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

Research Plan

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Energy: Sustainable Materials & Design

Section A- Rationale

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 MFCs. They harvest energy in the same way that a MFC does but instead the anode is placed in anaerobic sediment rather than water, and the cathode is 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).

Section B

Research Questions

How can we engineer and optimize the production of energy of a plant microbial fuel cell (PMFC) used with *Brassica rapa*?

Hypotheses

Alternative:

If the alternative hypothesis is supported, the PMFC will yield the largest amount of energy with square shaped electrodes (largest surface area), placed 3 cm apart (least internal resistance), and with *Escherichia coli* k-12 inoculum. The addition of *Citrus sinensis* peels and connection of PMFCs in a series configuration will increase the overall total energy output.

Null:

If the null hypothesis is supported, electrode shape, distance between electrodes, and inoculation of anode with *E.coli* k-12 will have no effect on PMFC energy generation. The addition of waste material, and connection of PMFCs in a series configuration will have no effect on total energy output.

Expected Outcomes(Prasad and Tripathi, 2018)

The PMFC will be able to generate a measure of approximately 1 volt individually after optimization and around 3 volts when optimized and connected in a series configuration. Three volts would be sufficient enough to power a small LED. It is expected that the optimal conditions for PMFC function will be with the smallest distance between the electrodes, as there will be the least amount of internal resistance, the electrode shape that provides the most surface area to

form a biofilm (square), the inoculation of anode with *Escherichia coli* k-12 (increasing the amount of electrons available at the anode), the presence of waste material (increases the amount of free electrons), and when PMFCs are connected in a series configuration.

Section C-

- **Procedures**

Experiment Overview

All trials will last for the duration of 20 days and multimeter readings will be taken every 24 hours.

Pilot Work

The pilot work will focus on setting up the system that will hold the PMFCs for the duration of the study. The purpose of the system is to prevent exposure to any unknown organisms that will be cultivated in the PMFCs. 12.5" x 11.5" x 11.75" (LxWxH) storage tote containers that can hold up to 4 PMFCs at a time will be utilized. A sprinkler system will be created with 1/2" PVC pipes and placed 9 cm above the fuel cells. The PVC pipe will be attached to a water pump located outside of the container which will allow the PMFCs to be watered. 1/2" holes will be drilled - plastic trays with 3/8" holes will be placed in each corner of the tote and PMFCs will subsequently be placed on top of the trays to allow for effective drainage. 5/64" holes will be drilled to connect each PMFC with alligator clips to a Vernier Energy Sensor. The sensor will be connected to a LabQuest Pro for data collection, and a 40 watt fluorescent light lamp controlled by an intermatic timer will be placed on the top cover of the system. There will be no openings in the system throughout the duration of each trial.

Phase 1

Phase 1 of the study will focus on creating PMFCs. 32 ounce containers will act as individual PMFCs. A 2" hole will be cut into the bottom of each container to allow for drainage and cathode oxidation. A cathode will be placed on top of the hole; sifted soil will be placed on top of the cathode, and the anode will be placed on top of the soil. Additional soil will be sifted and placed on the anode. The amount of soil in the system will be varied depending on the distance between electrodes be tested.

Phase 2

Phase 2 will focus on optimizing factors that affect the efficiency of the PMFC function from Phase 1. Variables like the distance between electrodes, electrode shape, and the inoculation of the anode with *Escherichia coli* k-12 will be looked at. By altering the distance between electrodes the internal resistance is being altered; distances of 3, 6, and 9 cm will be tested by altering the amount of soil between the electrodes. By testing different electrode shape the effects of electrode surface areas on PMFC function will be observed. The different electrode shapes will be circular, square, or octopus shaped. The anode be inoculated with *Escherichia coli* k-12 to observe the effects of the bacteria on voltage output.

Phase 3

Using the design form Phase 1 and optimal factors from Phase 2, Phase 3 will look at ways to increase PMFC energy output. The addition of waste material and connection of PMFCs in series connection will be looked at. The effect of waste

material will be tested through the addition of *Citrus sinensis* peels. The addition of waste material will provide additional material for the bacteria to decompose which will increase the amount of electrons being transferred to the anode. Three PMFCs that will be connected in a series configuration to increase the energy output.

Obtaining Materials

For the enclosed system, a plastic container 12.5" x 11.5" x 11.75" and a 80 gallons per hour water pump will be 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 will be obtained from Home Depot.

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

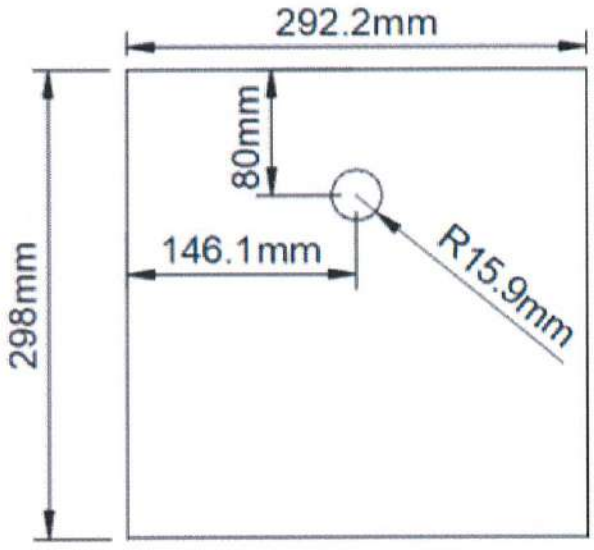

Figure 1 Side View schematic of the input hole that will allow water to be pumped into the enclosed system. (Image by author)

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 will be drilled. 60 cm of the 1/2" vinyl tubing will be attached to the opening of a 1/2" tee. The other end of the tubing will be attached to a 80 gallons per minute pump which will be 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 will be drilled into the bottom of the container for drainage. One hole will be drilled 14.5 cm from a short edge and 13.5 cm from the long edge. The other two holes will be drilled 7.25 cm to the left and right of the first hole. A plastic container 12.5" x 11.5" x 11.75" will be placed and taped under the tote. It will collect the water that drains out of the system and will be emptied at the end of each trial.

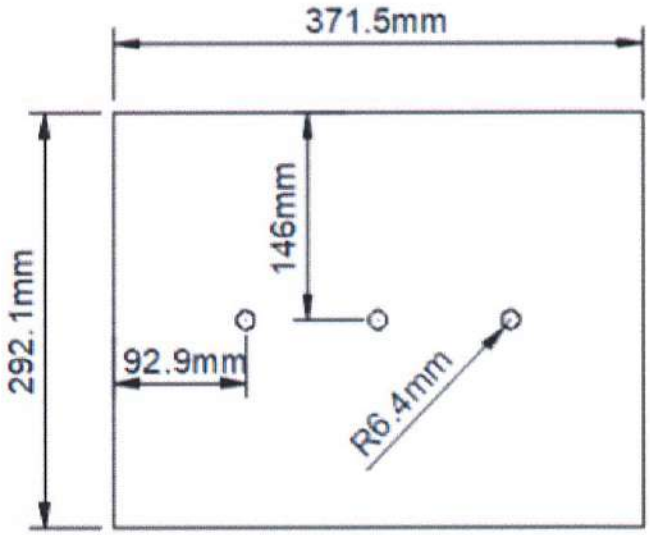

Figure 2 Bottom view schematic of tote. Three holes with radius 6.4mm will be drained to allow for drainage. (Image by authors)

Building the Watering System (The Family Plot)

Left Side Short Edge

4.45 cm of the 1/2" PVC pipe will be cut, with the 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 will be attached so that the shorter end of the tee is facing to the right. 11.5 cm of PVC pipe will be

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 will be 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 will be attached, and at the end of the PVC pipe another elbow with an open end facing to the right will be attached.

Right Side Short Edge

4.45 cm of the 1/2" PVC pipe will be 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 will be attached so that the shorter end of the tee is facing to the left. 4.45 cm of PVC pipe will be 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 another tee will be attached so that the shorter end of the tee is facing to the right. At the opposite end of the attached tee another 4.45 cm of PVC pipe will be attached, and at the end of the PVC pipe another elbow with the open end facing to the left will be attached.

Both Long Edges

To connect the two short sides two 27 cm of PVC pipe will be cut out

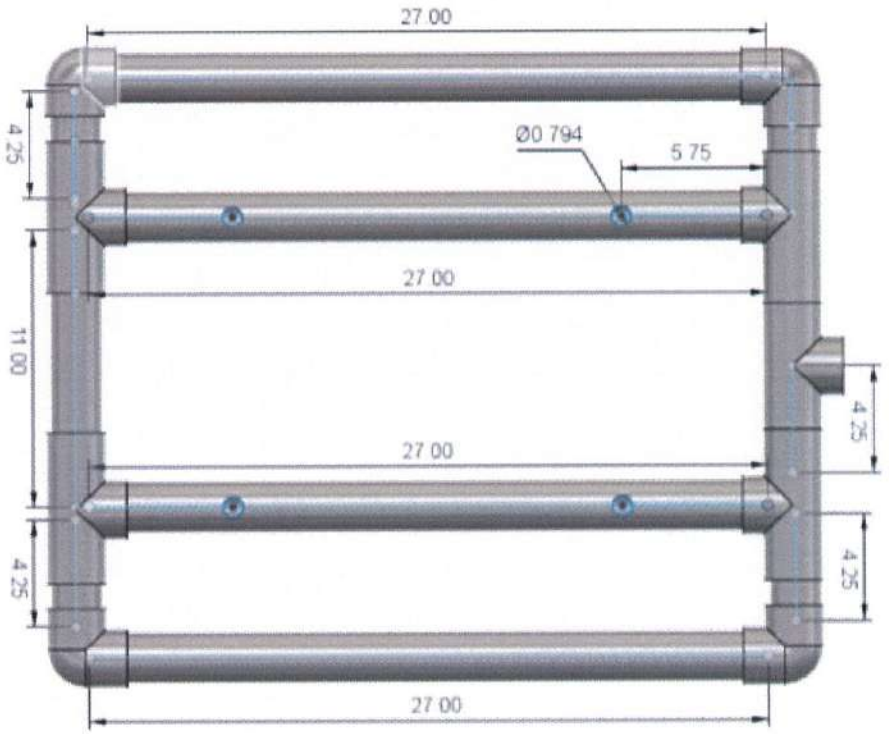

Figure 3 Bottom view schematic of self watering system in centimeters. (Image by author)

and attached to the elbows. The watering system will then be placed into the container to check if the sizing and positioning is correct, and adjustments will be made as needed.

Sprinklers

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

Phase 1

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

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

Setting Up Lights (Carolina Biological, 2001)

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

Using the Vernier Energy Sensor

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

Phase 2

Creating Electrodes (Tapia et al. 2017)

Circular

The 40x40cm graphite felts will be cut into electrodes that are 7.5 cm in diameter which is approximately 5 electrodes per sheet. On a piece of paper using a ruler, a line 3.75 cm in length will be drawn and using a compass, with a marker, a circle with a radius of 3.75 cm will be drawn. The paper circle will be placed on top of the graphite felt and will be traced and cut out or directly cut out with scissors. Total surface area of the electrode will be 89 cm^3. 7cm of an 18 cm piece of titanium wire will be 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 will be inserted from the edge of an electrode disk.

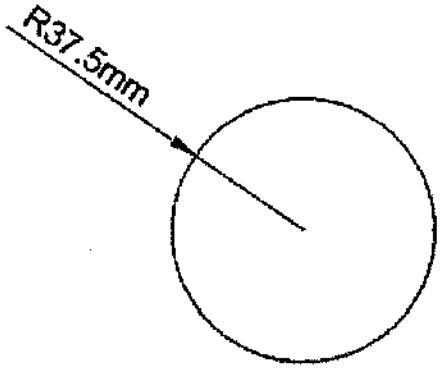

Figure 4 Top view of circle electrode in millimeters; radius of 37.5 mm and thickness 0.56mm. (Image by author)

Square

From the 40x40cm graphite felt electrodes 7.5 cm in length and with, will be cut out. Using a ruler and marker a square that is 7.5 by 7.5 cm will be drawn and with scissors cut out. The paper square will be placed on top of the graphite felt and will be traced and cut out or directly cut out with scissors. Total surface area of the electrode will be 132 cm^2. 7cm of an 18cm piece of titanium wire will be inserted from an edge of an electrode disk, this will serve as an anode. For the cathode cut 7cm of a 27 cm piece of titanium wire will be inserted from the edge of an electrode disk.

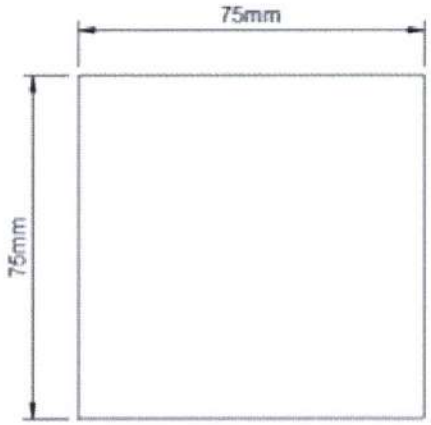

Figure 5 Top view of square electrode in mm; 75mm x 75mm and a thickness of 0.65mm.
(Image by author)

Octopus

From the 40x40cm graphite felt electrodes 7.5 cm in length will be cut out. Using a ruler, marker, and compass a circle with a radius of 1.25 cm will be drawn and eight petals 2.5 cm in length and 1 cm in thickness will be drawn and cut out. This octopus shape will be placed on top of the graphite felt and will be traced and cut out or directly cut out with scissors. The total surface area of the electrodes will be 91 cm^3. 7cm of a18cm piece of titanium wire will be inserted 4.5 cm from one of the

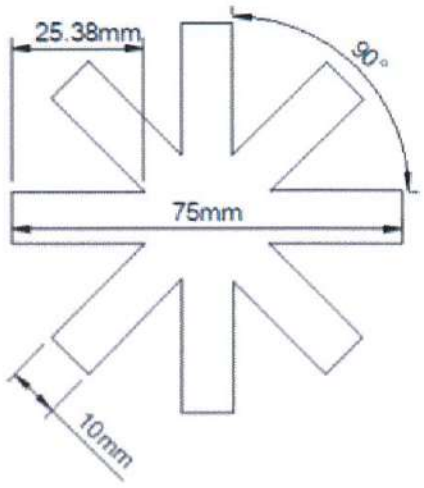

Figure 6 Top view of octopus electrode in millimeters; center has a radius of 125mm.
(Image by author)

petals through the circular center, this will serve as an anode. For the cathode cut 7cm of a 27 cm piece of titanium wire will be inserted from the edge of an electrode disk.

Germination of *Brassica rapa* (Vicrotio, 2016)

4 grams of *Brassica rapa* seeds will be soaked in 200 mL tap water for 2-3 hours and then scooped equally into each of the four seed trays (1g per tray). The trays will then be stacked on top of one another with the water basin at the bottom. 473 mL of water will be poured into the top seed trays and allowed to siphon to the bottom. The trays will be watered 3 times a day with 473mL of water (at 7:30, 10:30, and 1:30). The seeds will grow until the stem of the plants are about 5 cm (about 5 days).

Transplanting *Brassica rapa*

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

Distance Between Electrodes (Tapia et al. 2017)

When creating the PMFC the distance between electrodes will be altered by changing the amount of soil between the electrodes. The soil being used will not be inoculated with *Escherichia coli* k-12. The amount of soil between electrodes that will be tested consist of 3 cm, 6 cm, and 9 cm. To increase the accuracy of the amount of soil between electrodes with each trial, the mass of the initial amount of soil used for each of the distances will be obtained by

using a scale.

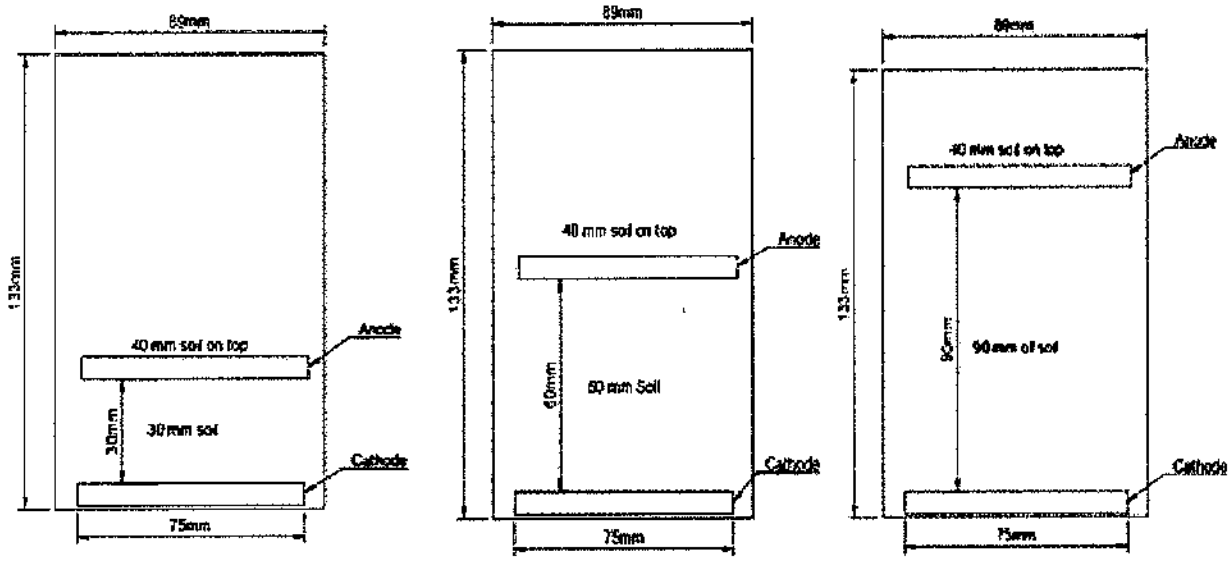

Figure 7 Side view of PMFCs. Distance between electrodes (left to right): 3 cm, 6 cm, 9cm. (Images by Author)

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

Work surfaces will first be sterilized by spraying down the surface with a 10% bleach solution and wiping it dry. An inoculating loop will sterilized by rinsing the loop with 70% isopropyl alcohol solution and placing over a Bunsen Burner flame. The sterilized loop will be used to pick up bacterial colonies from the plates of *Escherichia coli* k-12, and the bacteria will then be inoculated in test tubes containing Luria Broth. The bacteria will also be subcultured on LB agar plates using a sterilized wire loop and the streak plate method. The plates will be 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 will be left overnight at 22°C to allow for bacterial growth. Using sterile technique, absorbance values for the sample will be measured

using a UV-VIS spectrophotometer. If absorbance at 600 nm is between 0.7 and 0.8, the bacterial solution can be prepared for inoculation onto anode of the PMFC. If absorbance has not reached 0.7 after the first day, the test tube culture will be 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 will be pipetted onto the anode of the PMFC. 1 mL of bacterial solution will be pipetted onto each side of the anode.

Assembling PMFC

The cathode,will be placed into the bottom of the container. 6 cm of sieved potting soil will be added on top of the cathode while ensuring that the wire attached to the cathode does not get buried. The anode will then be added on top of the soil and 4 cm of soil on top of it while ensuring that the wire connected to the anode is not buried. Six *Brassica rapa* seeds will be placed equidistant from each other on the surface of the soil around the center of each PMFC. The cathode wire will be connected to the red alligator clip, and the anode wire to the black alligator clip.

Phase 3

Addition of Waste Material

The rinds from one *Citrus sinensis* (orange) will be obtained by peeling a *Citrus sinensis* obtained from a local supermarket. The rinds will be washed with distilled water and wiped dry with paper towels. Using an onion chopper obtained from Amazon the peels will be cubed into

0.68 cm pieces. Peels will then be placed in the Excalibur dehydrator, Model 029743350029 at 110°C for 24 hours. Peels will be massed out on a scale and placed on the surface of the soil between the anode and cathode so that they just cover the surface of the soil. The mass of rinds added will be taken and this mass will be used for creating the other trials. The anode will then be placed on top of the rinds.

Connecting PMFCs in Series (Prasad and Tripathi 2018)

Three PMFCs will be connected in a series using 16 gauge titanium wire. This will be 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 is connected to the red alligator clamp and the anode of the rightmost PMFC is connected to the black alligator clamp.

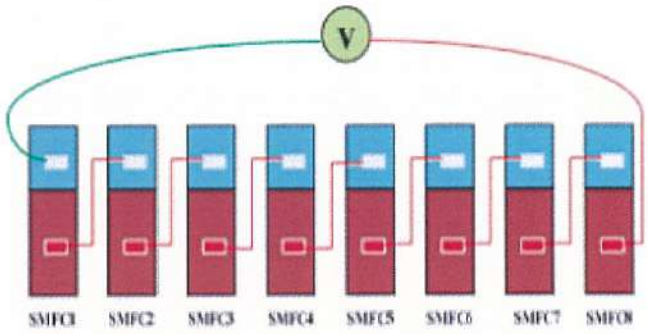

Figure 8 A series configuration of sediment microbial fuel cells (SMFC). (Prasad and Tripathi, 2018)

- **Risk and Safety**

1. ***Human participants research**: N/A*
2. ***Vertebrate animal research**: N/A*
3. **Potentially Hazardous Biological Agents (PHBA):**

   As per ISEF rules and guidelines, this study involves BSL-1 organisms that are exempt from prior SRC review and require no additional forms:

   (ISEF Rulebook Page 16, section 1e and 2f)

   As such, Escherichia coli k-12, outlined below, will be listed on ISEF Form 3.

Organism Name: ***Escherichia coli* k-12 Strain**

a. Source of Organism: Carolina Biological

BSL assessment determination: BSL- 1

b. Detail safety precautions you plan to take

- The student researchers will be trained by the designated supervisor in all safety protocols associated with working with microbes, sterile technique and proper handling and disposal of the bacteria. The designated supervisor will directly supervise the student researcher when working with the bacteria. Goggles, lab apron and nitrile gloves will be worn during experimentation. Prior to use, all surfaces will be wiped with 10% bleach solution. All surfaces will also be wiped down with 10% bleach after experimentation.
- To avoid contact with skin and eyes a lab apron, gloves, and goggles will be worn. In the case of an emergency eye wash stations and safety showers will be available for use.
- Hands and other exposed areas will be washed before and after handling of the chemical.
- 10% bleach will be used to to kill the bacteria. 10% bleach will be incorporated onto the NGM agar plate/luria broth/contaminated soil and the container will be sealed with parafilm and disposed of.

https://www.neb.com/-/media/7317f9f136b344399610f784444df887.pdf

4. Hazardous Chemicals and Devices

Name of chemical/activity/device: **Sodium Hypochlorite Solution (NaClO) (1L, 10%)**

- Sodium Hypochlorite is classified as a Category 1B skin corrosion, Category 1 serious eye damage, and Category 1 acute aquatic toxicity.

- All handling of sodium hypochlorite, at all concentrations, will be supervised under a certified research mentor or teacher.
- To avoid contact with skin and eyes a lab apron, nitrile gloves, and goggles will be worn. In the case of an emergency eye wash stations and safety showers will be available for use. Hands and other exposed areas will be washed before and after handling of the chemical.
- Excess sodium hypochlorite solution will be disposed of by an approved disposal plant.

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=425044&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2F425044%3Flang%3Den

Name of chemical/activity/device: **Isopropanol (16 fl oz. 70%)**

- This chemical is classified as a Category 2 flammable liquid, Category 2 eye irritation and category 3 specific target organ toxicity.
- Isopropanol will be handled with caution, and under the supervision of a certified teacher or mentor.
- For protection from exposure to the chemical, goggles, nitrile gloves and a lab apron will be worn during usage. Hands will be washed before and after usage, and eyewash stations will be readily available for use if needed. When not in use, the 70% isopropanol will be stored in a locked flammables cabinet.
- This chemical will be sent to a licensed waste disposal company for disposal after use.

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=563935&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Drubbing%2Balcohol%26interface%3DAll%26

N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

Name of chemical/activity/device: **Speedway 3/8" Variable Speed Reversible Drill Model# 45137**

- The power drill will be handled with caution when in use. If not used with caution damage can be caused to human tissue or surrounding material.
- All handling of the power drill will be done under supervision by a mentor or teacher.
- To avoid debris, goggles and aprons will be worn when handling the power drill.
- The device will be used in a well lit, clutter free area to avoid any potential accidents. The device will be unplugged before any adjustments are made to the device. To prevent unintentional injuries switches will be ensured to be in the off position before any handling or movement of the device.
- Instructions, according to the user manual, will be followed when handling this device.

https://images.homedepot-static.com/catalog/pdfImages/8d/8da03b90-3741-450b-bbc0-224351f21037.pdf

Name of chemical/activity/device: **PVC Cutter Armour Line Model# RP77131**

- The PVC Cutter will be handled with caution. The device has a blade and if not handled with caution serious injury could result.
- All handling of the device will be done under the supervision of a teacher or mentor.
- To avoid debris, goggles, and aprons will be worn.
- When not in use the device will be closed and the safety clamps will hold the handle shut.

https://www.homedepot.com/p/Armour-Line-Up-To-1-1-2-in-Dia-Pipe-and-Tubing-Cutter-Red-RP77131/306655882

Name of chemical/activity/device: **Autoclave**

- The autoclave will be handled with caution, as it reaches very high temperatures and is pressurized.
- All handling of the device will be under the supervision of a qualified teacher or mentor.
- Heat-protective autoclave gloves, aprons and goggles will be worn while placing objects into and out of the autoclave. The device will be tightly shut while in use to prevent any exposure to high temperature materials. Care will be taken to only open the autoclave after it is fully depressurized to avoid steam burns.
- The device will be closed and turned off when not in use.

https://www.fishersci.com/shop/products/heidolph-tuttnauer-benchtop-sterilizers-m-series-9/p-4906613

Name of chemical/activity/device: **Bunsen Burner**

- This device will be utilized with extreme caution, as it involves being in close contact with flames.
- Handling of this device will be supervised by a qualified teacher or mentor.
- Nitrile gloves, goggles and aprons will be worn for protection while working with this device. Long hair will be tied back and dangling articles of clothing will not be worn. The device will be utilized in a clutter-free area to avoid potential accidents.
- The device will be disconnected from the gas pump when not in use and the gas will be turned off when not in use.

https://www.fishersci.com/shop/products/eisco-bunsen-burners-tirrill-3/p-6714054#?keyword=bunsen+burner

- **Data Analysis**

Voltage readings taken with the Vernier Energy Sensor will be stored in the LabQuest Pro, and data will then be obtained from the LabQuest Pro. Analysis will be run on groups of data based off each variable (ex. data from each electrode shape will be 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) will be graphed with Microsoft Excel, and statistical significance will be denoted with asterisks. Significance will be measured for the variables shape, distance between electrodes, and inoculation of *E.coli* k-12; based on the variable being analyzed, and respectively, it will be 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 will then allow for determining the optimal factors of a PMFC. With the most optimal PMFC design, statistical significance will then be calculated between different light exposure, irrigation frequency and waste material groups, with this data used to design the PMFCs that will be connected in a series.

Section D- Bibliography

Carolina Biological. 2001. Wisconsin Fast Plants: Growing Instructions. http://abacus.bates.edu/acad/depts/biobook/FastPGro.pdf

Deng, Huan, Zheng Chen, and Feng Zhao. "Energy from Plants and Microorganisms: Progress in Plant-Microbial Fuel Cells." , vol. 5, no. 6, 2011, pp. 1006–1011., doi:10.1002/cssc.201100257.

Ewing, Timothy, Phuc Thi Ha, Jerome T. Babauta, Nghia Trong Tang, Deukhyoun Heo, Haluk Beyenal. "Scale-up of Sediment Microbial Fuel Cells." , vol. 272, 2014, pp. 311–319., doi:10.1016/j.jpowsour.2014.08.070.

Helder, M., Strik, D.P.B.T.B., Hamelers, H.V.M., Kuhn, A.J., Blok, C., Buisman, C.J.N. – Concurrent bio-electricity and biomass production in three Plant-Microbial Fuel Cells using Spartina anglica, Arundinella anomala and Arundo donax – 2010 – Bioresource Technology 101 (10) , pp. 3541-3547

Huarachi-Olivera Ronald , Alex Dueñas-Gonza, Ursulo Yapo-Pari, Patricia Vega, Margiht Romero-Ugarte, Juan Tapia, Luis Molina, Antonio Lazarte-Rivera, D.G. Pacheco-Salazar, Mario Esparza, "Bioelectrogenesis with microbial fuel cells (MFCs) using the microalga Chlorella vulgaris and bacterial communities", *Electronic Journal of Biotechnology,* Volume 31, 2018, Pages 34-43, ISSN 0717-3458, https://doi.org/10.1016/j.ejbt.2017.10.013.

Jang Jae Kyung, The Hai Pham, In Seop Chang, Kui Hyun Kang, Hyunsoo Moon, Kyung Suk Cho, Byung Hong Kim, "Construction and operation of a novel mediator- and membrane-less microbial fuel cell", *Process Biochemistry*, Volume 39, Issue 8, 2004, Pages 1007-1012, ISSN 1359-5113, https://doi.org/10.1016/S0032-9592(03)00203-6.

Khaled, Firas, Olivier Ondel, Bruno Allard. "Microbial Fuel Cells as Power Supply of a Low-Power Temperature Sensor." *Journal of Power Sources*, vol. 306, 2016, pp. 354–360., doi:10.1016/j.jpowsour.2015.12.040.

Kumar, Vikash & Ansari, Mohd & Ramjan, Md & Angami, Theja & Shankar, Kripa. (2019).Rooftop Vegetable Garden-A new concept of urban agriculture. *Agriculture & Food: e-Newsletter,* 1(4), 109-112.

Lindsey, Rebecca. "Climate Change: Atmospheric Carbon Dioxide." *Climate Change: Atmospheric Carbon Dioxide | NOAA Climate.gov*, 1 Aug. 2018, www.climate.gov/news-features/understanding-climate/climate-change-atmospheric-carbon-dioxie.

Logan, Bruce E. "Scaling up Microbial Fuel Cells and Other Bioelectrochemical Systems." , vol. 85, no. 6, 2009, pp. 1665–1671., doi:10.1007/s00253-009-2378-9.

Nitisoravut, Rachnarin, and Roshan Regmi. "Plant Microbial Fuel Cells: A Promising Biosystems Engineering." , vol. 76, 2017, pp. 81–89., doi:10.1016/j.rser.2017.03.064.

Prasad, Jeetendra, and Ramesh Kumar Tripathi. "Scale up Sediment Microbial Fuel Cell for Powering Led Lighting." , vol. 7, no. 1, 2018, p. 53., doi:10.14710/ijred.7.1.53-58.

Rahimnejad, Mostafa, Arash Adhamia, Soheil Darvaria, Alireza Zirepoura, Sang-Eun Oh. "Microbial Fuel Cell as New Technology for Bioelectricity Generation: A Review." *Alexandria Engineering Journal*, vol. 54, no. 3, 2015, pp. 745–756., doi:10.1016/j.aej.2015.03.031.

Tapia Natalia F. , Claudia Rojas, Carlos A. Bonilla, Ignacio T. Vargas, "Evaluation of Sedum as driver for plant microbial fuel cells in a semi-arid green roof ecosystem", *Ecological Engineering*, Volume 108, Part A, 2017, Pages 203-210, ISSN 0925-8574, https://doi.org/10.1016/j.ecoleng.2017.08.017.

Victorio. (2016). Kitchen Crop. https://victoriostore.com/pub/media/wysiwyg/product_manuals/VKP1014_Sprouter_Manual_July_2018.pdf

Wang, De-Bin, Tian-Shun Song, Ting Guo, Qinglu Zeng, Jingjing Xie. "Electricity Generation from Sediment Microbial Fuel Cells with Algae-Assisted Cathodes." , vol. 39, 24 July 2014, pp. 13224–13230.

Yadava, Asheesh Kumar , Purnanjali Dasha, Ayusman Mohantya, Rouzbeh Abbassi, Barada Kanta Mishra."Performance Assessment of Innovative Constructed Wetland-Microbial Fuel Cell for Electricity Production and Dye Removal." , vol. 47, 2012, pp. 126–131., doi:10.1016/j.ecoleng.2012.06.029.

"Building an Inexpensive Aeroponics System." Family Plot, 18 Aug. 2017, www.familyplotgarden.com/building-an-inexpensive-aeroponics-system.html.

Research Plan: Project Summary

No addendums exist. No changes were made.