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

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Abstract

According to the EPA, approximately $25 billion is annually spent on domestic wastewater treatment, largely due to the aeration technology as it consumes 45-75% of wastewater treatment plant costs. A microbial fuel cell (MFC) is an apparatus that uses bacteria as the catalysts to oxidize organic and inorganic matter and generate current, used as an alternative for wastewater treatment. The purpose of this study was to create a novel MFC that would 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 using an algae bioreactor. The control treatment consisted of still artificial wasetwater, the aeration reactor consisted of artificial wastewater circulated by an aquarium diffuser, and the MFC consisted of two chambers (cathode and anode) joined together by a proton exchange, or Nafion membrane. All three reactors were tested for the increase in dissolved oxygen by 90%, which shows complete remediation of the water. The control treatment took 11 days to remediate, the aeration reactor took 3 days, and the MFC took 22 hours to remediate. The MFC was capable of producing voltage as it produced 0.62 V at its max. The algae bioreactor used the growth of *Anabaena* biomass in the remediated wastewater as a method to reduce nitrates. As shown in this study, MFC treatment holds promise for a more electrically and cost efficient method for treating wastewater.

Introduction

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 was *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 was compared to determine which one has the highest rate of efficiency similar to Huggins et al. in 2013. The control treatment was solely the artificial wastewater, the aeration technology was constructed using an aquarium diffuser circulating the wastewater, and the novel MFC with a commercially available cation exchange membrane was constructed of a double chamber MFC separated by a Nafion membrane. Tests in this study were overall wastewater treatment, hydraulic retention time, DO levels, electricity generation, and nitrogen and phosphorus removal. This study focused on optimizing energy generation as well as pollutant, or nitrogen and phosphorus removal.

The purpose of this study was to determine whether a novel MFC was more efficient than current aeration technologies and a control treatment, and whether it could 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, dissolved oxygen, nitrate removal, and electricity generation of the microbial fuel cell should be higher than the aeration and control reactors. In order for the null hypothesis to be supported, dissolved oxygen, nitrate removal, and electricity generation of the microbial fuel cell would be insignificant as compared to the control and aeration reactors. The engineering goal of this study is to develop a dual chambered microbial fuel cell with a remediation chamber and a denitrification chamber.

Methodology

Culturing of

E. coli K-12 was ordered from Carolina Biological and was 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

The artificial wastewater solution was 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

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

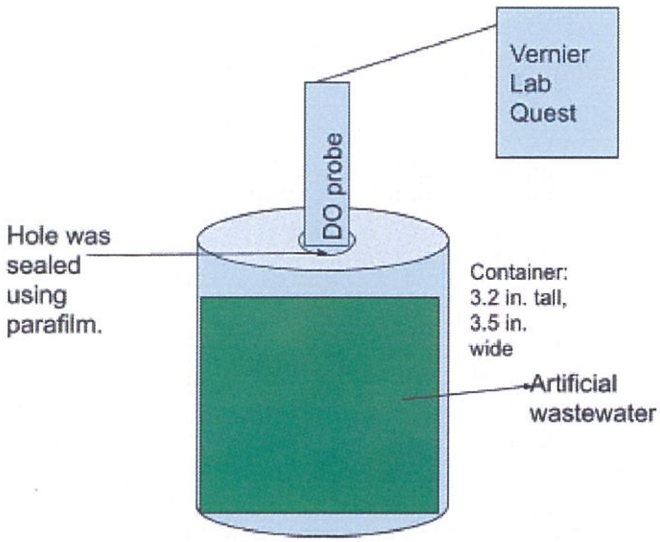

Figure 1: control reactor setup (diagram by authors)

Construction of the Aeration System

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

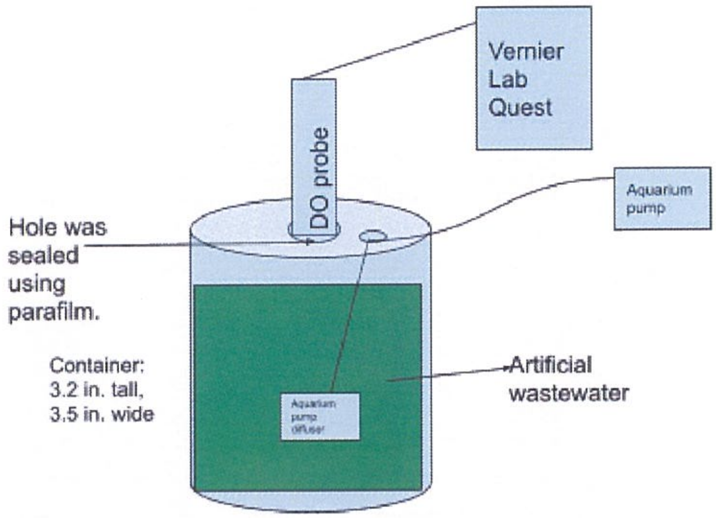

Figure 2: Aeration system setup (diagram by authors)

Construction of Microbial Fuel Cell

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

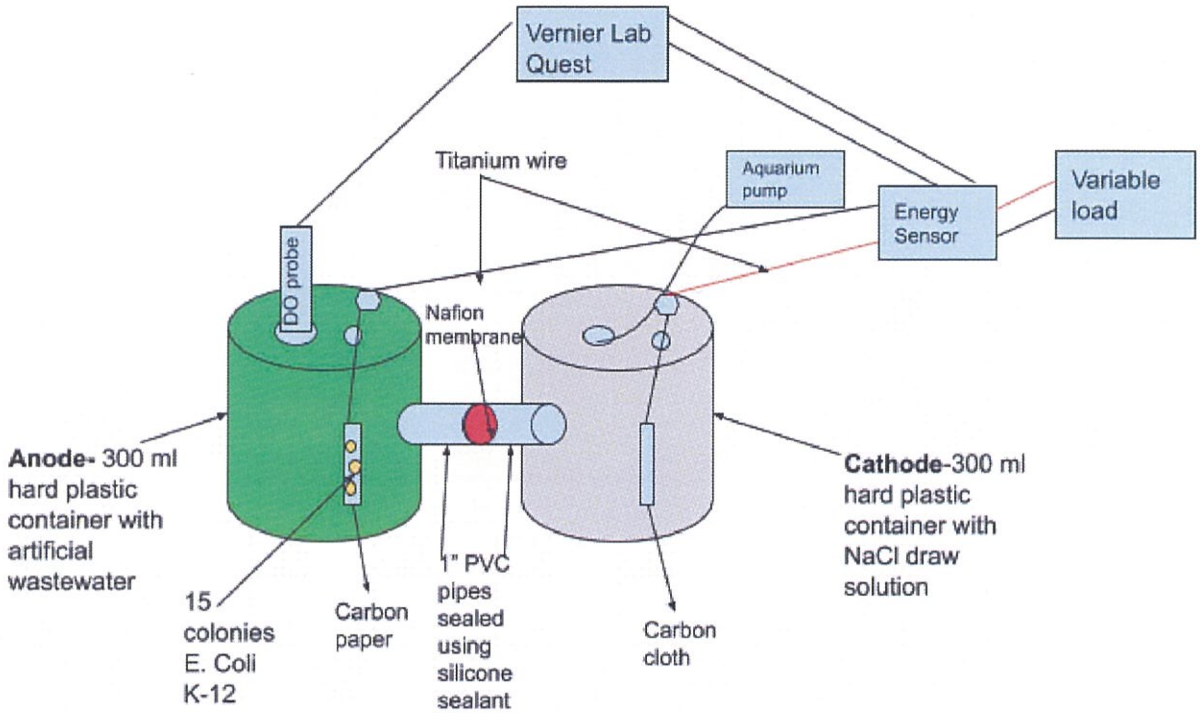

Figure 3: Schematic of Microbial Fuel Cell setup (diagram by authors)

Operation of the Microbial Fuel Cell

The fuel cell was operated in the fume hood at room temperature. The anode was fed with the artificial wastewater solution containing 15 colonies of *E. Coli* K-12, while the cathode chamber was operated in continuous mode with NaCl dissolved in distilled water as the draw solution, which was prepared by dissolving 15g of NaCl in 300 ml of distilled water. The Vernier LabQuest took 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 was 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 was ordered from Carolina Biological containing 20 ml of cyanobacteria solution. The 20ml of cyanobacteria solution was added to 900 ml of spring water and 50 ml of Algae Gro Concentrate (Carolina Biological) in an Erlenmeyer flask which was connected to an aquarium pump diffuser which allowed the cyanobacteria to reproduce.

Denitrification Compartment

After 90% dissolved oxygen increase is observed in the MFC, the remediated wastewater was treated with cyanobacteria as they are capable of removing residual nutrients such as nitrates due to their enzyme nitrogenase as they remediated nitrate into atmospheric nitrogen by increasing in biomass. Sodium nitrate solution at a concentration of 100 mg/L was added to a 250ml sample of the remediated wastewater from the anode chamber of the MFC. 250ml of *Anabaena* and the 250ml water sample was added to a recycled plastic water bottle and mixed to create a homogenous solution. The water bottle was exposed to natural sunlight to aid the algae to create biomass. Each day, the nitrate level was 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 was removed from the solution using a French press.

Results

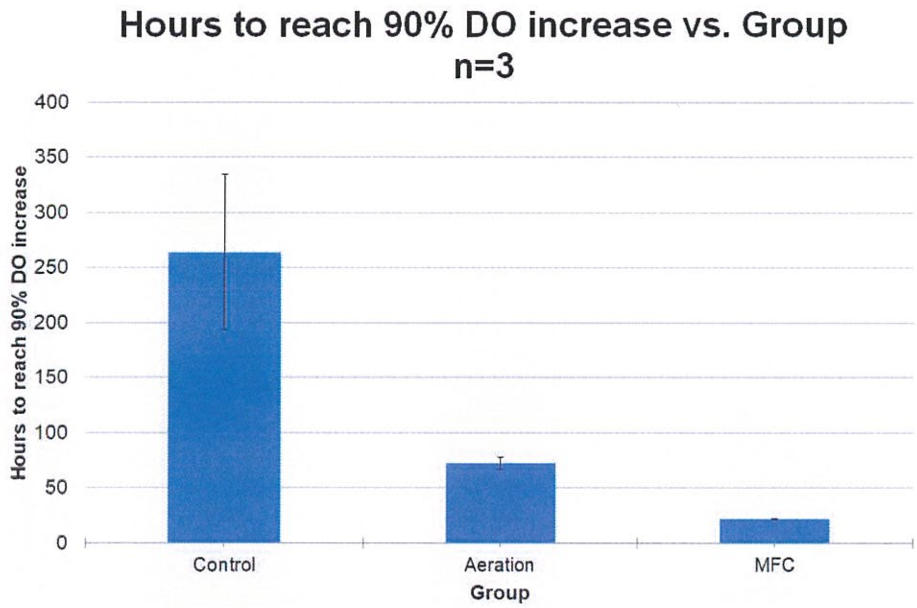

Figure 4: The amount of time, in hours, it took the Control reactor, Aeration reactor, and Microbial Fuel Cell to reach a 90% increase in DO from the initial readings. (Graph by authors)

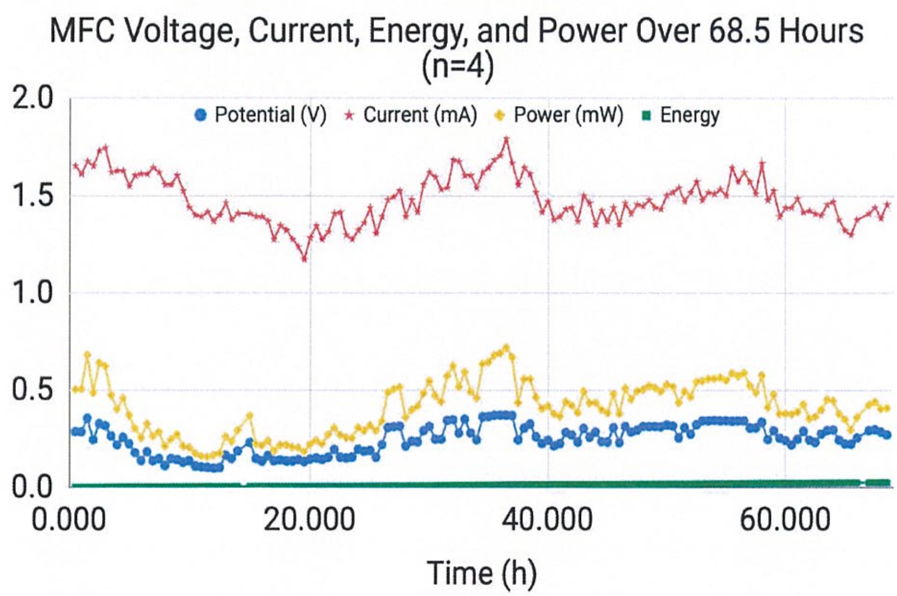

Figure 5: Novel MFC testing over 68.5 hours for variables including voltage (blue), current (red), power (yellow), and energy (green). All variables experienced fluctuation, reaching a maximum at approximately hour 35 of operation. (Graph by authors)

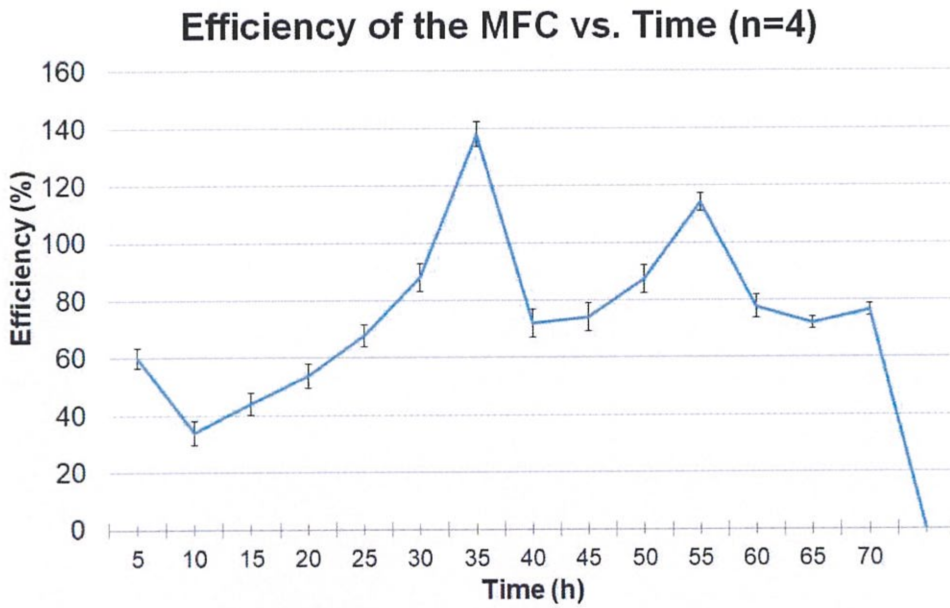

Figure 6: The efficiency of the MFC system as time increased from 0 to 70 hours. Power input and power output readings were used to determine efficiency percentage. (Graph by authors)

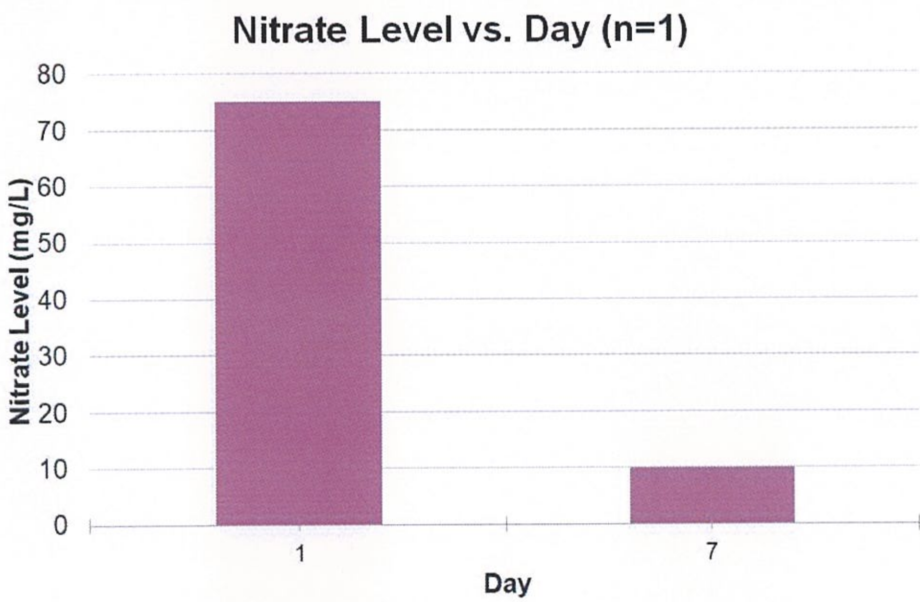

Figure 7: Nitrate removal by the algae bioreactor over a span of 7 days. 10 mg/L of nitrate is considered a safe drinking water standard. (Graph by authors)

Discussion

The purpose of this study was to determine the efficiency of a novel MFC to treat wastewater in terms of electricity generation and pollutant removal. In order for the wastewater to be considered clean enough to allow life to survive in it (EPA, 2012), there must be a 90% increase of DO from the original recorded value, as it indicates that enough dissolved oxygen has entered the water to overcome the effects of hypoxia and eutrophication. The DO probe was the chosen equipment to carry out this procedure since it is used to measure how much oxygen is present in an environment, and the data obtained was used to determine if life would be able to survive in the particular type of system being worked with in this study. In this study, the control reactor consisted of 300mL of artificial wastewater, which was kept in a closed system, while no other factors acted upon it. The control group experienced a 90% increase in DO at an average of 11 days (figure 4). This slow increase in DO may be due to the lack of circulation in the water. The aeration reactor experienced a 90% increase in DO at an average of 3 days (figure 4). The aeration reactor derived its source of oxygen from the bubbles in addition to the circulation of the water created by the aquarium pump diffuser.

This study incorporated elements of both Mustakeem, 2015 and Zhang et al., 2011 to create an optimized MFC. This study altered Zhang et al.'s design to allow for all parts of the MFC to be closed, and instead of a forward osmosis membrane being used, a Nafion membrane was used. Nafion membranes are commercially available and do not require specific storage conditions, as it can be kept and used at a range of temperatures unlike the forward osmosis membrane, making the Nafion membrane more practical in everyday wastewater treatment. Also, Zhang et al.'s study used NaCl as the draw solution for the cathode when operated in batch mode, but this study integrated the NaCl draw solution in continuous mode allowing it to be implemented in a closed system.The MFC group experienced a 90% increase in DO at an average of 1.58 days (figure 4). The MFC had a membrane in between the anode and cathode remediating the water by filtering out particulates, and *E. Coli K-12* was responsible for transferring hydrogen ions to the cathode to combine with oxygen molecules from the air (Logan, 2019), which facilitates the transfer of oxygen from the air to the water, hence increasing the DO. The MFC group was capable of producing an increased voltage of 0.126V on average(figure 19), so *E. Coli K-12* is a valid exoelectrogenic microbial agent which concurs with Sugnaux et. al, 2013 as it created an electrical current between the anode and cathode, by oxidizing the organic substrate of the wastewater (Logan, 2019). The MFC was more electrically efficient than Puig et. al in 2011, as electricity was observably produced at hour 10 compared to day 15 which can be due to the use of the Nafion membrane and cost efficient electrodes as studied by Mustakeem, 2015.The MFC reactor was more efficient (figure 6) than current aeration reactors as it exhibited an average of 75.02% efficiency while aeration reactors are only 40% efficient (Frankel, 2019) because of the high consumption of electricity required to run the aeration pumps. The control and MFC data concurred with the findings of Huggins et al., 2013.

Limitations

There were some limitations in this project, but the design of both the control and MFC reactors were altered to account for the limitations. For the control reactor, the dissolved oxygen probe produced fluctuating readings due to the varying temperature from daytime to nighttime in the lab, when the control reactor was kept in the fume hood. To solve this limitation, the control reactor was brought into ambient temperature, where it was sealed with Parafilm, so it could remain a closed system. Also, while constructing the MFC, leaks were experienced between the vantage points of the PVC pipe and container, and the PVC pipe and the membrane. To resolve these limitations, silicone sealant caulk was used to seal the PVC pipe to the hole in the plastic container so water would not leak from any gaps between the holes in the anode and cathode. For the PVC pipe and membrane joint, EZ fuze tape was used to firmly secure it. However, the MFC contained *E. Coli K-12,* so it had to remain in the fume hood, causing for fluctuations in dissolved oxygen for the microbial fuel cell reactor. In the aeration reactor, problems were encountered with evaporation as the pump diffuser produced a high rate of bubbles even at a low flow rate of the aquarium pump.

Conclusion:

The alternative hypothesis was accepted as the rate of the remediation of wastewater (90% DO increase) was significant in the MFC as compared to the control group. The MFC design was capable of producing voltage, which could be generated into power and energy, indicating a preservation of energy through wastewater treatment. The electrodes used in the experiment were determined to be the most cost and energy efficient.The MFC indicated a higher efficiency and conservation of power to treat wastewater as opposed to current aeration technologies. Wastewater treatment can benefit from MFC treatment reducing the burden on fossil fuels and reducing the 130% increased electricity consumption projection by 2040.

Future Studies

In the future, different electrodes such as graphite brush or carbon paper can be tested for energy and cost comparison for the MFC. Also, wastewater from treatment plants can be tested instead of artificial wastewater due to previously existing microbes and nutrients in industrial wastewater. In addition, a larger scale model of the MFC, control, and aeration reactors may be tested to determine the real world application of the small scale model observed in this study. Different types of algae for the bioreactor, e.g. *Chlorella Vulgaris* and *Microcystis* can be used to compare the nitrate removal efficiency as compared to *Anabaena.*

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Appendix

Flow Chart

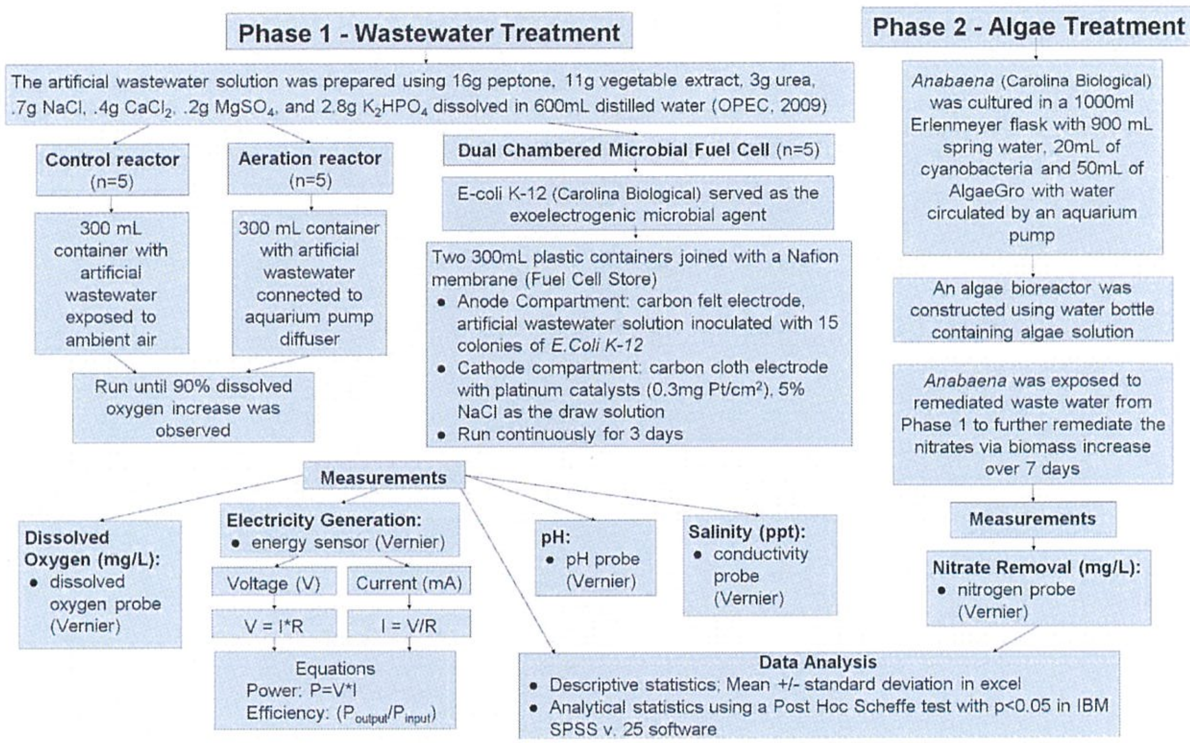

Figure 1: Flow Chart of the Methodology