The Creation and Optimization of a Plant Microbial Fuel Cell for Energy Generation with *Brassica rapa*

Research Paper

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Abstract

In 2017, 25% of the world's energy came from renewable sources, with 50% of the renewable energy coming from bioenergy. As the global consumption of energy is expected to increase by 28% by 2040, the demand for renewable and biological energy will increase as well. The purpose of this study was to create and optimize a Plant Microbial Fuel Cell (PMFC) using *Brassica rapa*, as a potential alternative source of energy. PMFCs were created in 13-ounce containers with graphite felt attached to titanium wire for electrodes. Square, circle, and octopus-shaped electrodes, distances of 3, 6, and 9 cm between the electrodes, inoculation of the anode with *Escherichia coli* k-12, the addition of *Citrus sinensis*, and connection of PMFCs in a series configuration were experimental variables tested to optimize the PMFC. Data was collected over a 360-hour period for each trial, and the results showed a PMFC with circle electrodes inoculated with *E. coli* k-12, separated by 3cm, and with the addition of *Citrus sinensis* yielding the greatest average potential. Circle electrodes yield greater consistency in readings due to the more efficient surface area use, and inoculation with E. coli increases the electron output due to the bacteria's electrogenic properties. A distance of 3 cm increases efficiency by minimizing internal resistance, and the addition of waste material increases the amount of organic material available for decomposition- this increases the number of free electrons in the system.

I. Introduction

In the last 60 years carbon dioxide (CO_2) levels have been increasing 100 times faster than they have since the previous natural increase in levels (the last ice age 11,000-17,000 years ago). The increase in CO_2 in the atmosphere is responsible for about two-thirds of the total energy imbalance causing Earth's temperature to rise. This large increase in CO_2 levels is due to the high demand for the burning of fossil fuels to generate energy/electricity. CO_2 is a greenhouse gas, it absorbs heat, too much in the environment results in negative effects like an increase in ocean acidity and in ocean water temperatures. Both factors make it more difficult for marine organisms to maintain homeostasis and survive. Increasing ocean temperatures also leads to melting ice sheets which in turn causes the release of more CO_2 into the atmosphere creating a positive feedback loop. The melting ice sheets also lead an increase in sea levels which result in habitat loss. (Lindsey 2018)

Sustainable energy has been produced through the use of wind, solar, geothermal, and hydroelectric power all of which decrease CO_2 footprints. However, such methods face disadvantages like landscape transformation, energy-intensive processes, and geographic limitations. Alternative energy sources that face less limitations while also decreasing the amount of greenhouse gases being emitted are needed. (Nitisora vut and Regmi 2017)

Microbial fuel cells (MFCs) could serve as this alternative source of energy. One continuously fed MFC can efficiently and continuously power a small sensor like a temperature sensor. (Khaled et al. 2015) MFCs use active microorganisms as a biocatalyst to speed up the anaerobic reaction at the anode. MFCs consist of a cathode normally in water and an anode as a single or double chamber with a proton exchange membrane (PEM). (Rahimnejad et al. 2015) Microorganisms that live in close contact with the anode oxidize the organic matter near the anode creating a biofilm layer around the anode (Aelterman et al. 2006). The electrons that are lost during oxidation can be exchanged through and external circuit that connects the anode to the cathode. The difference in voltage between the cathode and anode combined with the electron flow in the outer circuit generates electrical power. Two-chamber MFC consist of aerobic (cathode) and anaerobic (anode) compartments separated by a PEM membrane or salt bridge. The protons generated diffuse through the PEM into the cathode chamber, where they combine with electrons and oxygen to form water. (Yadava et al. 2012) A PEM or salt bridge in MFCs serve as electrolytes they allow protons to pass through to the cathode (Jang et al. 2004). To increase the efficiency of the electron transfer in the MFC, bacteria like *Rhodoferax ferrireducens*, *Bacillus subtilis*, *Geobacter sulfurreducens*, and *Escherichia coli* k-12 can be used to inoculate the electrodes. The addition of the bacteria allow for the creation of a mediator-less MFC. (Huarachi-Olivera et al. 2018) MFCs are not only limited to the generation of electricity. Additional voltage can be added to a fuel cell allowing for the generation of various products at the cathode such as hydrogen, methane, and hydrogen peroxide. Membranes can be used to facilitate desalination, while simultaneously generating electrical power. (Logan 2009)

Sediment microbial fuel cells (SMFCs) are a type of MFC. These fuel cells harvest energy in the same way that an MFC does, but the anode is placed in anaerobic sediment rather than water, and the cathode is placed in overlying aerobic water. SMFCs have the advantage of

maintenance-free operation, long-term power generation, and the ability to power devices in remote areas. (Wang et al. 2014) Organic compounds in sediment serve as nutrients to microbes that end up inoculating on the anode surface. Microbes that oxidize organic matter and create electrons and protons as byproducts are known as electrogenic. Like in a MFC, through an external circuit, electrons are taken up by the anode and are transferred to the cathode. At the cathode oxygen as the electron acceptor is reduced. Since the reaction is thermodynamically favorable, electricity is generated when the electrons are transferred through the circuit. (Prasad and Tripathi 2018)

In order to generate more energy and scale up SMFCs, Ewing et al.2014 looked at a parallel scale up set up in comparison to a single SMFC of the same size. In the study the parallel system consisted of 4 single-equivalent SMFCs the scaled-up SMFC was made of 4 smaller cathodes and anodes in one SMFC that amounted to the same size as one of the cathodes and anodes in the equivalent set up. Both systems were connected to a power management system (PMS). With the single-equivalent SMFC allf 4 channels of the PMS were used with the scaled-up SMFC only one channel was used. The PMS harvest energy from the SMFC(s) using non-regulated boost converters. The boost converters increased the voltage from the input into the system to the output. The channels gathered the energy from the SMFC and outputted them into one storage capacitor. Results showed that after the first 155 days the single scaled-up SMFC produced significantly more power than the 4 SMFCs that were parallely connected. The larger generation of power by the of the smaller electrodes shows that the answer to scaling up microbial fuel cells may not be physically scaling up the size of electrodes, but rather through the use of electronics.

Prasad and Tripathi 2018, stacked SMFCs and compared the energy generated from just a series connection of SMFC to a hybrid of both series and parallel connections. To link all the SMFCs together in a series, SMFCs were set up in a single line and the cathode of each electrode was connected to the anode of the SMFC that followed it. The cathode of the last cell was connected to the anode of the last cell. In the hybrid the same mechanism of cathode connected to anode was used but a parallel connection was incorporated by connecting an anode with an anode and a cathode with a cathode. The study found that output voltage and current increased with the hybrid SMFC configuration, whereas only voltage output increased in the SMFC series configuration. However, the series configuration ended up generating a maximum voltage greater than that of the hybrid; the max voltage being 8.150 volts (V). It was concluded that through the use of series configuration voltage can be boosted enough for real world electrical application.

Scaling up SMFCs using a hybrid, series, or parallel configuration are all novel ideas that are more economically feasible than physically scaling up electrodes. More research on scale up methods needs to be done in order to address this problem faced by SMFCs. Another possible method boost power production is the addition of plants into SMFCs making plant microbial fuel cells (PMFCs). Since plants produce root exudates (excess nutrients that are released into soil) they provide more nutrients for microorganisms in the sediment.(Deng et al 2012) The addition

of organic material has shown to increase power output, because there is more organic material for the microorganisms to decompose. However, the addition of organic material must be done cautiously because the organic material could promote fermented bacteria which can decrease power output by outcompeting electrogenic microorganisms for electrones. (Nitisoravut and Regmi 2017) Many plants that are utilized are usually aquatic, because of the overlaying aerobic water generally used for the oxidation of the cathode (Deng et al 2012).

II. Methodology

II.1 Experiment Overview

All trials lasted for a duration of 20 days and multimeter readings were taken every 24 hours. Control trials consisted of non-inoculated circle electrodes placed 6 cm apart with 4 cm of soil on top of the anode. Constant variables throughout all trials were the use of 6 *B. rapa* seeds, 14 hours of light with 10 hours of darkness, and 50 mL of tap water as irrigation every other day. 3 trials were conducted for every experimental variable being tested.

Pilot Work

The pilot work focused on setting up the system that held the PMFCs for the duration of the study. The purpose of the system was to prevent exposure to any external unknown agents that could cultivate in the PMFCs. 12.5" x 11.5" x 11.75" (LxWxH) storage tote containers with a capacity of 4 PMFCs each were utilized. A sprinkler system was created with 1/2" PVC pipes and placed 9 cm above the fuel cells. The PVC pipe system was attached to a water pump located outside of the container to all for watering the PMFCs. 1/2" holes were drilled- plastic trays with 3/8" holes were placed in each corner of the tote and PMFCs were subsequently placed on top of the trays allowing for effective drainage. 5/64" holes were drilled into the totes to connect each PMFC to a Vernier Energy Sensor using alligator clips. The sensor was connected to a LabQuest Pro for data collection, and a 40 watt fluorescent light lamp controlled by an intermatic timer was placed on top of the cover of the system. There were no openings in the system throughout the duration of each trial.

Phase 1

Phase 1 of the study focused on creating the PMFCs. 32 ounce containers acted as individual PMFCs. A 2" hole was cut into the bottom of each container to allow for water drainage and cathode oxidation. A cathode was placed on top of the hole; sifted soil was placed on top of the cathode, and the anode was placed on top of the soil. Additional soil was sifted and placed on the anode- 6 pre-germinated *B. rapa* seeds were planted in a circle in this soil. The total amount of soil in the system varied depending on the experimental distance between the electrodes that was being tested.

Phase 2

Phase 2 focused on optimizing factors from Phase 1 that could have a potential effect on the efficiency of the PMFC function. Variables including the distance between electrodes, electrode shape, and the inoculation of the anode with *Escherichia coli* k-12 were tested and

observed. By altering the distance between the electrodes, the internal resistance was altered. Distances of 3, 6, and 9 cm was tested by altering the amount of soil between the electrodes. By testing different electrode shapes, the effect of electrode surface area on PMFC function was observed. The different electrode shapes were circular, square, and octopus. The anode was inoculated with *Escherichia coli* k-12 to observe the effects of the bacteria on voltage output.

Phase 3

Using the design from Phase 1 and the most optimal variables from Phase 2, Phase 3 incorporated these factors to look at ways to increase PMFC energy output. The addition of waste material to individual PMFCs and the connection of PMFCs in a series configuration were then observed as well. The effect of waste material was tested through the addition of *Citrus sinensis* peel. The addition of waste material provided additional material for the bacteria to decompose, increasing the amount of electrons being transferred to the anode. Three PMFCs were connected in a series configuration as another variable to increase the energy output.

II.2 Obtaining Materials

For the enclosed system, a plastic container 12.5" x 11.5" x 11.75" and an 80 gallon per hour water pump were obtained from Amazon; 1/2" PVC elbows, 1/2" PVC tees, and 1/2" PVC pipes, 360° sprayers, a PVC pipe cutter, power drill, and 1/2" vinyl tubing were obtained from Home Depot.

For the PMFC, *Escherichia coli* k-12 and 32 ounce plastic containers- 13.3 centimeters (cm) in height and a top diameter of 8.9 cm- were obtained from Carolina Biological. Graphite felt was obtained from the Fuel Cell Store. Potting soil was obtained from Home Depot. Titanium wire was obtained from Amazon. *Brassica rapa* seeds, Vernier Energy Sensors, LabQuest 2, and alligator clips were obtained from school. *Citrus sinensis* peels were obtained by peeling oranges from local supermarkets.

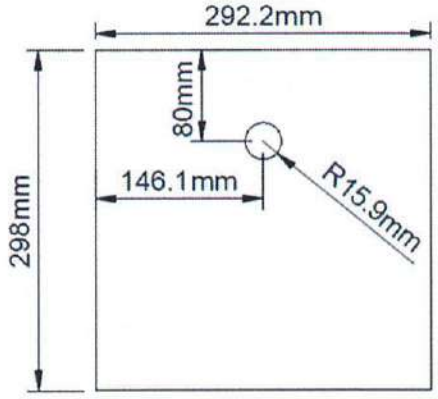


Figure 1 Side View schematic of the input hole that will allow

II.3 Pilot

Water Supply Input

To allow for the input of tap water into the watering system, on a short edge of the container 14.05 cm from the left and 8 cm from the top of the container, a 1 1/4" hole was drilled. 60 cm of the 1/2" vinyl tubing was attached to the opening of a 1/2" tee. The other end of the tubing was attached to an 80 gallon per minute pump which was placed in a container of water; allowing water to be pumped into the system.

Creating the Drainage for the Enclosed System

Three 1/2" holes were drilled into the bottom of the container for drainage. One hole was drilled 14.5 cm from a short edge and 13.5 cm from the long edge. The other two holes were drilled 7.25 cm to the left and right of the first hole. A plastic container 12.5" x 11.5" x 11.75"

was placed and taped under the tote. It collected the water that drains out of the system and was emptied at the end of each trial.

Building the Watering System (The Family Plot)

Left Side Short Edge

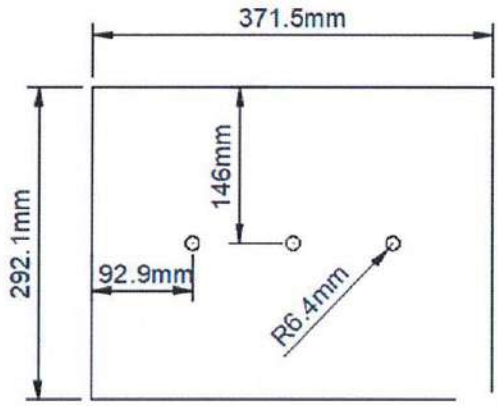


Figure 2 Bottom view schematic o
tote. Three holes with radius 6.4mn

4.45 cm of the 1/2" PVC pipe was cut using a PVC cutter, and attached to an elbow so that the open end of the elbow faces to the right. At the end of the PVC pipe, a tee was attached so that the shorter end of the tee was facing to the right. 11.5 cm of PVC pipe was attached to the end of the tee that is opposite of the end already attached to a PVC pipe. At the end of the 11.5 cm PVC pipe, another tee was attached so that the shorter end of the tee is once again facing to the right. At the opposite end of this new tee, 4.45 cm of PVC pipe was attached, and at the end of the PVC pipe, another elbow with an open end facing to the right was attached.

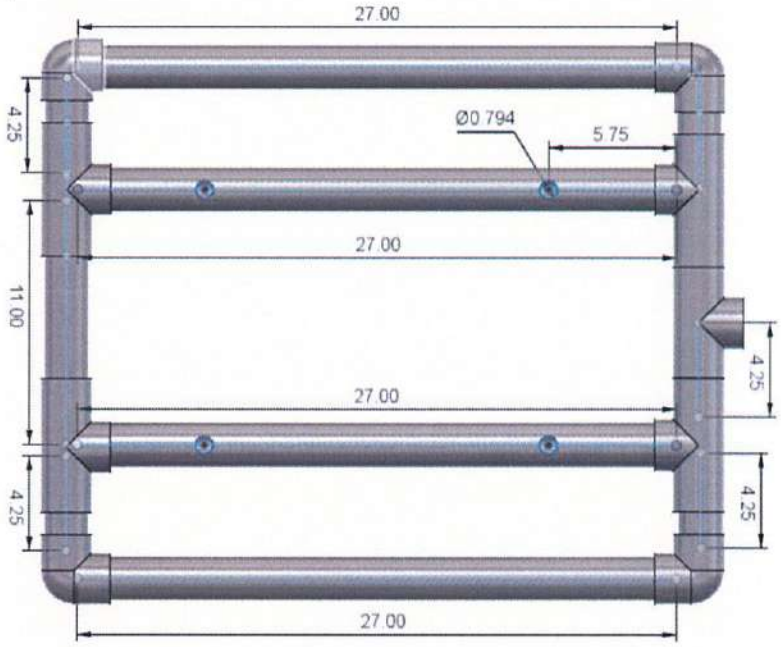


Figure 3 Bottom view schematic of self watering system in

Right Side Short Edge

4.45 cm of the 1/2" PVC pipe was cut with the PVC cutter and attached to an elbow so that the open end of the elbow faces to the left. At the end of the PVC pipe, a tee was attached so that the shorter end of the tee is facing to the left. 4.45 cm of PVC pipe was attached to the end of the tee that is opposite of the end already attached to a PVC pipe. At the end of the 6.5 cm PVC pipe, a tee was attached so that the shorter end of the tee is facing to the right. Then, at the opposite end of the attached tee, another 4.45 cm of PVC pipe was attached, and at the end of the PVC pipe, an elbow with the open end facing to the left was attached.

Both Long Edges

To connect the two short sides of the pipe system, two 27-cm PVC pipes were cut out and attached to the elbows. The watering system was then placed into the container to check if the sizing and positioning is correct, and adjustments were made as needed.

Sprinklers

Two 27 cm PVC pipes were cut and attached to the remaining shorter ends of the tees. The watering system was checked once again for fitting and positioning in the container, and adjustments were made as needed. The holes for the sprinklers were then drilled with a 3/16" thread attachment. The sprinklers were attached to the inner two PVC pipes of the system. One hole was drilled 5.75 cm from the protruding end of the tee, and the second hole was drilled 5.75 cm from the protruding end of the opposite tee. When repeated on the second inner PVC pipe, there were a total of 4 holes. A 360° sprayer was screwed into each hole.

II.4 Phase 1

Creating the PMFC container (Tapia et al., 2017)

One hole 2 cm in diameter was cut on the bottom of the 32 oz plastic containers (4.45 cm from the edge); the hole served as drainage and provide oxygen needed for the cathode reaction.

Setting Up Lights (Carolina Biological, 2001)

A 40 watt fluorescent light was placed on top of the lid of the enclosed container (15 cm away from the surface of the PMFCs), and was switched on for 14 hours and off for 10 through the use of a Intermatic Timer. The light was placed as close to the enclosed system as possible for the most efficient use of light.

Using the Vernier Energy Sensor

A LabQuest Pro was turned on and each of the wires of the energy sensor was plugged into separate LabQuest Pro channels. Black alligator clips was attached to the black input of the energy sensor and the other end of the clip was connected to the anode wire of the PMFC. The red alligator clip was connected to the red input of the energy sensor and the other end was connected to the cathode wire of the PMFC. Data collection on the LabQuest was set to take readings every 15 minutes for 360 hours (15 days).

II.5 Phase 2

Creating Electrodes (Tapia et al. 2017)

Circular

The 40x40cm graphite felts were cut into electrodes 7.5 cm in diameter. On a piece of paper, a circle with a radius of 3.75 cm was drawn using a compass and ruler. The paper circle was placed on top of the graphite felt to be traced and cut out with scissors. Total surface area of the electrode was 89 cm^3. 7cm of an 18 cm piece of titanium wire was inserted from the edge of an electrode disk, this makes one anode. For the cathode cut 7cm of a 27 cm piece of titanium wire was inserted from the edge of an electrode disk.

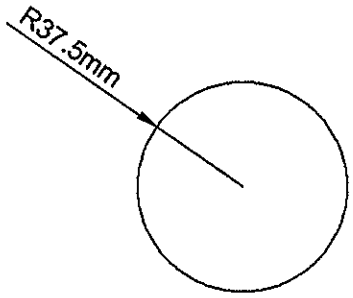


Figure 4 Top view of circle electrode in millimeters; radius of 37.5

Square

From the graphite felt, electrodes 7.5 cm in length and width were cut out. Using a ruler and marker, a 7.5 by 7.5 cm square was drawn and cut out. The paper square was placed on top of the graphite felt to be traced and cut out. The total surface area of the electrode was 132 cm^2. 7 cm of an 18 cm piece of titanium wire was inserted into the electrode from the edge of the electrode, this served as an anode. For the cathode, 7 cm of a 27 cm piece of titanium wire was bent and inserted from the edge of another electrode.

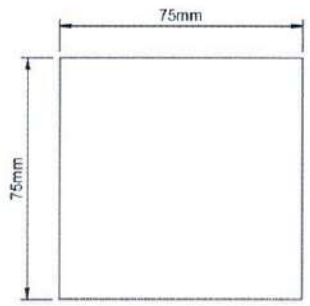


Figure 5 Top view of square electrode in mm.

Octopus

From the 40x40cm graphite felt, electrodes 7.5 cm in length were cut out. Using a ruler, marker, and compass a circle with a radius of 1.25 cm was drawn and eight petals 2.5 cm in length and 1 cm in thickness was drawn and cut out. The octopus shape was placed on top of the graphite felt to be traced and cut out. The total surface area of the electrodes was 91 cm^3. 7 cm of a18 cm piece of titanium wire was inserted 4.5 cm from one of the petals through the circular center, this served as an anode. For the cathode, 7 cm of a 27 cm piece of titanium wire was bent and inserted from the edge of the electrode.

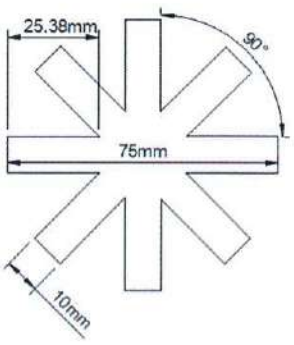


Figure 6 Top view of octopus electrode in

Germination of *Brassica rapa* (Vicrotio, 2016)

4 grams of *Brassica rapa* seeds were soaked in 200 mL tap water for 2-3 hours and then scooped into each of the four seed trays (1g per tray). The trays were stacked on top of one another with the water basin at the bottom. 473 mL of water was poured into the top seed trays, which then siphoned to the bottom tray. The trays were watered 3 times a day with 473mL of water (at 7:30am, 10:30am, and 1:30pm), rotating the bottom tray to the top before every water. The seeds grew until the stem of the plants were about 5 cm (about 3-5 days).

Transplanting *Brassica rapa*

Six holes equidistant from one another and about 1/4" deep were made in the soil. Six of the pre-germinated *Brassica rapa* plants, each with a stem length of 5 cm, were each planted into a hole.

Distance Between Electrodes (Tapia et al. 2017)

When creating the PMFC, the distance between electrodes was altered by changing the amount of soil between the electrodes. The soil used was not inoculated with *Escherichia coli* k-12. The experimental distances between the electrodes were 3 cm, 6 cm, and 9 cm, with the soil measured to create these distances. To increase the accuracy of the amount of soil between electrodes in each trial, the masses of soil were measured with a scale and kept constant for each

distance.

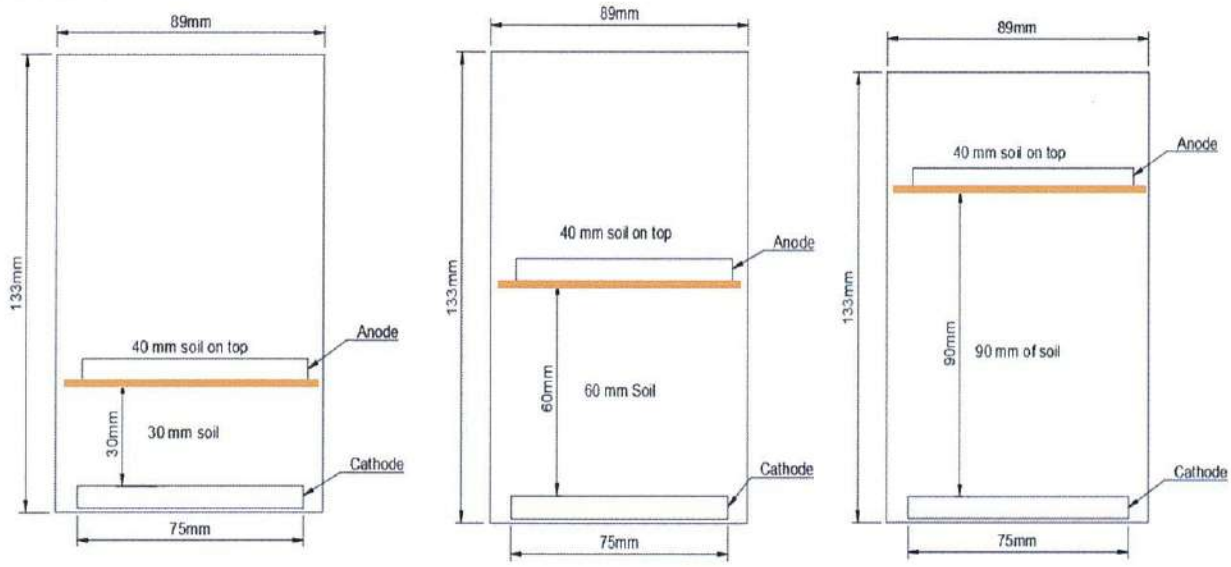


Figure 7 Side view of PMFCs. Distance between electrodes (left to right): 3 cm, 6 cm, 9cm. Orange line indicates addition of Citrus sinensis when applicable.

Culturing *Escherichia coli* k-12 (Carolina Biological Bacteria Care Guide)

Work surfaces were first sterilized with a 10% bleach solution and wiped dry. An inoculating loop was sterilized by rinsing the loop with 70% isopropyl alcohol solution and holding it over a Bunsen Burner flame. The sterilized loop was then used to pick up bacterial colonies from plate cultures of *Escherichia coli* k-12, and the bacteria was inoculated in test tubes containing Luria Broth. The bacteria was also subcultured on LB agar plates using a sterilized wire loop and the streak plate method. The plates were kept at room temperature.

Incubating and Growth of *Escherichia coli* k-12 (Sigma Aldrich Microbial Growth Protocols)

Test tube cultures of *Escherichia coli* k-12 were left overnight at 22°C to allow for bacterial growth. Using sterile technique, absorbance values for the sample were measured using a UV-VIS spectrophotometer. If absorbance at 600 nm was between 0.7 and 0.8, the bacterial solution was prepared for inoculation onto anode of the PMFC. If absorbance had not reached 0.7 after the first day, the test tube culture was incubated for another night and reassessed for absorbance the next morning.

Inoculating the Anode with *Escherichia coli* k-12

Using sterile technique, *Escherichia coli* k-12 cultures from the test tube were pipetted onto the anode of the PMFC. 1 mL of bacterial solution was pipetted onto each side of the anode, fully covering both surfaces of the electrode.

Assembling PMFC

The cathode was placed into the bottom of the container. 6 cm of sieved potting soil was added on top of the cathode while ensuring that the wire attached to the cathode did not get buried by the soil. The anode was then added on top of the soil, followed by 4 cm of soil on top

of the anode, also ensuring that the wire connected to the anode did not get buried. Six *Brassica rapa* seeds were placed equidistant from each other on the surface of the soil in a circle around the center of each PMFC. The cathode wire was connected to the red alligator clip, and the anode wire to the black alligator clip.

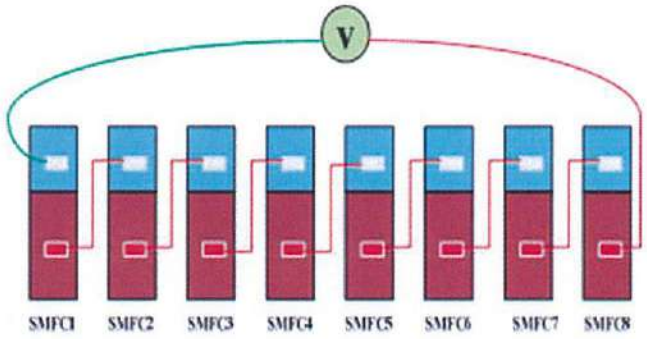
II.6 Phase 3

Addition of Waste Material

The rinds from one *Citrus sinensis* (orange) were obtained by peeling a *Citrus sinensis* obtained from a local supermarket. The rinds were washed with distilled water and wiped dry with paper towels. Using an onion chopper obtained from Amazon, the peels were cubed into 0.68 cm pieces. Peels were then placed in the Excalibur dehydrator (Model 029743350029) at 110^{O}C for 24 hours. Peels were massed out on a scale and placed on the surface of the soil between the anode and cathode so that they just covered the surface of the soil. The mass of the rinds were held constant for all trials The anode was then placed on top of the rinds.

Connecting PMFCs in Series (Prasad and Tripathi 2018)

Three PMFCs were connected in a series using 16 gauge titanium wire. This was done by connecting the anode of the leftmost PMFC to the cathode of the middle PMFC, and the anode of the middle PMFC to the cathode of the rightmost PMFC. The cathode of the leftmost PMFC was connected to the red alligator clamp and the anode of the rightmost PMFC was connected to the black alligator clamp.



II.7 Data Analysis

Voltage readings taken with the Vernier Energy Sensor were stored in the LabQuest Pro, and data was obtained from the LabQuest Pro. Analysis was run on groups of data based off each variable (ex. data from each electrode shape was compared with each other) using a One-Way ANOVA followed by a Post-Hoc Scheffe with $p<0.05$ on IBM SPSS version 25. All descriptive statistics (mean +/- standard deviation) were graphed with Microsoft Excel, and statistical significance were denoted with asterisks. Significance was measured for the variables shape, distance between electrodes, and inoculation of *E.coli* k-12; based on the variable being analyzed, and respectively, it was measured for each shape compared to the other shapes, each distance compared to the other distances, or inoculation of the bacteria compared to no inoculation. This analysis allowed for the determination of the optimal factors of a PMFC. With the most optimal PMFC design, statistical significance was calculated between waste material groups, with this data used to design the PMFCs that were connected in a series.

III. Results

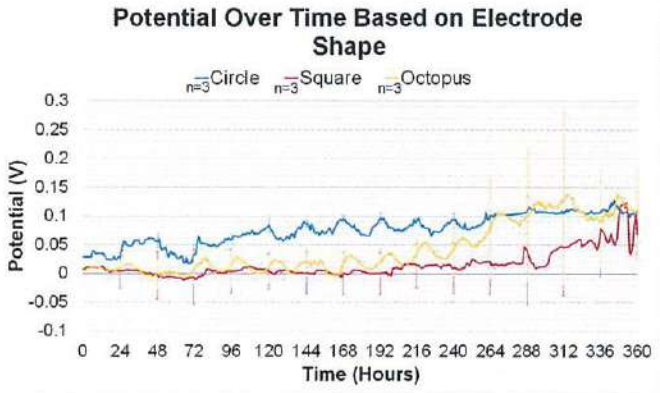


Figure 9 Average potential of electrode shapes over a 360 hour period. All shapes indicate a cyclical pattern with gradual increases over time. The circular electrodes consistently displayed the highest readings until around 265 hours.

Figure 9 shows the results in the change in potential depending on whether electrodes were circular, square, or octopus shaped over the 360 hour period. The anodes were not inoculated with *E.coli* k12 in these trials. All shapes show gradual increases in potential over time, with the circle electrode group consistently showing the highest readings until about 265 hours. Large error bars/large variations in readings can be seen in the square and octopus trials as duration of trials increased. The greatest potential readings for circle, square, and octopus readings are as follows: 0.1287114284V, 0.07766666667V, 0.136293619V.

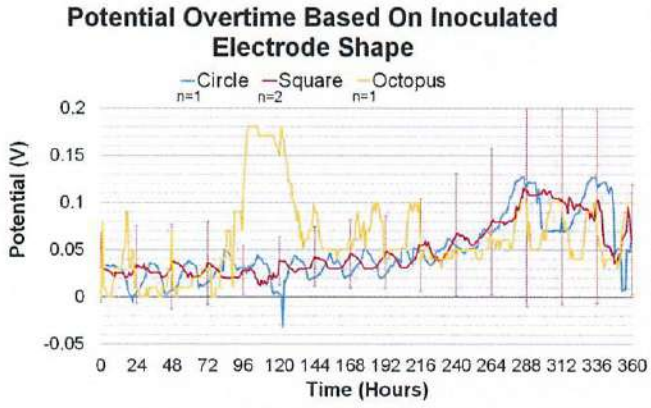
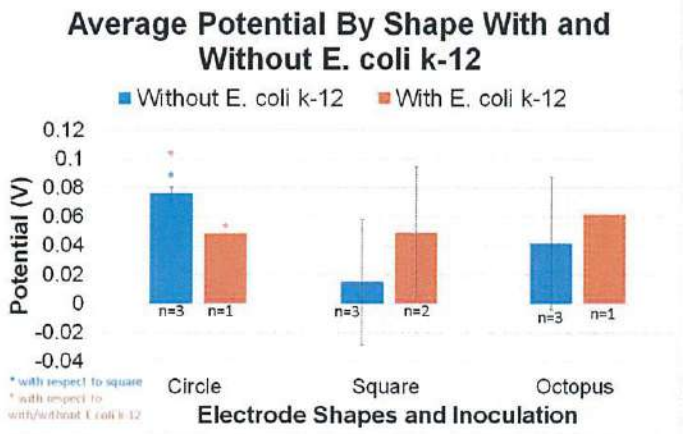


Figure 10 Average potential of each electrode group with the *E.coli* k-12 inoculum on the anode over a 360-hour period. Shapes exhibited a cyclic pattern over time, with circle and square displaying gradual increases in potential, and octopus reaching peak voltage (0.18 V) at hour 99.

Figure 10 shows the change in potential over time of the three differently shaped electrodes inoculated with *E.coli* k-12. Results are similar to those of trials without *E.coli* k12 they display a gradual increases over time. Each electrode group show cyclical patterns as they increase over time, with abrupt spikes at almost regular intervals. An upwards trend in potential is also not evident until the 240 hour mark for all electrode groups.The circle and square electrodes peak around hour 290 with readings of 0.121651448V and 0.1204734485V. The octopus electrodes reach a peak at hour 99 with a reading of 0.18V.

Figure 11 Comparison of the average potentials of the shapes with and without *E.coli* k-12 inoculum.



Circle without *E.coli* k-12 is significantly greater than the square without the inoculum, as well as circle with *E.coli* k-12.

Figure 11 compares the average potentials of each electrode shape with and without *E.coli* k-12. The circle electrode without *E.coli* k-12 shows significantly higher potentials than the circle with *E.coli* k-12 and the square without *E.coli* k-12. There is no significance for the other shapes with and without *E.coli* k-12, and asterisks denote statistical significance between groups.

IV. Discussion

As seen, all electrode groups, regardless of shape or the presence of *E.coli* k-12, displayed a cyclical pattern over time that corresponds directly to the cycle of daylight and irrigation frequency. As the plants receive exposure to sunlight, they photosynthesize and produce more excess nutrients available for decomposition, therefore increasing the amount of electrons available for transfer. Spikes also followed irrigation in PMFCs without any *E.coli* k-12, as the increased water carried a greater number of protons from the anode to the cathode as it trickled down due to gravity. These increased number of protons at the cathode would expedite the oxidation process which inturn would increase the rate that electrons are being transferred to the cathode. The light cycle was reset every 24 hours and the plants were irrigated every 48 hours, which correspond to the abrupt spikes in the potential that appeared about every 24 hours. The more sudden upwards trend of potential after 240 hours can be accounted for by the fact that plants were still growing up until that period, and had not reached the point where they could consistently release large amounts of excess nutrients. In order to explicate these 240 hours and optimize time use we decided to shift to pre-germinating plants, implementing them into the system when their stems grew about 10 cm long.

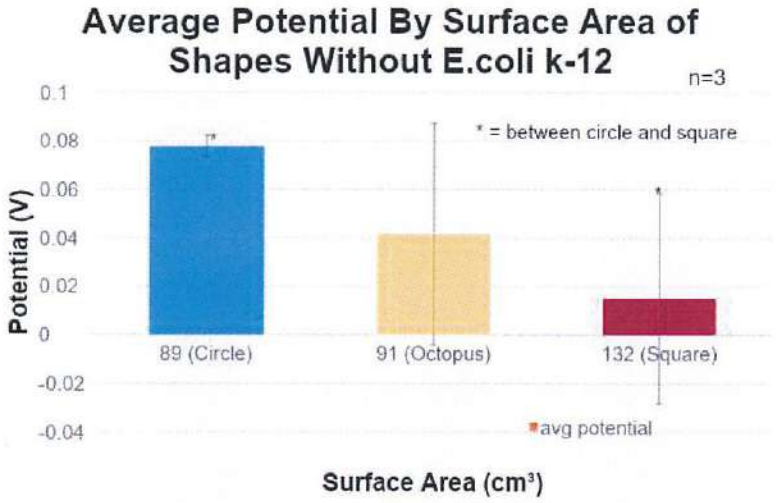


Figure 12 The greater the surface area of the electrodes the lower the average potential. Asterisks denote significance.

Phase 1 of the study found the circle electrode to be the most efficient shape, due to its greater consistency in potential outputs even though it had the smallest surface area. This can be explained by the fact that, although the square had the largest surface area, it did not fit perfectly into the PMFC, with its corners up along the edges of the PMFC- this prevented the fuel cell from using its electrode surface area efficiently. The square electrode's large surface area could have also trapped some of the water from irrigation in the anaerobic zone of the fuel cell, preventing water from properly draining through the electrode to the bottom. In comparison, the circular electrode fit into the bottom of the PMFC and still had extra soil around it to allow for more efficient drainage of water and transfer of protons to the cathode. The octopus electrode had 2cm greater surface area then the circle, but did not perform as well likely due to the large amount of space between each arm and greater amount of surrounding soil, which would cause more excess nutrients to easily pass by the electrode- this would reduce the amount of electrons available for transfer and energy production.

The inoculation of the anodes with *E.coli* k-12 showed the PMFCs to receive the inoculation to exhibit higher initial spikes in potential. This is pertained to there initially being a greater number of electrodes available for transfer from the soil to the anode, as the bacteria was freshly exposed to the soil. However, once the PMFCs were irrigated these initial higher spikes grew smaller, and this was most likely because the *E.coli* k-12 did not have sufficient time to adapt to the environment and form a biofilm layer around the anode before it was washed out.

Limitations

Throughout the study, several limitations were faced. Temperature within the enclosed system could not be controlled, causing plants to be subjected to uncontrolled fluctuations in external temperatures. Furthermore, more efficient or advanced electrogenic bacteria than *E.coli* k-12 could not be included in the study for safety and health purposes, and it is likely that there were many unknown agents in the soil of the enclosed PMFcs that could not be detected or identified.

V. Conclusion

In summary, all electrode shapes in a PMFC, with and without the inoculation of *E.coli* k-12, produced gradual cyclical increases in voltage over a 360-hour period, with the pattern becoming apparent at the 240th hour. The null hypothesis stating that electrode shape and *E.coli* k-12 inoculum would have no significant impact on PMFC function was supported, though the PMFCs with circle electrodes and lack of *E.coli* k-12 produced significantly higher voltages than the circle electrodes with *E.coli* k-12 and the square electrodes without *E.coli* k-12.

VI. Future Studies

In the future, additional variables can be added for testing to optimize the PMFC. Varying irrigation frequencies and daylight lengths can be tested with the system, as well as different plants, as they may have more complex root systems that could potentially facilitate energy production. Moreover, the duration of the experiment can be lengthened to determine long-term results of this study and potential further increases in power output, or the experiment can be run in a non-enclosed environment. Lastly, stronger electrogenic bacteria, such as *Bacillus subtilis*, can be implemented into the system instead of *E.coli* k-12, as it may allow for more effective electron transfer.

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