V and 1.15 V, respectively. The two most significant spikes in the produced power and voltage, at 72 and 144 hours, are due to the periodic pumping of SL into the central chamber (**Figure 2** and **3**). When the SL was pumped, the bacteria metabolized the lactate through anaerobic respiration, producing electrons in the process (4). This led to the release of electrons and a voltage difference across the cell. Furthermore, the power output increased by 2.62 mW while the voltage output increased by 0.12 V from 72 hours to 144 hours, reflecting increases of 24.7% and 11.7%, respectively. This increased power and voltage over time aligns with our initial hypothesis and is likely due to the biofilm growing over time. Intermediate voltage spikes, apart from the aforementioned two, are also present, separated by a constant time interval of 25.26 ± 0.01 hours, likely due to the circulation of SL between pumping cycles.

	Milk Powder	Whey	Trehalose	None
Trial 1	0.75 g	0.30 g	0.75 g	0 g
Trial 2	0.75 g	0.30 g	0.75 g	0 g
Trial 3	0.75 g	0.30 g	0.75 g	0 g

Table 1: Lyophilization Pre Experiment. Cells marked in red in the table indicate that the biofilm used with the excipient in that trial died after lyophilization. Cells marked in green indicate that the biofilm survived the trial with that particular amount of excipient.

The power graph displays the power output of the MFC as calculated by the squared voltage across the load divided by its 100 Ω resistance (**Figure 3**). The MFC's power output peaked at 72 and 144 hours, reaching peaks of 10.61 mW and 13.23 mW.

The estimated accumulation of energy production, as approximated by a trapezoidal Riemann sum, rose somewhat steadily throughout the experiment's duration, reaching a total of 176.67 mJ after nearly eight days in microgravity (**Figure 4**). No power was produced by the cell following this eight-day peak.

DISCUSSION

The aim of our investigation was to assess the viability of an MFC as a means of power production in microgravity. Due to biofilm proliferation in microgravity and bacterial growth over time, we expected an increase in power production throughout the experiment's duration. Our observed 24.7% increase in power supports this hypothesis, providing evidence that the biofilm's accelerated growth over time did indeed cause an increase in power output.

Intermediate spikes in power and voltage - outside of SL pumping cycles - were likely due to SL which was recirculated into the growth medium bag before being fully utilized by the biofilm. This SL was then circulated back into the anodic chamber at regular intervals, leading to shorter spikes in the cell's energy production.

Lastly, the lack of energy production after eight days of testing can be best attributed to diluted growth medium or a lack of sufficient growth medium due to the experiment's size limitations. In this case, future research that supplies an increased amount of growth medium and SL to the cell's anodic chamber to reduce effects of dilution would be beneficial. However, the energy production of our MFC demonstrates that these fuel cells are a viable option for energy production in space. Furthermore, as spikes in power and voltage output increased over time with the growth of the *S. oneidensis* biofilm, this research provides evidence for a

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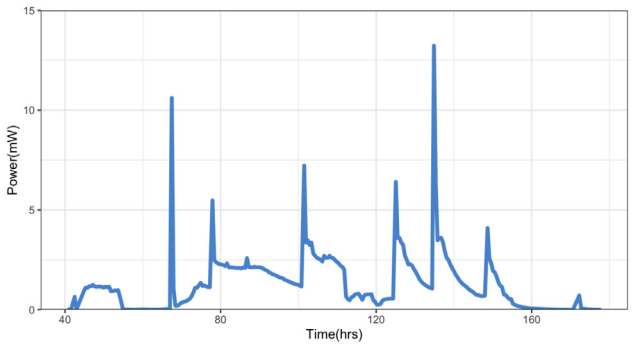

Figure 3: Calculated MFC Power in microgravity over time. Since the MFC was kept in series with constant resistance, the power shown is proportional to the square of the voltage. Power peaked at 13.23 mW after 144 hours.

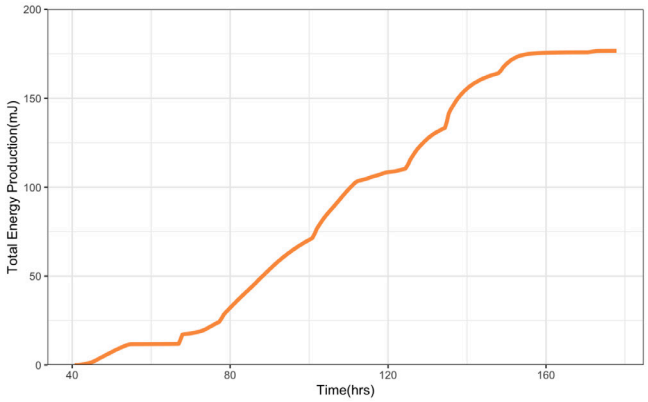

Figure 4: Estimated Total Energy Production over time. Accumulated energy was calculated as the integral of power with respect to time throughout the experiment's runtime. The total energy consistently increased as long as the biofilm remained alive.

correlation between biofilm growth in microgravity and the corresponding energy production of an MFC.

Because of the biofilm's increasing power output over time, this experiment provided evidence that MFCs are a viable avenue for small-scale sustainable energy production in microgravity. Sources of unwanted biomass, such as wastewater, can be used as an energy source for *S. oneidensis,* as shown by previous research, where these forms of biomass contain carbon sources that power anaerobic respiration, which leads to the eventual release of electrons (11). MFCs provide an important opportunity to utilize waste as a renewable resource; however, this opportunity must be determined to be scalable. The problem in scalability lies in the fact that energy production is determined by the surface area of the bacterial biofilm for reactions to take place while the energy source for the reactions takes up a certain volume. As the size of the cell increases, the energy efficiency of the cell will likely decrease due to this making it less scalable.

Potential future research in microgravity-based renewable energy production still exists within the field of biofilm MFCs. A greater understanding of the difference between biofilm MFC power production on earth and in microgravity could be established by more experimentation directly comparing power production between biofilms grown under varying gravitational conditions. While our research has shown the potential for small-scale power production of a *S. oneidensis* biofilm in microgravity, questions of the potential for other electrochemically active bacteria remain.

MATERIALS AND METHODS

Bacterial culture

First, the purchased *Shewanella oneidensis* MR-1 strain (ATCC, catalog #700550) was received in a lyophilized form, so it needed to be reactivated. A petri dish was prepared with 10 mL of tryptic soy broth (TSB) (Millipore Sigma, catalog #22092). Lyophilized *S. oneidensis* was placed into the petri dish. The petri dish was placed in an incubator for 48 hours at 30°C.

Bacterial culture on graphite felt

Next, a *S. oneidensis* biofilm needed to be formed on top of a graphite felt for placement into the MFC. A 10 mm x 10 mm square of graphite felt was submerged into the petri dish containing *S. oneidensis*. An additional 5 mL of TSB was added to allow for additional growth. The petri dish was placed into an incubator for another 24 hours at 30°C.

Lyophilization

The graphite felt containing the *S. oneidensis* biofilm was placed in a test tube containing 10 mL of TSB and 0.75 g of dissolved milk powder. The test tube was then shipped in a freezer to Lyohub for lyophilization.

Assembly of MFC

For assembly of the fuel cell, open-ended cylindrical cathodic and anodic chambers were 3D printed in Acrylonitrile Butadiene Styrene (ABS) plastic, both measuring 15.8 mm in diameter and 6 mm in height. A copper wire was placed through the center with one end inside of the cathodic chamber and one end on the outside. The cathodic chamber was then filled with 1.176 mL of 0.002 M potassium ferricyanide (Millipore Sigma, catalog #702587). A semi-permeable proton membrane was placed on to the open end of the cathodic chamber and epoxied down to close the chamber. A sterilized copper wire was threaded with a sterilized needle through the graphite felt containing the lyophilized *S. oneidensis* biofilm. The graphite felt was then placed on the semi permeable proton membrane in the anodic chamber. Three holes were drilled into the anodic chamber: two for the pumps and one for the copper wire of the graphite felt. The copper wire for the graphite felt was threaded through its corresponding hole in the anodic chamber which was then epoxied shut. The anodic chamber was epoxied to the semi-permeable proton membrane creating a sandwich of anodic chamber, proton membrane, and cathodic chamber (**Figure 1**). The copper wire of the anodic chamber and the copper wire of the cathodic chamber were attached to a 100 Ω resistor and soldered to their respective places on the circuit board of the microcontroller (**Figure 5**).

Two water bags laid in the center of the experiment and were connected to individual peristaltic pumps. One water bag was filled with 12 mL of TSB, and another water bag was filled with 10 mL of SL. The TSB bag had both an input and output connection to allow for the circulation of fluids through the bag. The SL bag only had an output connection meaning

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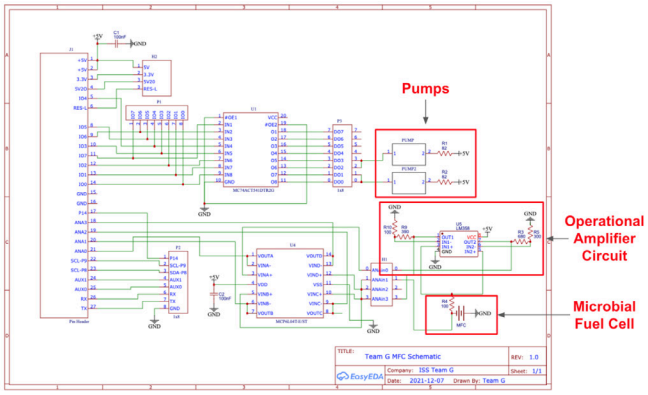

Figure 5: Electrical schematic. The operational amplifier circuit is displayed in the bottom right, which connects to the MFC to amplify the fuel cell's voltage so that the microcontroller can accurately read it. The copper wire of the anodic chamber was soldered to the 100 Ω resistor, and the copper wire of the cathodic chamber was soldered to the ground. Amplification factors of 4.9x and 3.27x were individually used on the fuel cell's voltage and linked to their own analog pins on the microcontroller. In addition, two pumps are displayed, each corresponding to an individual water bag.

that SL could only be pumped into the chamber, used by the biofilm, and then circulated through the TSB bag. The MFC chamber was placed with the anodic chamber pointing towards the two water bags to allow for shorter tubing (**Figure 6**).

Operating MFC in ISS

After the experiment was plugged in aboard the ISS, an automated program ran over 30 days as follows. The pump attached to the TSB water bag ran for 24 hours to activate the lyophilized biofilm and start its growth process by circulating all of the TSB through the chamber. The pump attached to the SL water bag was then turned on for 25 seconds pumping 0.5 mL. The following loop was then run for the remainder of the 30 days. The TSB bag was pumped for 72 hours then turned off. The SL bag was then pumped for 15 seconds for 0.5 mL of SL Photos of the chamber and voltage readings were taken every 30 minutes for the entire duration of the experiment (**Figure 7**).

In the bottom right of the electrical schematic is the operational amplifier circuit that connects to the MFC to amplify the fuel cell's voltage for it to be accurately read by the microcontroller. The copper wire of the anodic chamber was soldered to the 100 Ω resistor, and the copper wire of the cathodic chamber was soldered to ground. Amplification factors of 4.9x and 3.27x were individually used on the fuel cell's voltage and linked to their own analog pins on the microcontroller. In addition, two pumps are displayed, each corresponding to an individual water bag (**Figure 5**).

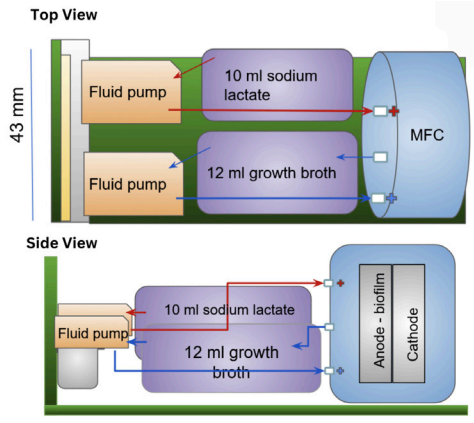

Figure 6: Mechanical block diagram. The water bags, laid in the center of the experiment, were connected to individual peristaltic pumps. The tryptic soy broth bag had both input and output connections to allow for the circulation of fluids through the bag. The sodium lactate bag only had an output connection, meaning that sodium lactate could only be pumped into the chamber, used by the biofilm, and then circulated through the tryptic soy broth bag. The MFC chamber was placed with the anodic chamber pointing towards the two water bags to allow for shorter tubing.

Analysis

For analysis of the data, voltage measurements were taken every 30 minutes. The power of the MFC was calculated through the formula $P = V^2/R$. The load attached to the fuel cell was a 100 Ω resistor. Each voltage reading was squared and divided by the resistance to find the output power of the MFC (**Figure 3**). The energy produced by the MFC was estimated through an approximation of the right-hand side of the equation:

$$E(t) = \int_0^t P(t) \cdot dt$$

E(t) is the total energy produced by the fuel cell up till time, *t*, and *P(t)* is the power of the fuel cell at time *t*. A trapezoidal approximation of the integral was performed, which estimated the integral of two consecutive voltage reading points in time, t_1 and t_2, as

$$\int_{t_1}^{t_2} P(t) \cdot dt \approx \frac{P(t_1) + P(t_2)}{2} \cdot (t_2 - t_1)$$

This approximation was used to generate a series of estimated total energy production points at each time when a voltage reading was taken (**Figure 4**).

Experiment Code

The code used in this manuscript can be found at: https://github.com/ISS-TeamG-2021/Microbial-Fuel-Cell. The code for the experiment was written in PBASIC.

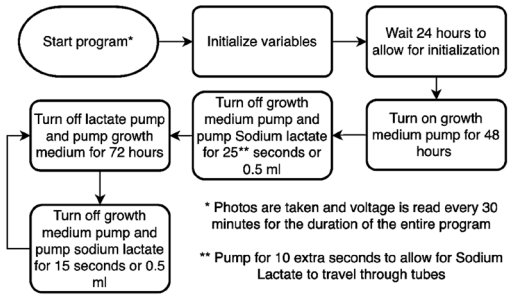

Figure 7. Software flowchart.

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Our work also used *S. oneidensis* MR-1 bacterial strains cultivated by the John D. Coates Lab at UC Berkeley. In addition, Dr. Coates and his lab were instrumental in teaching our team how to grow a *S. oneidensis* biofilm and the basics of an MFC.

Our team also worked with the company, Lyohub at Purdue University, run by Dr. Alexeenko and Dr. Topp who lyophilized our grown *S. oneidensis* biofilm pro bono prior to it being sent up to the ISS. Furthermore, Dr. Alexeenko, Dr. Topp, and research scientist Drew Strongrich explained to our team the steps on how to test lyophilization with our biofilms using different excipients to ensure their survival post-lyophilization on the ISS.

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