Research Plan

Sanjna Kedia and Emily Ma
Advanced Science Research
Ms. Huenger

Remediation of Wastewater Using a Microbial Fuel Cell with Optimized Electricity Generation and an Algae Bioreactor

Energy: Sustainable Materials and Design

A. Rationale

Approximately $25 billion is annually spent on domestic wastewater treatment, and another $202 billion is needed for improving publicly owned treatment works in the United States (United States Environmental Protection Agency (EPA), 2008). Also, 3% of the United States' electrical load is due to wastewater treatment, which is equivalent to the electricity use of 9.6 million households (McCarty et al., 2011). The cost and electrical load of treating wastewater is largely due to the aeration technology as it consumes 45-75% of wastewater treatment plant costs (Huggins et al., 2013). Current wastewater treatments prove to be ineffective in treating nitrogen and phosphorus in the wastewater so when the "clean" water is released, nutrient pollution is highly probable (EPA, 2019). Although aeration technologies prove successful in remediating wastewater, microbial fuel cell technologies may prove as an alternative as they occur spontaneously, and even produce energy instead of consuming it.

Microbial fuel cells (MFC) are devices that use bacteria as the catalysts to oxidize organic and inorganic matter and generate current. Exoelectrogenic bacteria produce electrons from the substrates, usually being wastewater or biomass, which flow from the anode to cathode compartment, linked by a conductive material including a resistor which produces electricity. Generated by the flow of electrons from anode to cathode, reversely, a positive current flows from the cathode to anode, allowing for a system to be established. Since this process occurs spontaneously the device must be capable of oxidizing the substrate and replenishing the anode, continuously or intermittently. (Logan et al., 2018)

Wastewater from sewage treatment plants often contains decaying organic matter and debris which can use up the dissolved oxygen in a lake so fish and other aquatic biota cannot survive (USGS, 2018). Oxygen in water is measured as dissolved oxygen, and when more oxygen is consumed by the microorganisms instead of produced, sensitive animals may move away, weaken, or die (EPA, 2012). Dissolved oxygen is an important contributor to water quality as it directly indicates an aqautic resource's ability to support aquatic life (EPA, 2016). The biological oxygen demand test is used to determine how much oxygen is being depleted from a body of receiving water as a result of bacterial action (Woodard and Curran Inc., 2006) Dissolved oxygen is inversely related to BOD or biologically disolved oxygen, as the higher the

demand for oxygen, the lower the amount of dissolved oxygen in the wastewater. As opposed to BOD, the dissolved oxygen test simply requires a probe that is dependent on temperature so it must be recalibrated before every use. The DO test does not require any chemical substrates, which makes it the easiest and least harmful to use.

Phosphorus and nitrogen are important nutrients but if present in excess, they can serve as pollutants for lakes, streams, and wetlands. Nitrates, a form of nitrogen can occur as a dissolved gas in water, which at elevated levels can affect humans and animals. Phosphorus is also linked to excessive algae growth and degraded lake water quality. Phosphorus builds up sediments in a lake where it is then processed chemically and biologically, allowing it to be released into the water and taken up by algae. The pollution in water is caused by algal blooms due to consumption of the excess nitrate and phosphate (Minnesota Pollution Control Agency, 2008).

Puig et al. (2011) developed a novel air-cathode MFC, which treated domestic wastewater as well as simultaneously producing electricity and removing nitrogen. The strategy used was to gradually increase the organic loading rate when the effluent was below 0.60 kg COD $m^{-3}d^{-1}$, so that the biodegradable organic matter was increased and more available for the exoelectrogenic bacteria. This mechanism proved to be capable of remediating the wastewater and eventually producing energy, as energy production was only observable after 15 days of the cell operating. As electricity generation was gradual in this study, the reduction of time span is necessary for practical MFC usage. Nitrogen levels were observed to be decreased, but a quantifiable method for determining the effects of the simultaneous denitrification and nitrification processes could not be measured. (Puig et al., 2011). To solve this problem,readings of the nitrogen content of the water before and after nitrogen treatment can be taken.

Zhang et al. (2011) integrated forward osmosis into microbial fuel cell technology for treating wastewater. They developed a novel OsMFC, or osmotic microbial fuel cell, where a forward osmosis membrane acted as the separator between the anode and the cathode chambers of the MFC, allowing for water flux to be tested as well. The efficacy of the novel OsMFC was compared to a conventional microbial fuel cell with a cation exchange membrane and it was found that the OsMFC produced more electricity in both the NaCl solution (batch operation) and

the artificial seawater group (continuous operation) which was likely accounted to the enhanced proton transport through the forward osmosis membrane. (Zhang et al., 2011) However, the handling of the forward osmosis membrane including storage was very specific and not practical, so membranes requiring less manual adjustment are looked more favorably upon in wasteatwater treatment as well as what is readily available.. (Zhang et al., 2011)

Huggins et al. in 2013 compared the performance of the microbial fuel cell and aeration treating of wastewater. The aeration and microbial fuel cell tretament, and a control treatment were observed for 90% chemical oxygen demand removal, calculating which treatment took the least amount of time, known as hydraulic retention time. In this study it was determined that the aeration treatment was the most efficient as it took 8 days to reach 90% COD removal whereas the microbial fuel cell took 10 days. However, the microbial fuel cell was more efficient than the control treatment. (Huggins et al., 2013) This study indicates that the optimization of microbial fuel cells is still required as it is not up to the effiency standard of aeration technologies currently in use, and efficiency must be further improved before microbial fuel cells can be put into practical use.

In this study, the species of bacteria responsible for the transfer of electrons will be *E. coli* K-12. Logan (2009), showed how *E. coli* was a gram-positive strain of bacteria capable of producing current. A control treatment, aeration technology, and a novel MFC will be compared to determine which one has the highest rate of efficiency similar to Huggins et al. in 2013. The control treatment will be solely the artificial wastewater, the aeration technology will be constructed using an aquarium diffuser circulating the wastewater, and the novel MFC with a commercially available cation exchange membrane will be constructed of a double chamber MFC separated by a Nafion membrane. Tests in this study will include overall wastewater treatment, hydraulic retention time, DO levels, electricity generation, and nitrogen and phosphorus removal. This study will focus on optimizing energy generation as well as pollutant, or nitrogen and phosphorus removal.

The purpose of this study is to determine whether a novel MFC will be more efficient than current aeration technologies and a control treatment, and whether it can remediate the water as well as produce electricity efficiently, with subsequent treatment of pollutants using an

algae bioreactor. As studied through the literature of Puig et al. in 2011 and Werner et al. in 2013, in order for the alternative hypothesis to be supported, remediation of the wastewater through COD removal, nitrate removal, and electricity generation of the microbial fuel cell must be significant as compared to the control and aeration reactors. In order for the null hypothesis to be supported, wastewater treatment, pollutant removal, and electricity generation through the microbial fuel cell will be insignificant as compared to the other two groups. The engineering goal of this study is to develop a dual chambered microbial fuel cell with a remediation chamber and a denitrification chamber. The microbial fuel cell should display the highest rate of efficiency in remediating the wastewater as compared to the aeration and control treatment, due to the enhancement of the Nafion membrane. Also, pollutant removal specifically of nitrates should be observed in the MFC-algae bioreactor system as the cyanobacteria will remove the nitrates found in the water using the enzyme nitrogenase. Electricity generation should also be significant in the MFC as a current should be observed when the electrons are transferred from the anode to the cathode spontaneously.

B. Hypothesis/Engineering Goals

Research Questions: Can a novel MFC serve as a more cost efficient device to remediate wastewater into usable drinking water equipped with a denitrification process?

Alternate hypothesis: As studied through the literature of Puig et al. in 2011 and Werner et al. in 2013, dissolved oxygen, nitrate removal, and electricity generation of the microbial fuel cell should be higher than the aeration and control reactors.

Null hypothesis: Dissolved oxygen, nitrate removal, and electricity generation of the microbial fuel cell will be insignificant as compared to the control and aeration reactors.

Engineering Goal: To develop a dual chambered microbial fuel cell with a remediation chamber and a denitrification chamber.

Expected Outcomes: The novel microbial fuel cell should display the highest rate of efficiency in remediating the wastewater as compared to the aeration and control treatment. Also, pollutant removal specifically of nitrates should be observed in the MFC-algae bioreactor system as the

cyanobacteria will remove the nitrates found in the water using the enzyme nitrogenase. Electricity generation should also be significant in the MFC as a current should be observed when the electrons are transferred from the anode to the cathode spontaneously.

C. Methodology

- **Procedures**

Culturing of *E. coli K-12* bacteria (Hauser, 2006)

E. coli K-12 will be ordered from Carolina Biological and will be cultured using the isolation streaking method by transferring the ordered bacteria onto a new petri dish with nutrient agar base.

Preparing the Artificial Wastewater Solution (OECD, 2009)

The artificial wastewater solution will be prepared using 16g peptone, 11g vegetable extract, 3g urea, 0.7g NaCl, 0.4g $CaCl_2$, 0.2g $MgSO_4$, and 2.8g K_2HPO_4 ordered from Sigma Aldrich dissolved in 1L distilled water, then split into two plastic containers.

Construction of the Control System (Huggins et al., 2013)

A 300 ml hard plastic container will be used for the control system. Instead of using its soft plastic lid, a petri dish cover of similar size to the lid will be used. To measure remediation of the artificial wastewater solution, dissolved oxygen or DO of the water will be measured. To measure DO, the Vernier DO probe will be linked to the Vernier Lab Quest, and an initial reading will be taken in mg/L. For the probe to fit through the petri dish lid, a hole will be cut using a circular heat source to the measurement of the diameter of the DO probe. Then, the DO probe will be inserted through the hole, and will be sealed using Parafilm to create a sealed system. The control system will be operated at room temperature. In order for the control trial to be completed, the initial reading will have to increase by 90% of its original value.

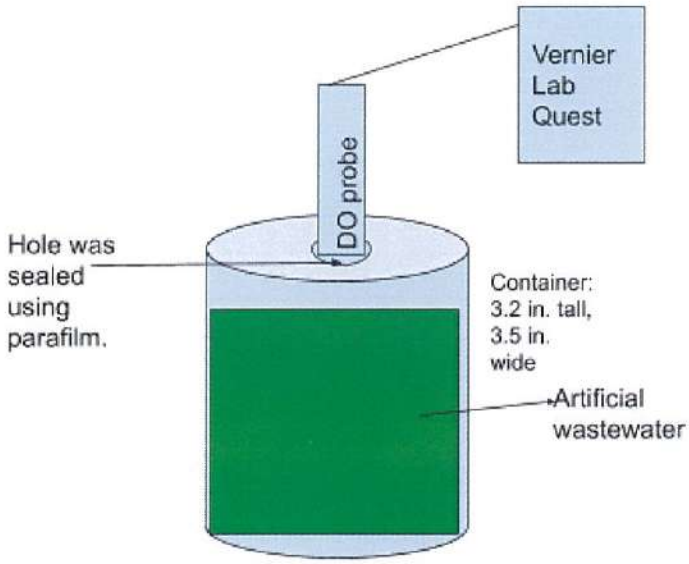

Figure 1: control reactor setup (diagram by authors)

Construction of the Aeration System

This setup will be similar to the control system construction. A 300 ml hard plastic container will be used, with the replacement of its plastic lid with a petri dish cover. 3 holes will be made in the petri dish cover: 1 for the dissolved oxygen probe, 1 for the aquarium tubing, and 1 for the pressure release. All three holes will be cut to their respective diameters using a circular heat source. The aquarium tubing will be connected to an aquarium diffuser which will be placed at the bottom of the container with the artificial wastewater. The other end of the aquarium tubing will be connected to an aquarium pump which will be connected to the nearest outlet. To measure DO, the Vernier DO probe will be linked to the Vernier Lab Quest, and an initial reading will be taken in mg/L. The control system will be operated at room temperature. In order for the control trial to be completed, the initial reading will have to increase by 90% of its original value.

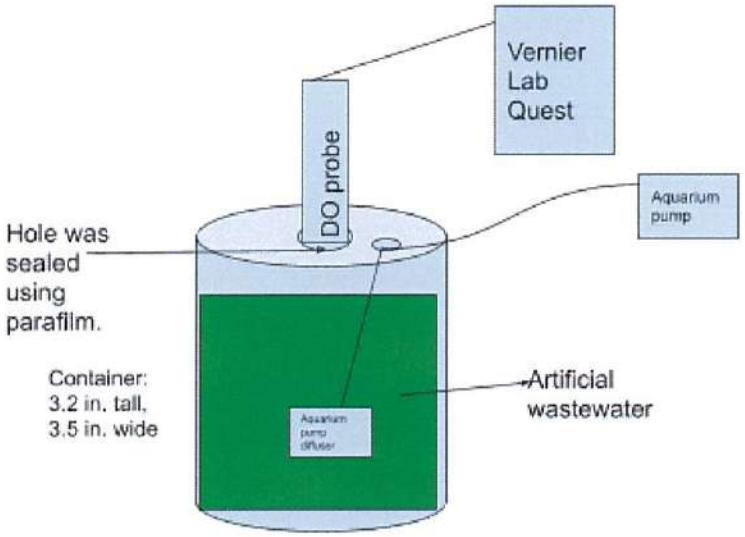

Figure 2: Aeration system setup (diagram by authors)

Construction of Microbial Fuel Cell (Zhang et al., 2011)

The Microbial fuel cell system will consist of one cathode and one anode with the total liquid volume in each compartment being 300 ml. The compartment will include clear, hard plastic containers that will be connected by a circular Nafion membrane with a 1in. radius made of proton-conductive polymer film which will be purchased from the Fuel Cell Store. The anode electrode will be 25 cm^2 carbon felt. The cathode electrode will be made from 25 cm^2 carbon cloth with platinum as catalysts at a concentration of 0.3mg Pt/cm squared purchased from the Fuel Cell Store. In order to join the anode and cathode compartments, a hole with a 1in diameter will be made using a drill and will be smoothed out using a file on both the plastic containers at the same location and height. PVC pipes of 1inch diameter will be fit into the holes and will be sealed using silicone gel spread by a caulking gun. Then, the Nafion membrane will be placed in between the pipes of the anode and cathode compartment. Before the Nafion membrane will be used, it's cover film and backing will be removed using two perpendicular pieces of tape to peel it off. The Nafion membrane will be compressed between the two PVC pipes and will be secured using EZ fuze tape. The carbon paper and carbon cloth in their respective compartments will be connected to 3 inches of titanium wire which will be the source for the Vernier energy sensor to be connected to. Artificial wastewater containing 15 colonies of *E. Coli K-12* (Carolina Biological) will be used as the substrate and for biofilm formation on the anode, as oxidation of

the carbon paper will be carried out using *E. Coli K-12* bacteria to transfer the electrons, and therefore clean water to the cathode compartment. The MFC system will be entirely sealed and operated under a fume hood.

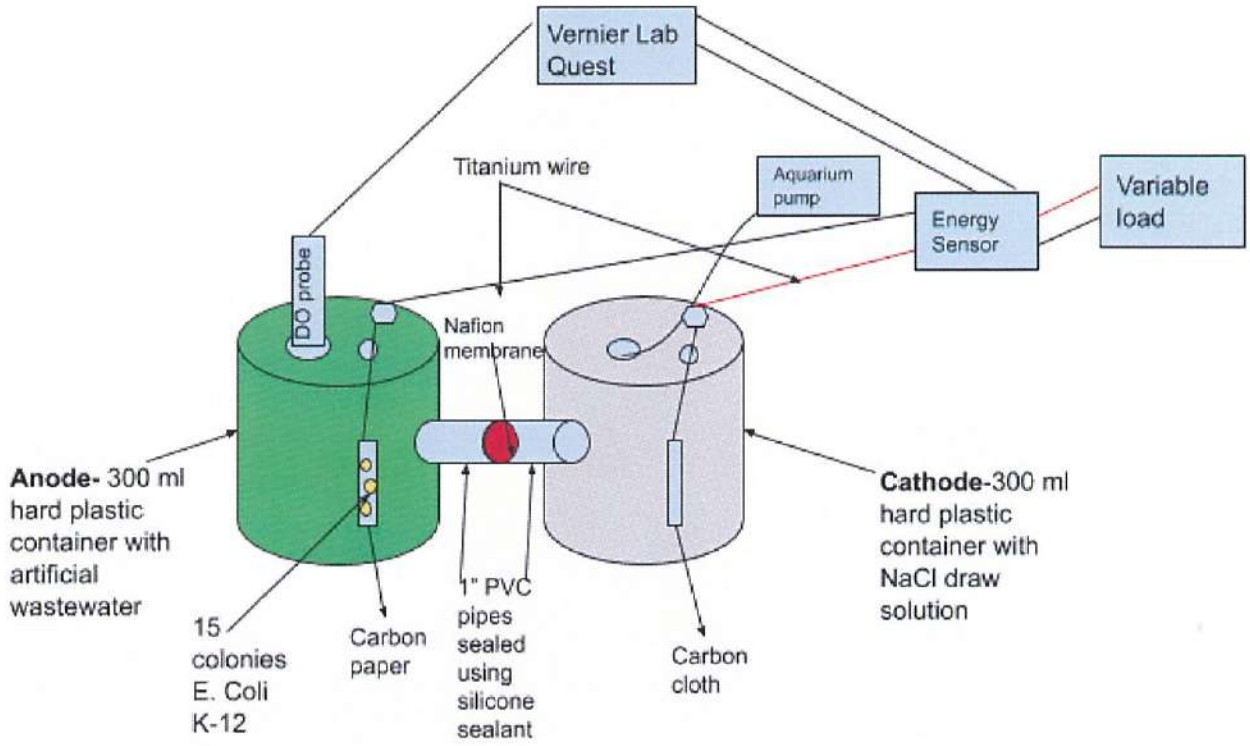

Figure 3: Schematic of Microbial Fuel Cell setup (diagram by authors)

Operation of the Microbial Fuel Cell (Zhang et al., 2011)

The fuel cell will be operated in the fume hood at room temperature. The anode will fed with the artificial wastewater solution containing 15 colonies of *E. Coli* K-12, while the cathode chamber will be operated in continuous mode with NaCl dissolved in distilled water as the draw solution, which will be prepared by dissolving 15g of NaCl in 300 ml of distilled water. The Vernier LabQuest will take readings of voltage and current from the energy sensor and dissolved oxygen from the DO probe in a time interval of 30 minutes for 3 days. Air will be supplied to the cathode at a flow rate of 15 cm^3/min using an aquarium pump diffuser under the testing conditions.

Culturing of Cyanobacteria

Anabaena will be ordered from Carolina Biological containing 20 ml of cyanobacteria solution. The 20ml of cyanobacteria solution will be added to 900 ml of spring water and 50 ml of Algae Gro Concentrate (Carolina Biological) in an Erlenmeyer flask which will be connected to an aquarium pump diffuser which will allow the cyanobacteria to reproduce.

Denitrification Compartment

After 90% dissolved oxygen increase is observed in the MFC, the remediated wastewater will be treated with cyanobacteria as they are capable of removing residual nutrients such as nitrates due to their enzyme nitrogenase as they will remediate nitrate into atmospheric nitrogen by increasing in biomass. Sodium nitrate solution at a concentration of 100 mg/L will be added to a 250ml sample of the remediated wastewater from the anode chamber of the MFC. 250ml of *Anabaena* and the 250ml water sample will added to a recycled plastic water bottle and mixed to create a homogenous solution. The water bottle will be exposed to natural sunlight to aid the algae to create biomass. Each day, the nitrate level will be monitored using a nitrate ion-selective electrode probe until a nitrate level of 10 mg/L is observed, which is the standard for nitrate in drinking water (EPA, 1991). After a nitrate level of 10 mg/L is observed, the algae biomass will be removed from the solution using a French press.

- **Risk and Safety**

1. *Human participants research: N/A*
2. *Vertebrate animal research: N/A*

3. Potentially Hazardous Biological Agents (PHBA)

a. Organism name: *E. coli K-12*

As per ISEF rules, studies involving E. Coli K-12 and studies with microbial fuel cells are exempt from prior SRC and require a Form 3.

Source of Organism: Carolina Biological

BSL assessment determination: BSL-1

b. When working with E. coli K-12, hands will be washed beforehand. Access to the bacteria will be restricted while working with the organisms. Eating and drinking will be prohibited. Lab coats, nitrile gloves, and goggles for eye protection will also be worn. There will be supervision by a scientist with general training in microbiology and sterile technique using mechanical pipetting devices to handle the organisms. To dispose of the bacteria, the *E. coli k12* will be treated with a 10% bleach solution, sealed with parafilm, and thrown into the garbage. Student researchers will be trained in sterile technique and that all surfaces will be cleaned with a 10% bleach prior to and after use.

a. Organism name: *Anabaena*

As per ISEF rules, Anabaena is a nitrogen-fixing bacteria (now classified as bacteria) and is therefore exempt from form 6A.

BSL assessment determination: BSL-1

Source of organism: Carolina Biological

b. When working with *Anabaena*, hands will be washed beforehand. Access to the algae will be restricted while working with the organisms, and eating, drinking or smoking will be prohibited. Lab coats, gloves, and goggles for eye protection will also be worn. There will be supervision by a scientist with general training in microbiology and sterile technique. To dispose of the algae, they will be treated with 10% bleach solution for 24 hours. The solution will be rinsed down the drain until a bleach odor can no longer be detected. I will be trained in sterile technique and that all surfaces will be cleaned with 10% bleach prior to and after use.

https://www.carolina.com/teacher-resources/Interactive/living-organism-care-guide-algae/tr10458.tr

4. Hazardous Chemicals, Activities, and Devices

Peptone- 16g dissolved in 1L water

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=70173&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldr

ich.com%2Fcatalog%2Fsearch%3Fterm%3DPeptone%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

- This product has no classified hazards.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

Urea- 3g dissolved in 1L water

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=U5378&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Durea%2Bpowder%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

- This product has no classified hazards.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company

NaCl (Sodium Chloride)- 0.7 g dissolved in 1 L water

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=S9888&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigma

aldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dnacl%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

- This product has no classified hazards.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

K_2HPO_4(Potassium phosphate dibasic)- 2.8 g. dissolved in 1 L water

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=P3786&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dpotassium%2Bphosphate%2Bdibasic%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

- This product has no classified hazards.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

$CaCl_2$ (Calcium Chloride)- 0.4 g dissolved in 1 L water

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=C1016&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dcacl2%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

- This product can cause serious eye irritation.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

$MgSO_4*7H_2O$(Magnesium Sulfate heptahydrate) - 0.2g dissolved in 1 L water

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=230391&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3DMagnesium%2Bsulfate%2Bheptahydrate%26interface%3DProduct%2520Name%26N%3D0%2B%26mode%3Dmode%2520matchpartialmax%26lang%3Den%26region%3DUS%26focus%3DproductN%3D0%2520220003048%2520219853286%2520219853269

- This product has no classified hazards.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

Sodium Nitrate Solution- 100 mg/L

http://www2.vernier.com/manuals/sds.no3-hst.pdf

- This product can cause serious eye irritation.
- A teacher with scientific training will be in the lab for supervision while working with the substance.

- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

Bleach- 10% (~500 mL)

https://hasapool.com/wp-content/uploads/10-Sodium-Hypochlorite-Solution-SDS.pdf

- This product can cause serious eye damage and is toxic to aquatic life with long lasting effects.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- Safety goggles, nitrile rubber gloves and lab aprons will be worn while working with this chemical. Moreover, hands will be washed before and after experimentation, and smoking, eating and drinking will be prohibited in the lab.
- This product will be disposed of by a licensed disposal company.

Autoclave

https://med.stanford.edu/content/dam/sm/medfacilities/documents/Autoclave_Safety.pdf

- Autoclaves pose hazards including physical hazards (e.g. heat, steam and pressure) and biological hazards.
- A teacher with scientific training will be in the lab for supervision while working with the substance.
- While using this device, autoclave gloves will be worn to protect hands while taking things in and out of the autoclave. Lab aprons and goggles will also be worn as a precaution, and open containers of flammable or toxic liquids will not be placed into the autoclave. Care will be taken and student researcher will only open the autoclave once it has fully depressurized to avoid getting steam burns.

- **Data Analysis**

Descriptive statistics will be calculated by creating graphs in Excel measuring the mean +/- the Standard Deviation with the graphs comparing MFC, aeration, and control reactor treatment. Dissolved oxygen will be measured for all three groups using a Vernier dissolved oxygen probe. A Vernier nitrate ion-selective electrode probe will be used to determine pollutant removal in the algae bioreactor by measuring the nitrate level of the water in mg/L. Power generation will also be calculated by the equation P= V*I, as well as voltage(V) and current(I) in the microbial fuel cell. Statistical analysis will be run using a One-Way ANOVA test followed by a Post Hoc Scheffe ($p \leq 0.05$) determining the significance between the MFC versus the aeration treatment and control system.

D. Bibliography

Huggins, Tyler, et al. "Energy and Performance Comparison of Microbial Fuel Cell and Conventional Aeration Treating of Wastewater." *Journal of Microbial & Biochemical Technology*, 2013, doi:10.4172/1948-5948.s6-002.

Li, Wen-Wei, et al. "Towards Sustainable Wastewater Treatment by Using Microbial Fuel Cells-Centered Technologies." *Energy Environ. Sci.*, vol. 7, no. 3, 2013, pp. 911–924., doi:10.1039/c3ee43106a.

Logan, Bruce E, et al. "Microbial Fuel Cells: Methodology and Technology†." *Environmental Science and Technology*, vol. 40, no. 17, 7 June 2006, pp. 5181–5192., doi:10.1002/9781118255728.ch7.

Logan, Bruce E. "Exoelectrogenic Bacteria That Power Microbial Fuel Cells." *Nature Reviews Microbiology*, vol. 7, no. 5, 2009, pp. 375–381., doi:10.1038/nrmicro2113.

Mccarty, Perry L., et al. "Domestic Wastewater Treatment as a Net Energy Producer–Can This Be Achieved?" *Environmental Science & Technology*, vol. 45, no. 17, 2011, pp.

7100–7106., doi:10.1021/es2014264.

Puig, S., et al. "Simultaneous Domestic Wastewater Treatment and Renewable Energy Production Using Microbial Fuel Cells (MFCs)." *Water Science and Technology*, vol. 64, no. 4, 2011, pp. 904–909., doi:10.2166/wst.2011.401.

Werner, Craig M., et al. "Wastewater Treatment, Energy Recovery and Desalination Using a Forward Osmosis Membrane in an Air-Cathode Microbial Osmotic Fuel Cell." *Journal of Membrane Science*, vol. 428, 2013, pp. 116–122., doi:10.1016/j.memsci.2012.10.031

Woodard, Frank. *Industrial Waste Treatment Handbook*. Butterworth-Heinemann, 2014.

Zhang, Fei, et al. "Integrating Forward Osmosis into Microbial Fuel Cells for Wastewater Treatment, Water Extraction and Bioelectricity Generation." *Environmental Science & Technology*, vol. 45, no. 15, 2011, pp. 6690–6696., doi:10.1021/es201505t.

5.2 Dissolved Oxygen and Biochemical Oxygen Demand." *EPA*, Environmental Protection Agency, 6 Mar. 2012, archive.epa.gov/water/archive/web/html/vms52.html.

"Clean Watersheds Needs Survey." *EPA*, Environmental Protection Agency, 1 Apr. 2019, www.epa.gov/cwns.

"Nutrients: Phosphorus, Nitrogen Sources, Impact on Water Quality." *Minnesota Pollution Control Agency*, Minnesota Pollution Control Agency, May 2008, www.pca.state.mn.us/sites/default/files/wq-iw3-22.pdf.

"OECD GUIDELINES FOR THE TESTING OF CHEMICALS." *OECD GUIDELINES FOR THE TESTING OF CHEMICALS*, Sept. 2009, www.oecd.org/chemicalsafety/testing/43735667.pdf.

"The Sources and Solutions: Wastewater." *EPA*, Environmental Protection Agency, 4 Feb. 2019, www.epa.gov/nutrientpollution/sources-and-solutions-wastewater.

Project Summary

NO ADDENDUMS EXIST