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Microbes as Proton Exchange Membranes in Microbial Fuel Cells

Andrew Timothy Stroud
University of South Carolina - Columbia

MICROBES AS PROTON EXCHANGE MEMBRANES IN MICROBIAL FUEL CELLS

by

Andrew Stroud

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Accepted by:

R. S. Norman, Director of Thesis

Alan Decho, Reader

Dwayne Porter, Reader

Lacy Ford, Vice Provost and Dean of Graduate Studies

DEDICATION

I dedicate my dissertation work to my family and wife. A special feeling of gratitude to my loving parents, John and Tammy Stroud whose words of encouragement and push for determination ring in my ears. My brother, Tyler, has never left my side and is very special. I will always appreciate all the advice my lab mates have done, Erin Fichot for her extensive knowledge of lab techniques and Eva Preisner for helping me stay on top of the graduate school paper work. Also, I would like to thank Dr. Sean Norman for taking me into his lab, mentoring and funding my ideas and work. Most of all, I dedicate this work and give special thanks to my best friend and wife, Jill Stroud, for being there for me throughout the entire master's program. You have been my biggest supporter through it all.

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ABSTRACT

Microbial fuel cells (MFCs) use proton exchange membranes (PEMs) to deliver protons to the cathode for electricity production. MFCs have been limited to low power generation because of complex ionic media characteristic of MFCs using the conventional Nafion-based PEM with high attraction for the competing ions. Benthic microbial fuel cells (BMFCs) use the marine sediment between the anode, embedded in marine sediment, and cathode, overlying in water, as a PEM. BMFCs have been shown to generate high power densities representing high proton permeability by the marine sediment layer between the anode and cathode. However, there is limited knowledge about this BMFC marine sediment layer. In this study, marine sediment from Charleston, South Carolina will be used to perform a metagenomic microbial community analysis of 16S rRNA genes. Microbial proton exchange membranes (MPEM) will be created by placing nylon membranes directly onto sediment and then extracting them for subsequent generations of MPEMs under conditions designed to isolate optimal organisms. After growing first generation microbes on nylon membrane, the microbes in the membrane will be fluorescently labeled for enriched. From the isolated first generation microbes, a second generation MPEM will be replicated, characterize and evaluated just as the first generation. A comparative analysis will be completed of first and second generations to further identify patterns of selection through time.

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C Of or relating to a temperature scale that registers the freezing point of water as 0° and the boiling point as 100° under normal atmospheric pressure.

g A unit of mass in the metric system, equal to 0.001 kilogram or 0.035 ounce.

mL A unit of liquid volume or capacity in the metric system equal to 0.001 liter.

pH The degree of acidity or alkalinity of a solution; it is the logarithm of the reciprocal of the hydrogen-ion concentration in gram equivalents per liter of solution.

List of Abbreviations

AEM ..... Anion Exchange Membrane

BMFC ..... Benthic Microbial Fuel Cell

CEM ..... Cation Exchange Membrane

MFC ..... Microbial Fuel Cell

PEMs ..... Proton Exchange Membranes

UFM ..... Ultrafiltration Membrane

CHAPTER 1

INTRODUCTION

Microbial fuel cells has been of research interest for the past couple of decades with specific interest in creating renewable energies that can provide public benefits, environmental improvements, and regional economic development benefits. Developing efficient renewable sources of energy by using microbes not only can help with our energy deficiencies but also with degradation of sewage and organic waste (Logan, 2008). Potter in 1911 is given credit for first observing electrical current being generated by bacteria (Potter 1911). It was not until the 1990's that interest in MFCs expanded (Allen & Bennetto 1993). During this time we have seen great progress in power increase in MFCs from 0.1 W/m^2 before 2001 to recently 2400 mW/m^2 (Logan et al. 2007). But MFCs are still well below their theoretical power densities and leave room for improvement from further research (Logan, 2008).

1.1 Microbial Fuel Cells

Microbial fuel cells (MFC) convert organic matter into electricity (Potter 1911; Tender et al. 2002). The MFC comprises of an anode and a cathode. The anode is normally in anaerobic conditions and accepts electrons from the break down of organic material by microorganisms (Potter 1911; Tender et al. 2002). The anodes are highly conductive, non-corrosive, large area per volume, inexpensive, non-fouling and easily made material (Logan, 2008; Tender et al. 2002). Carbon-based electrodes, such as

carbon paper, carbon cloth and reticulated vitrified carbon are commonly used as materials for anodes (Logan, 2008). The cathode is placed in aerobic conditions and transfers electrons to an electron acceptor (Reimers et al. 2001; Tender et al. 2002). The reaction that occurs at the cathode is challenging to produce as the electrons, protons and oxygen must all meet at a catalyst. Again, the same materials used for the anode have been used most commonly in the cathode. The electrons move from the anode to the cathode by a connection that connects to directly to devices or uses a battery, which in turn provides electricity to meteorological monitoring devices, oceanography monitoring devices, or some other type devices that need to be powered by electricity (Reimers et al. 2001; Tender et al. 2002; Tender et al. 2008). Protons are moved across a membrane or separator that is essential for a two chambered MFC. These membranes are permeable to protons while impermeable to other ions (Logan, 2008). Figure 1.1 shows the basic schematic of Microbial Fuel Cell.

1.2 Benthic Microbial Fuel Cells

Benthic microbial fuel cells (BMFC) are similar to MFCs except that the anode is placed below the marine sediment and the cathode is positioned in the overlying water (Tender et al. 2008). Using the organic carbon from sedimentation of phytoplankton detritus, the marine sediment provides considerable energy reserves that sit on the seafloor (Tender et al. 2002). An important part of the BMFC is endurance, which is credited to constant supply of organic carbon without dependence on added electron-transfer mediators (Bond et al. 2002; Tender et al. 2008). Also, it is important to note that BMFCs are true microbial fuel cells (Tender et al. 2008). Microbes completing work at the anode, microbes moving electrons across the marine sediment between the anode

and cathode, then microbes on the cathode surface catalyzes oxygen reduction (Tender et al. 2008). BMFCs have implications for development of power supplies that could harvest energy from marine sediment for long-term sustained power-generation that have been shown before with marine oceanographic instruments (Reimers et al. 2001; Tender et al. 2002; Bond et al. 2002; Tender et al. 2008). Figure 1.2 shows the basic setup of a BMFC (US Dept. of Energy).

1.3 Proton Exchange Membranes

Proton Exchange Membranes (PEMs) are principally used in two-chambered MFCs. This allows the anode and cathode contents to stay separated (Logan, 2008). The PEMs need to be permeable to allow protons to pass through to the cathode chamber and impermeable to oxygen to keep the anode chamber ananerobic (Logan, 2008; Lovely, 2006). Each electron that is released onto the anode, a proton also must be transported to the cathode to maintain electricity production (Chae et al. 2008). The PEM transfers the proton from the anode chamber to the cathode chamber. An adverse effect of the PEM performance is usually a result of increased internal resistance in which will reduce power production (Logan, 2008). This is shown in Kim et al. (2007) Table 1. For MFCs, the proton exchange membrane must provide three main contributions, which include 1) functioning as ion transfer media; 2) separating reactant spaces, such as anaerobic and aerobic environments, which react at the cathode and anode, and 3) functioning as a catalyst support (Logan et al., 2007; Logan 2008; Reimers et al., 2001; Tender et al., 2002; Tender et al., 2008).

1.4 Nafion

Walther Grot discovered Nafion in the 1960's while working for DuPont. Nafion has unique ionic properties with perfluorovinyl ether groups terminated with sulfonated groups on a tetrofluorothelylene. This unique chemical structure allows high conductive properties. Essentially protons hop from one sulfonate group to another and do not allow anions (electrons) movement across the membrane. This has worked great for broad applications in fuel cells because of it thermal and mechanical stability. But Nafion has its disadvantages when used in MFCs. In complex ionic environments inherent to MFCs, Nafion is not proton specific and therefore not efficient for MFCs (Rozendal et al., 2006). Another disadvantage to using Nafion is that high cost being $1400 per square meter. These high costs would be prohibitive in large-scale application of MFCs (Logan, 2008; Du et al., 2007).

1.5 Anion Membrane

Anion exchange membranes (AEM) use chemicals as a pH buffer to balance the anode and cathode. This is shown by Kim et al. (2007) in Table 1 by the phosphate concentrations measured in the chambers on either side of the AEM. A disadvantage of using AEM is that the pH of the cathode chamber has been shown to increase versus using Nafion (Kim et al. 2007; Logan, 2008). An increase in pH can effect power production.

1.6 Ultrafiltration Membrane

Ultrafiltration membranes (UF) were developed for wastewater application to separate organic matter from water but have been shown as a PEM for MFCs (Kim et al.,

2007). UFs are permeable to small charged ions and are used as membranes in MFCs to separate fluid between the chambers. Kim et al. (2007) used three different UFs for power-generation and had high internal resistances produced less power than Nafion and AEM membranes (Logan, 2008).

1.7 Bipolar Membrane

The make up of a bipolar membrane entails an anion and cation membrane joined in series (Logan, 2008). The way the bipolar membrane works is as voltage accumulates, rather than protons passing the membrane, water is split (Logan, 2008). Anions are transported to the anode and cations to the cathode to balance charge (Logan, 2008). A disadvantage of using bipolar membrane is the energy needed for the water splitting reaction and a ferric iron catholyte is needed to keep the cathode chamber pH low.

Table 1.1 Internal resistance and maximum power density for Nafion, cation (CEM), anion (AEM), and ultrafiltration (UF) membranes tested in bottle and cube MFCs. Data from Kim et al. (2007).

	internal resistance (Ω)		maximum power (mW/m^2)	
membrane	B-MFC	C-MFC	B-MFC	C-MFC
no membrane	1230 ± 44	84 ± 3	*a*	*a*
Nafion	1272 ± 24	84 ± 4	38 ± 1	514
CEM	1308 ± 18	84 ± 2	33 ± 2	480
AEM	1239 ± 27	88 ± 4	35 ± 3	610
UF−0.5K	6009 ± 58	1814 ± 15	5 ± 1	*b*
UF-1K	1239 ± 52	98 ± 5	36 ± 0	462
UF-3K	1233 ± 46	91 ± 6	36 ± 0	*b*

[a] Not applicable. [b] Not measured.

1.8 Culture Independent Analysis of Microbial Communities

Culturing techniques in environmental samples will account for 1% or less of the diversity of bacteria in these sample (Riesenfeld et al., 2004). Using a metagenomic approach, which is the "functional and sequence-based analysis of the collective microbial genomes contained in an environmental sample", one can account for the bacteria in environmental samples that are not able to be cultured (Riesenfeld et al., 2004; Weisburg et al., 1991). Woese established the techniques for using the 16S rRNA in identification of bacteria and archaea since these regions are highly conserved between species (Woese et al., 1977). With these highly conserved regions, there are also hyper-variable regions that can be used to for species identification in bacteria using the 16S rRNA gene (Riesenfeld et al., 2004; Woese et al., 1977). This approach will support identification of the microbial community in the MFCs and on the microbial proton exchange membrane.

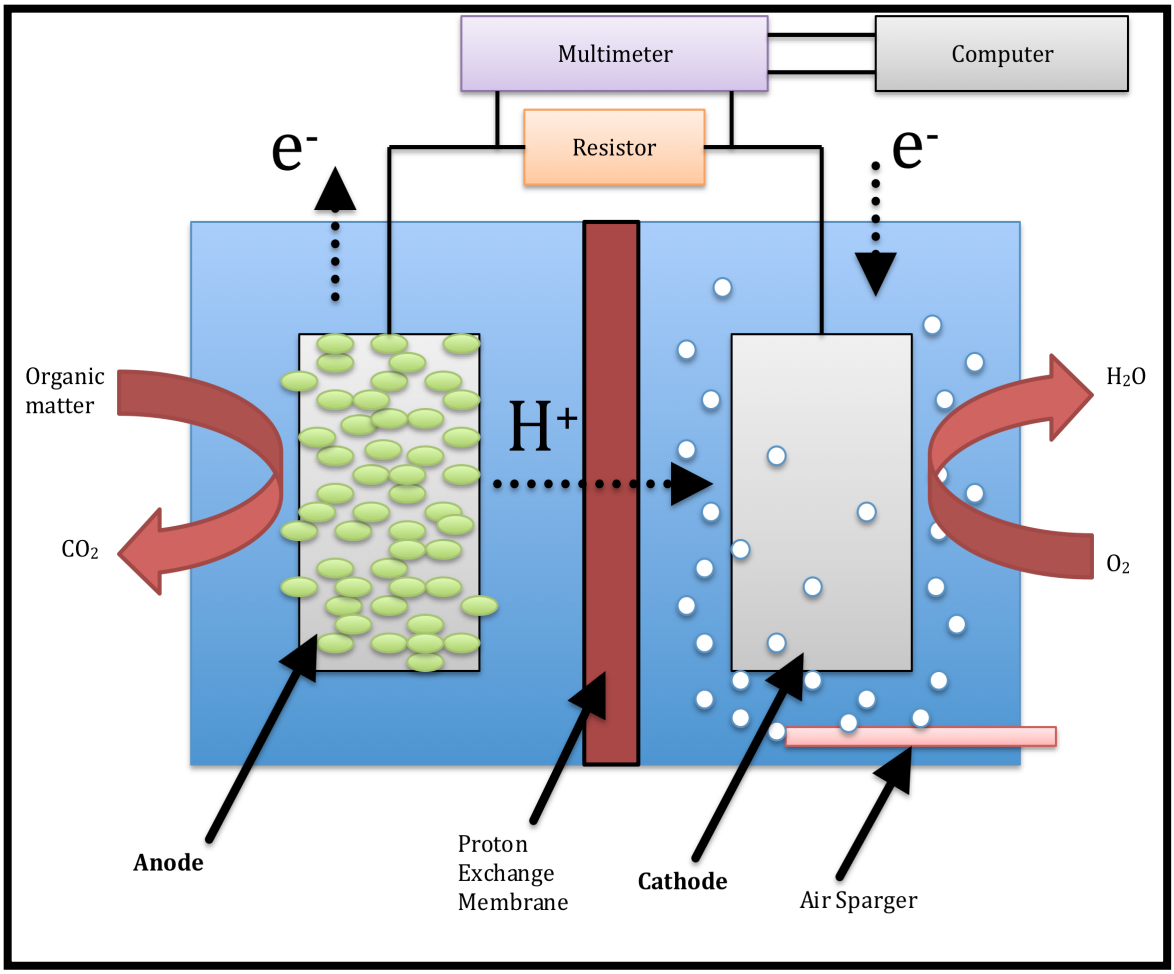

Figure 1.1 Basic schematic of a microbial fuel cell. The anode and cathode chambers are separated by a proton exchange membrane. Bacteria grow on the anode breaking down organic matter and releasing electrons to the anode. The cathode is sparged with air to provide dissolved oxygen for the reactions of electrons, protons and oxygen at the cathode. The system is shown with current determined based a multi-meter measurement hooked to a computer collecting the data.

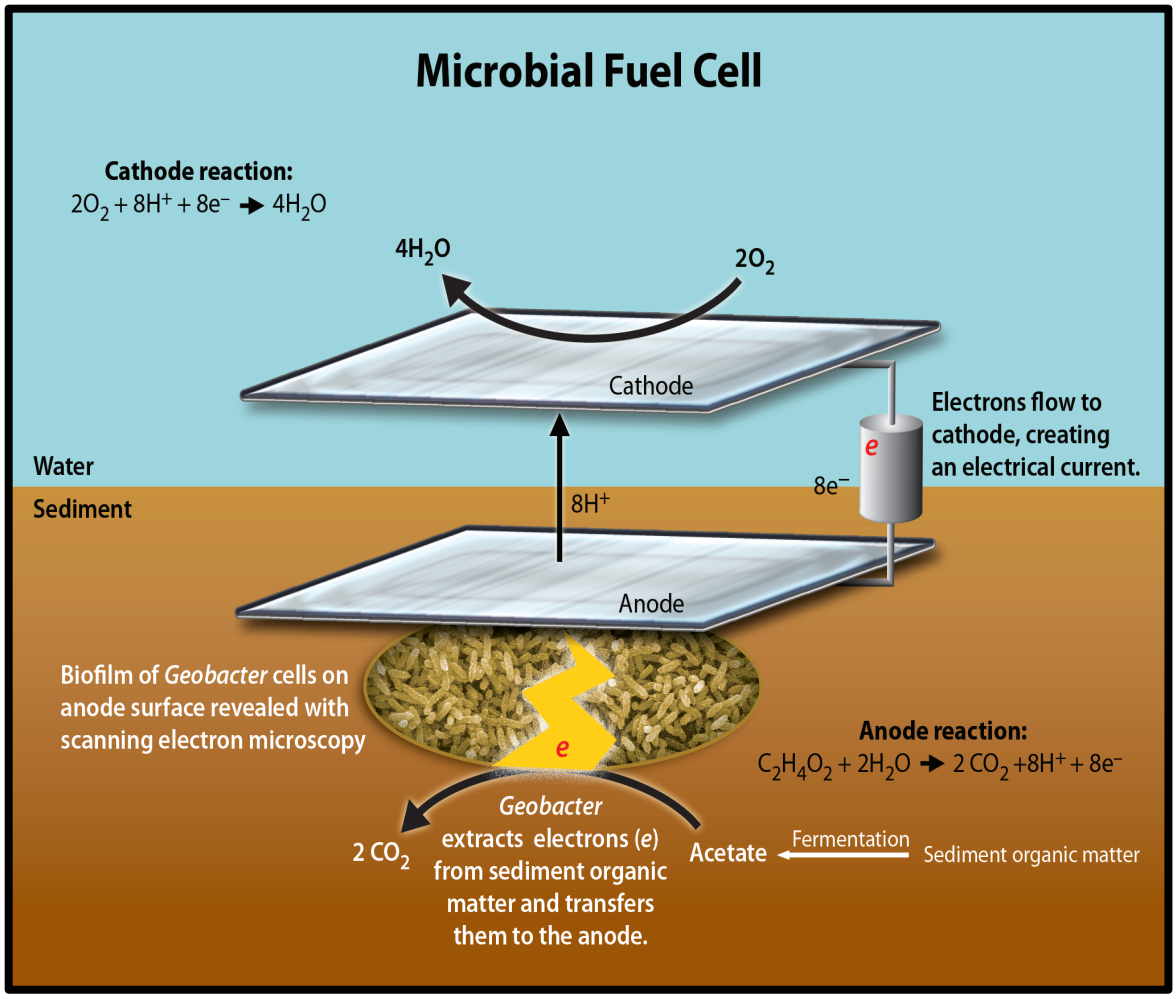

Figure 1.2 Basic schematic of a Benthic Microbial Fuel Cell. The anode is placed below the marine sediment while the cathode is positioned over the anode. The anode and cathode are separated by marine sediment, which serves as the PEM.

CHAPTER 2

HYPOTHESIS

It is well known that renewable energy sources are needed to replace our dependence on coal, oil and other fossil fuels. Our dependence on fossil fuels is unsustainable due to limited supplies of resources and pollution. One solution will not replace fossil fuels, but many different alternatives will be needed to fill the energy demand created by fossil fuels. Microbial fuel cell technology is one alternative that has been researched extensively as of late. Using the microbe's metabolism to produce an electrical current for low power marine instruments has been demonstrated as a viable replacement (Reimers et al., 2001; Tender et al., 2002; Tender et al., 2008). This project aims to improve proton exchange efficiency in microbial fuel cells with the microbial-based proton exchange membrane. MFCs have not been a practicable alternative because of the low-power output due to low proton exchange efficiency. This project also aims to fill data gaps by defining which microbes are involved in the proton exchange.

I hypothesize that membranes modified with biofilms comprised of microorganisms that naturally inhabit aerobic/anaerobic interfaces, such as those isolated from the sediment surface of marine environments may act as effective proton exchange membrane for use in microbial fuel cells. In this study I want to discover and characterize the microorganisms involved in proton exchange in benthic microbial fuel cells by using the 16S rRNA gene for sequencing.

CHAPTER 3

SELECTION OF MICROBES AS PROTON EXCHANGE MEMBRANES

3.1 Introduction

Benthic microbial fuel cells have been shown to harvest energy from marine sediment for long-term sustained power generation (Tender et al. 2002). BMFCs use the marine sediment as a proton exchange membrane (Reimers et al. 2001). This sediment layer is where the microbes are located that were extracted as a microbe-based proton exchange membrane. The overall goal was to develop biofilms on some type of permeable membrane and evaluate their performance as PEMs in MFCs. Our first task was to determine what type of permeable membrane we were going to use for biofilm development. Identifying several different types of materials, we initially tested sponge aquatic filter, blue aquatic filter, US Fabric 65, US Fabric 40, US Fabric 32, and muslin cloth. These potential membranes were tested for the microbe's ability to establish growth on the surface of the material. We wanted to provide a structure for the biofilm to develop.

3.2 Alginate Mixture Growth Experiment

Growing a biofilm on these potential membranes was first tested using two different concentrations of alginate. 2% and 4% alginate concentrations were use to give the biofilms some sort of substrate for the growth on the potential membrane. Microbes were extracted from the marine sediment and then placed on each potential membrane.

After giving the microbes two weeks to develop on the potential membranes, the membranes were analyzed for biofilm development. This biofilm development is important for the membrane to work as a PEM. Without a fully developed biofilm the membrane will not work efficiently and will generate low power.

3.3 Materials and Methods

The sediment container was selected and the water on top of the sediment was removed to make sediment removal more efficient and exact. The top 5mm of marine sediments was removed. 200 mL of artificial seawater was added to marine sediment. The mixture was poured into a laboratory blender and blended for 30 seconds at the lowest blending speed. After the 30 seconds of blending, blending stopped and the mixture was allowed to cool for 1 minute. This step was repeated 6 times. The mixture was then put into a centrifuge at low speed (500 g) to pellet the sediment. The supernatant was decanted to a new centrifuge container. 200 mL of artificial seawater was then added to the spun down sediment and the mixture was mixed well and the centrifuging of the sediment completed again. The supernatant was decanted into a new centrifuge bottle and this process was completed two more times. The supernatant that was collected was spun down at a high speed (25,000 g) for 20 minutes. The supernatant from this mixture was discarded and the cell pellet was diluted in 25 mL of seawater media.

Alginate was made using artificial seawater 25 mL and 1 g of aginic acid to make a 2% and 4% concentration of alginate.

The 25 mL diluted cell pellet was homogenized and added to the previously made alginate mix. A stirring magnet was used to stir in the diluted cell pellet solution. After 10 minutes of stirring, the alginate/cell mixture was added petri dishes with US Fabric 65 (Polypropylene), US Fabric 40 (Polypropylene), US Fabric 32 (Polypropylene), and muslin cloth (Cellulose) (See Figure 3.1). Seawater media was added to each petri dish. The petri dish was covered and wrapped in parafilm and put at 25° C.

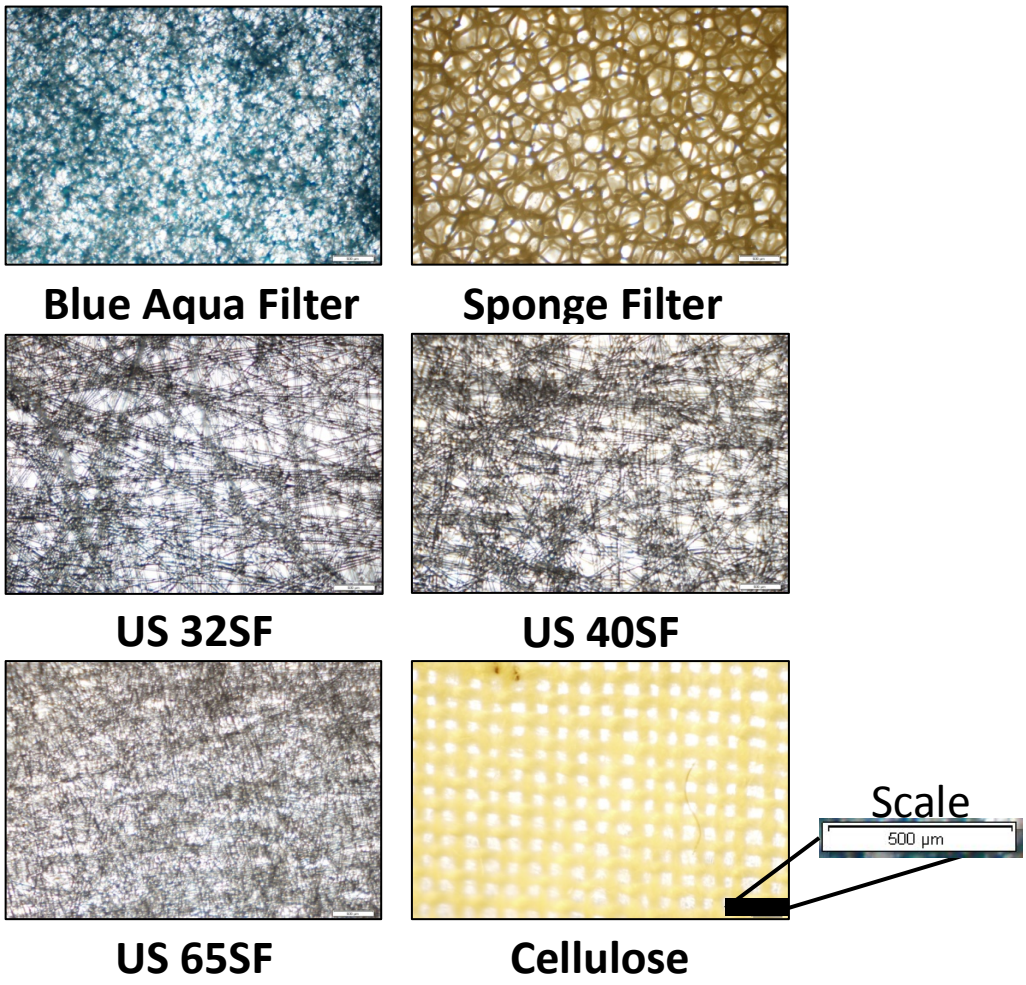

Figure 3.1 Different types of membranes tested in the alginate growth experiment.

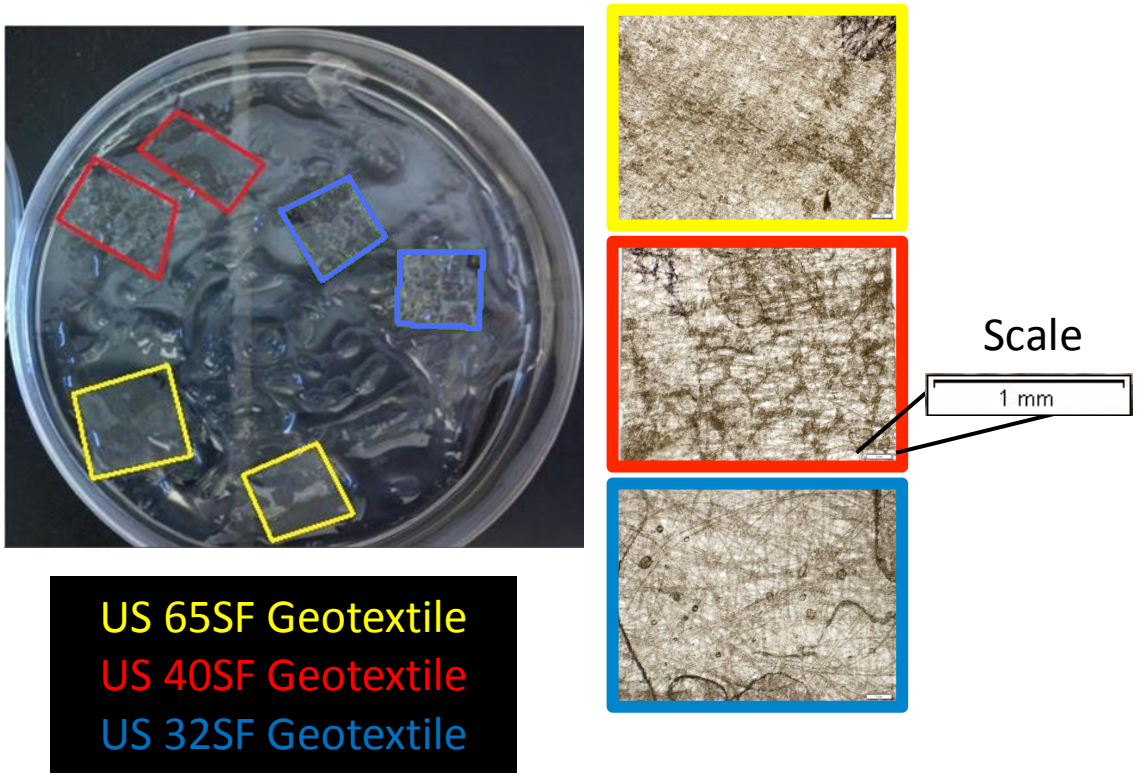

Figure 3.2 2% alginate coating on the US 65SF Geotextile, US 40SF Geotextile, US 32SF Geotextile.

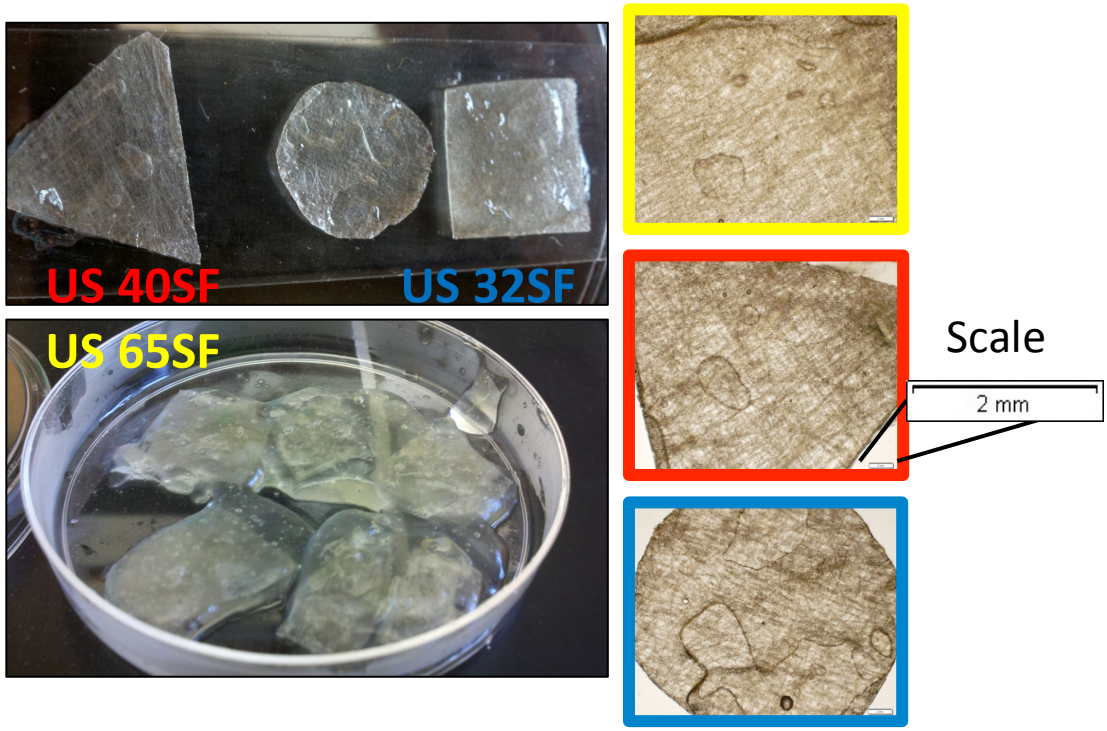

Figure 3.3 4% alginate coating on the US 65SF Geotextile, US 40SF Geotextile, US 32SF Geotextile.

Using the Electron Microscope Center at the University of South Carolina, I took images of the membranes where the biofilms developed. The first the cellulose membrane (muslin cloth) is fixed in 2.5% gluteraldehyde overnight at room temperature. The membrane was then washed 5 times in 0.1M of cacodylate buffer (7.2 pH). Post wash, in cacodylate buffer 1% osium tetroxide for 1 hour at 4°C. The membrane was then washed 3 times in 0.1M cacodylate buffer (7.2 pH). Next was the dehydration process, a series of ethanol washes starting with 50% for 10 minutes each then 70%, 80%, 95% and then 100% twice. The membrane was put in microporus vial and then put in a critical point dryer. The membrane samples were then mounted on a stub on the gold spatter and coated in gold. Membranes were then ready for scanning electron microscope.

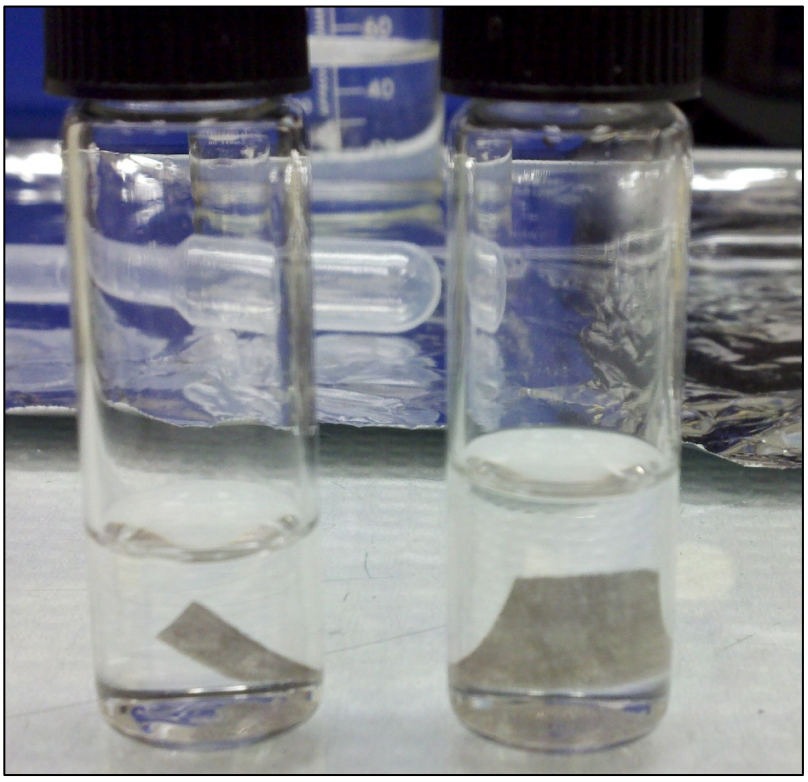

Figure 3.4 Scanning electron microscope preparation for membranes fixed in 2.5% gluteraldehyde.

3.4 First Generation Microbial Proton Exchange Membrane

After establishing that the muslin cloth would be used as our membrane, the next experiment was to establish a biofilm on the membrane using marine sediment. The experiment used a two-chambered fuel cell and created a BMFC. The anode side was filled marine sediment and cathode side artificial seawater. Separating the anode and cathode would be our selected muslin cloth membrane. Setting the up the cell as a BMFC would have the microbes grow on the membrane and conduct proton exchange. At the end of the experiment the membrane was analyzed for biofilm development and power production.

3.5 Methods and Materials

Marine sediment was collected from Charleston, South Carolina from the coastline at the Hollings Marine Laboratory. This sediment was collected using a shovel and stored in a five-gallon plastic bucket with a lid to seal the source material. The sediment was stored at room temperature in the sealed five-gallon bucket. 1 gallon of ocean water from the harbor was added to keep the marine sediment anaerobic.

250 ml two chamber fuel cells from Adams and Chittenden Scientific Glass were used as our MFCs. The anode chamber was filled to the top with marine sediment from Charleston, SC (See Figure 3.3). The marine sediment was homogenized before being added to the anode chamber. The cathode chamber was filled with artificial seawater and air was supplied into the chamber with an aquatic air pump. The two chamber fuel cells was separated by Nafion and muslin cloth (cellulose) membranes. Three sets of MFCs were set up in the incubator. Triplicate of Nafion 117 membrane, muslin cloth and an

heat-treated sediment with muslin cloth as the membrane. The heat-treated sediment was autoclaved and then cooled for 4 hours and this step was repeated three times to ensure all microbes in the sediment were eliminated. The MFCs were then setup and placed in an incubator at 30 degrees Celsius. A MAS-345 digital multi-meter was taking voltage readings every five minutes. The data was stored using DMM View Version 2 MAS-TEC software on a three Dell desktop computers. This allowed us to monitor the voltage record over time. The MFCs were broken down and the membranes were analyzed for biofilm development on the membranes.

Figure 3.5 Adams and Chittenden Scientific Glass 250 ml two-chamber MFC

3.6 Second Generation Microbial Proton Exchange Membrane

With the inconsistency and problems of the MFCs in first generation microbial proton exchange membrane, the design was rethought and the growth experiment reconfigured to try to eliminate inconsistencies in the experiment. A problem that

occurred in the first generation was the leaking of artificial seawater out of the cathode chamber.

3.7 Materials and Methods

Again using the same marine sediment that was collected for the first generation microbial proton exchange membrane was used for the second generation. 250 ml two chamber fuel cells from Adams and Chittenden Scientific Glass were used as our MFCs. The anode chamber was filled to the top with marine sediment from Charleston, SC. The marine sediment was homogenized before being added to the anode chamber. The cathode chamber was filled with artificial seawater and air was supplied into the chamber with an aquatic air pump. The two chamber fuel cells was separated by Nafion and muslin cloth (cellulose) membranes. Three sets of MFCs were set up in the incubator. Triplicate of Nafion 117 membrane, muslin cloth and an heat-treated sediment with muslin cloth as the membrane. The heat-treated sediment was autoclaved and then cooled for 4 hours and this step was repeated three times to ensure all microbes in the sediment were eliminated. The MFCs were then setup and placed in an incubator at 30 degrees Celsius. A MAS-345 digital multi-meter was taking voltage readings every five minutes. The data was stored using DMM View Version 2 MAS-TEC software on a three Dell desktop computers. This allowed us to monitor the voltage record over time.

The second-generation design was changed at the connection of the anode and cathode chamber to stop leaking of the artificial seawater. Two-inch rubber washers were used to create a seal between the anode and cathode chamber. The membranes were attached to the rubber washers using Gorilla Glue®. Using the two-inch rubber washers stopped the leaking that occurred in the first generation.

Another experimental design change was to slowly remove the sediment from the anode chamber of all the MFCs over time. Removing approximately 100 ml of sediment at two-week intervals and replacing the sediment with acetate media and keeping a record of the voltage. This removal is done until all the sediment is removed and the MFCs can be evaluated with the membranes operating with no sediment.

These MFCs will be the membranes used to complete DNA extraction, 16S rRNA amplification and from that complete a comparative community analysis of the membranes. This work is ongoing and the results are expected to come by the end of semester.

3.8 Results and Discussion

3.9 Alginate Growth Experiment Results

After comparison of the 2% and 4% alginate mixtures under the electron microscope, the results showed that the muslin cloth (cellulose) had a more fully developed biofilm versus the US Fabric 65. Figure 3.6 shows the comparison of 2% and 4% alginate mixtures with muslin cloth and Figure 3.7 shows the US Fabric 65. The US Fabric 65 does not have a well developed biofilm while the muslin cloth 4% alginate mixture has a well-defined biofilm. From this experiment, we decided to use the muslin cloth as our membrane for the microbial-based proton exchange membrane.

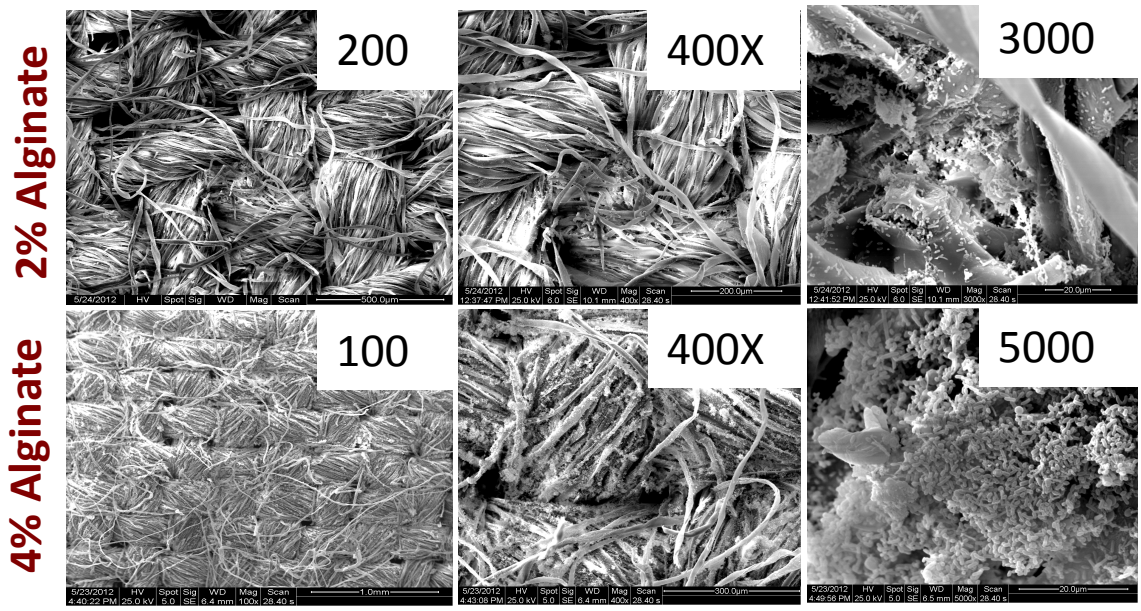

Figure 3.6 Comparison of the 2% and 4% Alginate growth experiment on muslin cloth (cellulose).

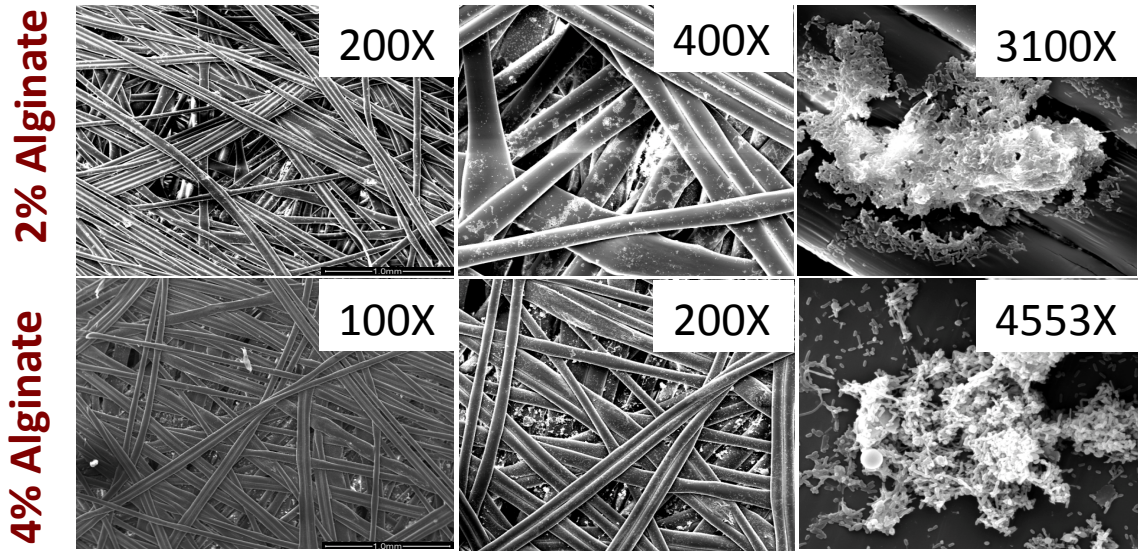

Figure 3.7 Comparison of the 2% and 4% Alginate growth experiment on US Fabric 65 (polypropylene).

3.10 First Generation Microbial Proton Exchange Membrane Results

The microbes were able to develop a well-defined biofilm on the cellulose membrane in comparison to the heat-treated and control cellulose membranes (See Figure 3.9). The cellulose membrane (CMFC 1) had a higher voltage output than Nafion during the growth experiment (See Figures 3.10, 3.11, 3.12). This suggests that the microbe based proton exchange membrane was more efficient and produced more power than the Nafion standard. But with the inconsistency of the other cellulose triplicate samples it is inconclusive to decisively state that the cellulose microbial proton exchange membranes worked more efficiently than the Nafion. A problem with the experiment was that the cathode chamber leaked in all of MFCs except the Nafion membranes. After this experiment, a second-generation microbial proton exchange membrane was designed to eliminate some of the inconsistencies and problem of the first generation.

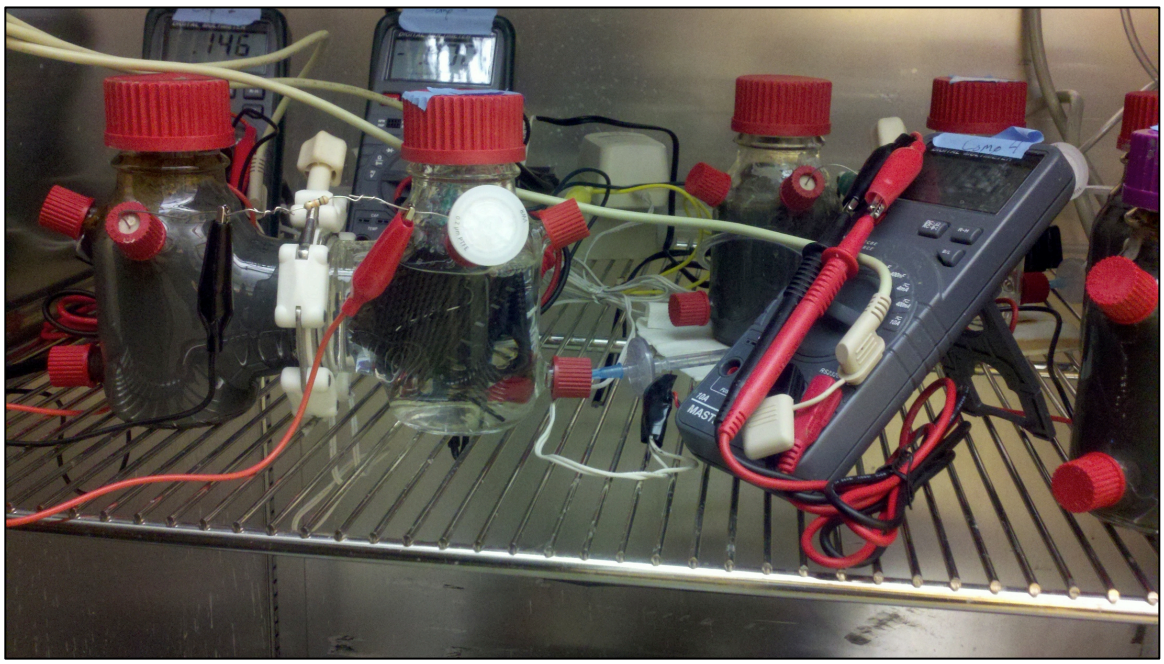

Figure 3.8 MFC growth experiment setup inside the incubator.

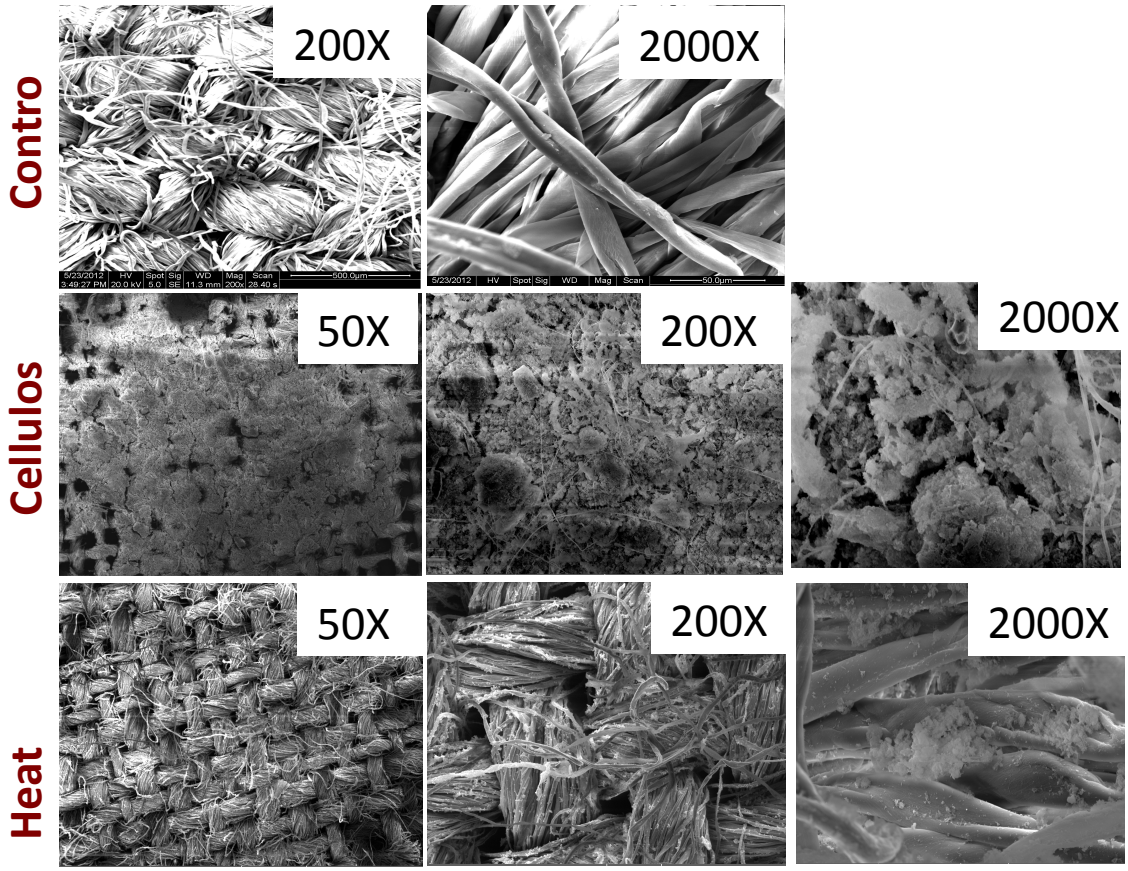

Figure 3.9 MFC growth experiment comparison of Control Cellulose (muslin cloth) membrane, Cellulose membrane and Heat Treated Cellulose membrane.

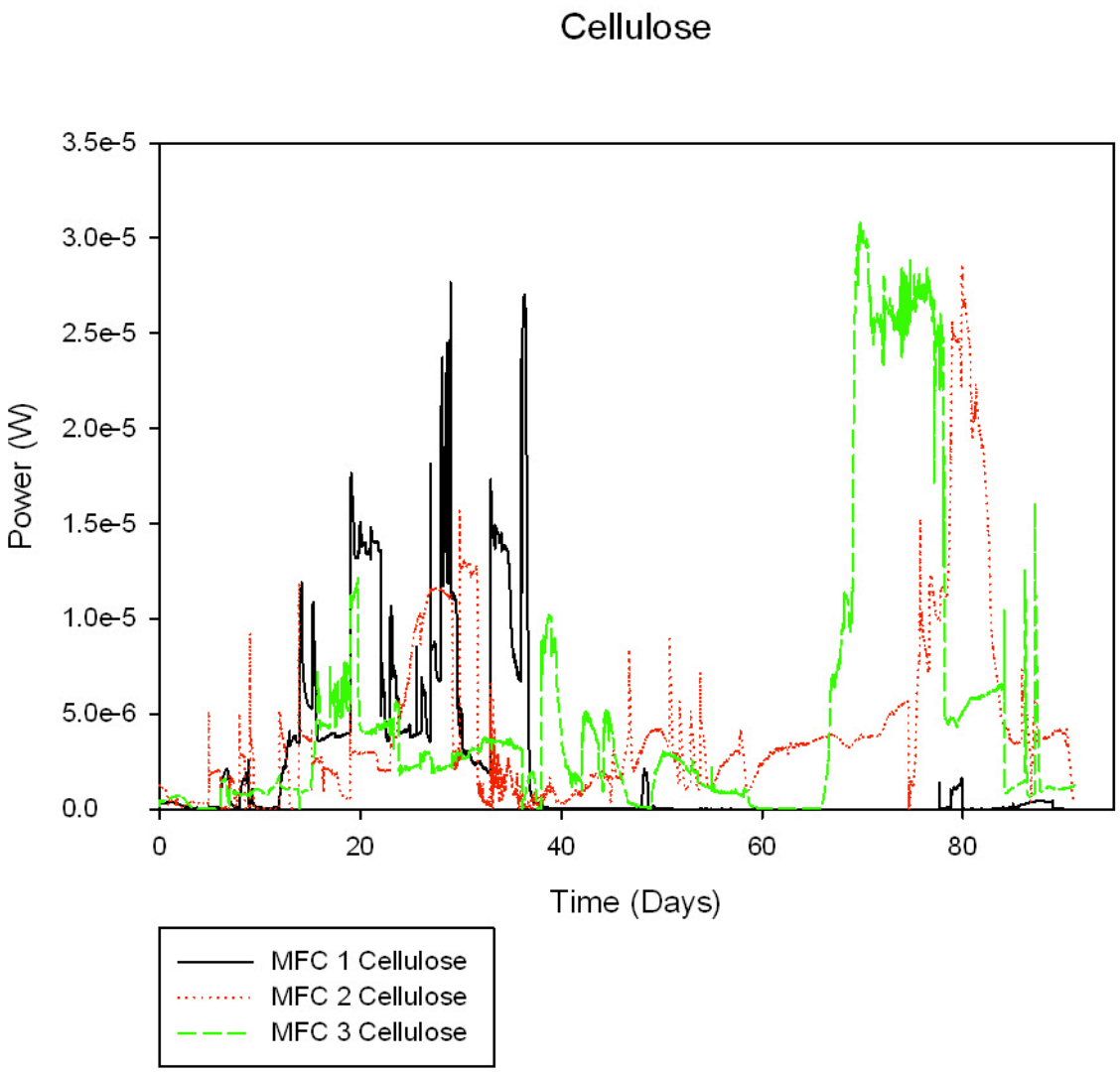

Figure 3.10 Record of voltage production during MFC growth experiment for Cellulose (muslin cloth) membrane.

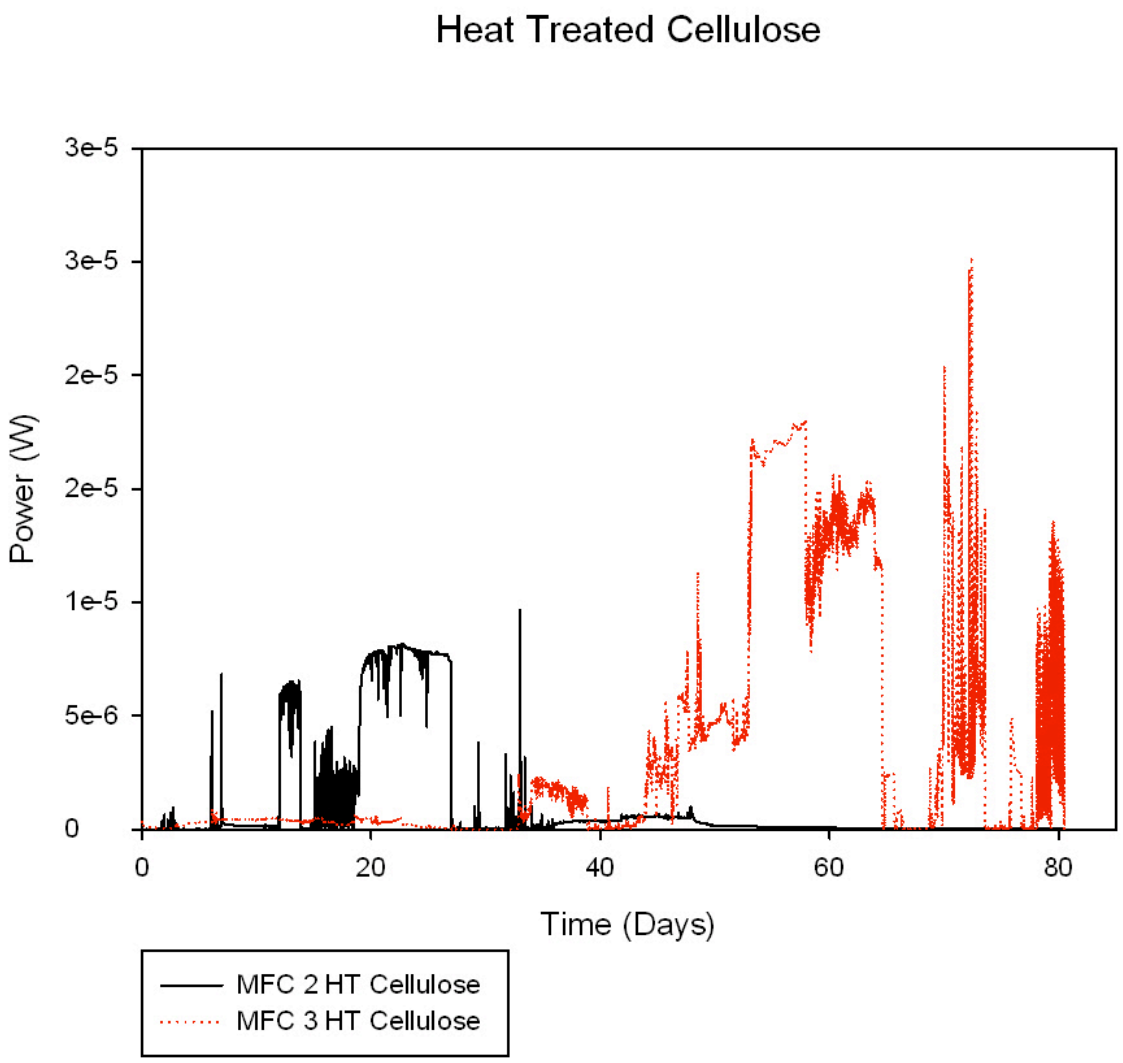

Figure 3.11 Record of voltage production during MFC growth experiment for Heat Treated Cellulose (muslin cloth) membrane.

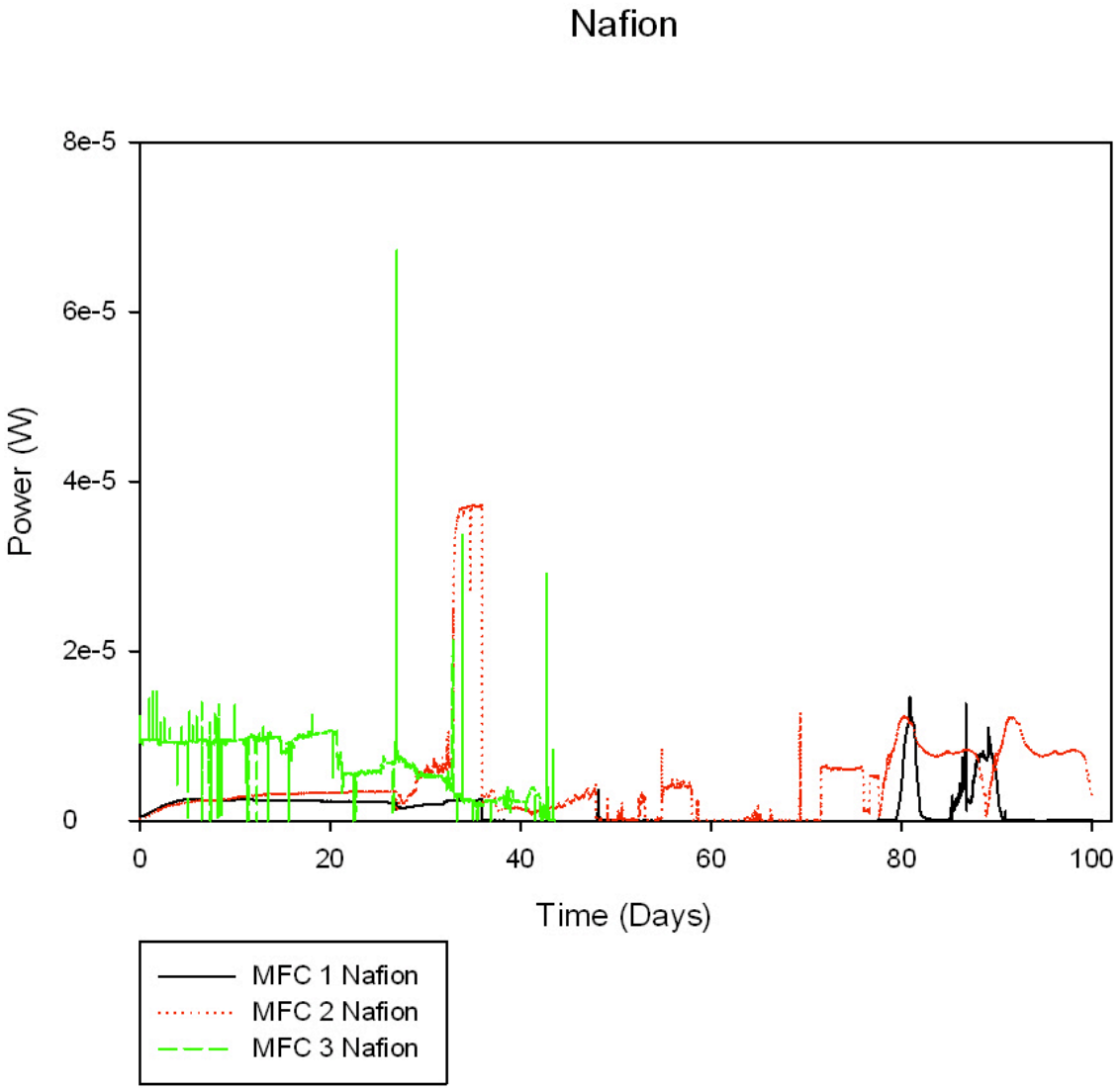

Figure 3.12 Record of voltage production during MFC growth experiment for Nafion membrane.

3.11 Second Generation Microbial Proton Exchange Membrane Results

This work is ongoing and the results are expected to come by the end of semester. As of right now the first sediment dilution has occurred and voltage is being recorded. These microbial fuel cells will be analyzed and their microbial evaluated to determine what microbes are on the membrane. This information will help lead us to determining

what microbes are involved in the proton exchange. Below are the voltage records for this ongoing experiment. Important to note, that the Nafion computer had a file corruption and only one set of data could be recovered.

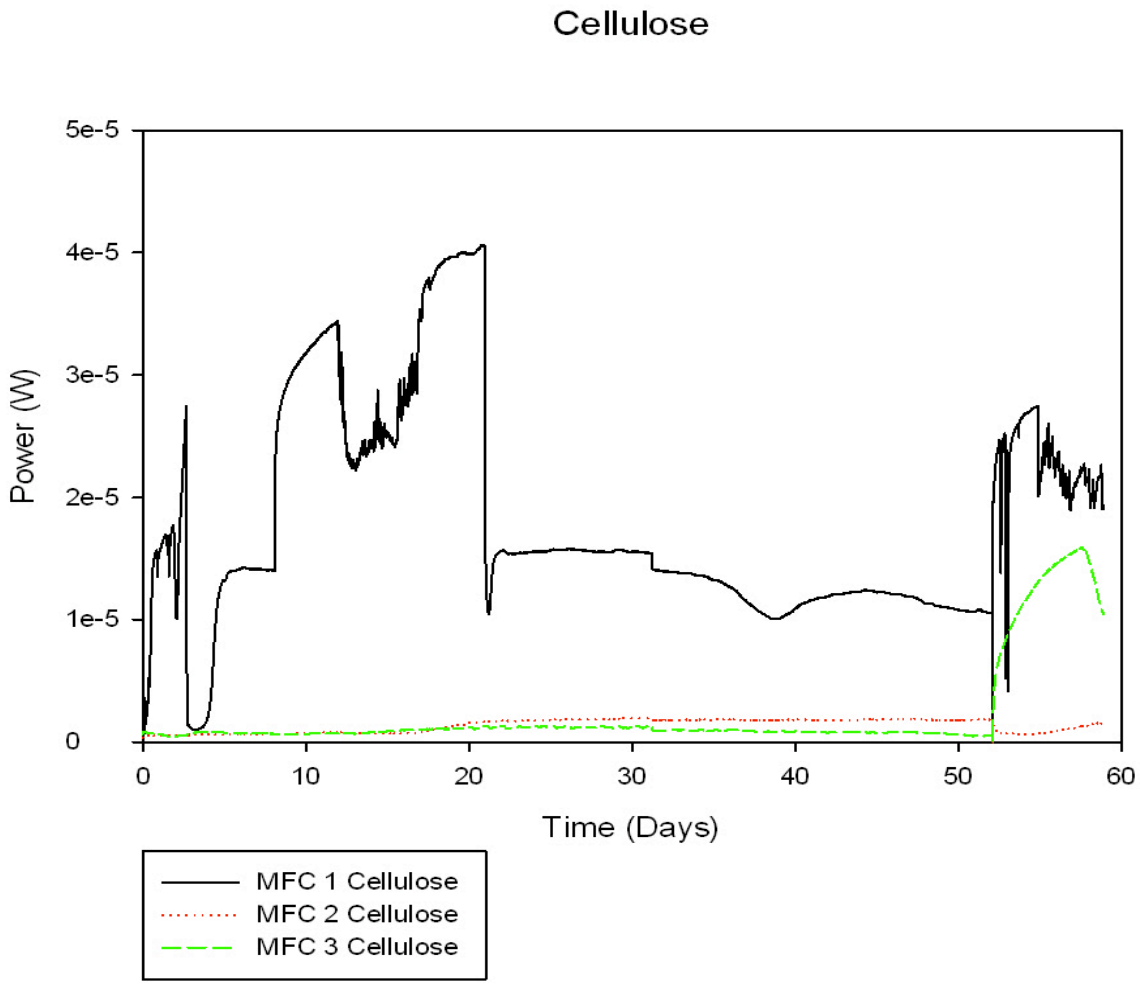

Figure 3.13 Record of voltage production during the Second Generation MFC growth experiment for Cellulose membrane.

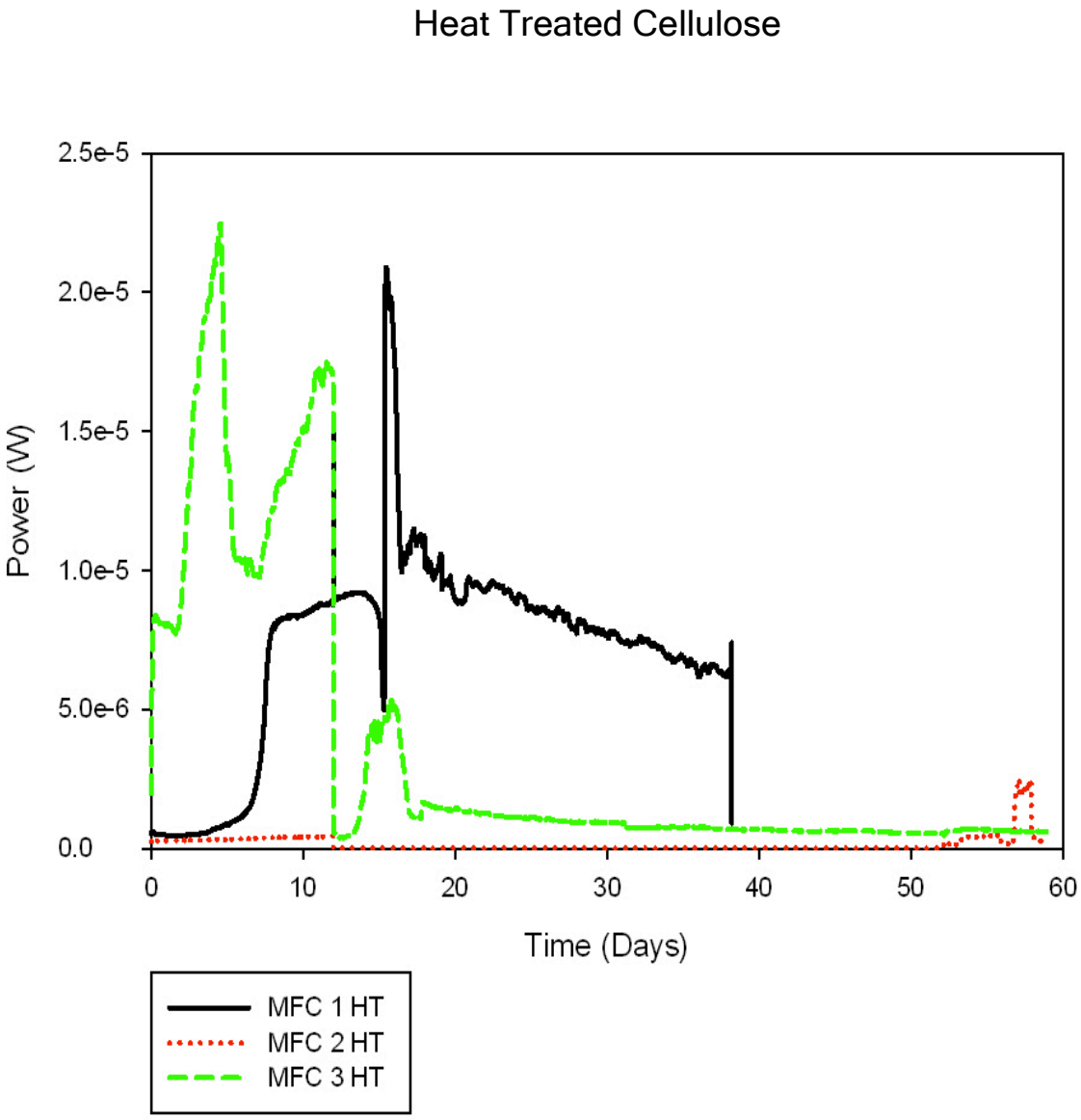

Figure 3.14 Record of voltage production during the Second Generation MFC growth experiment for Heat Treated Cellulose membrane.

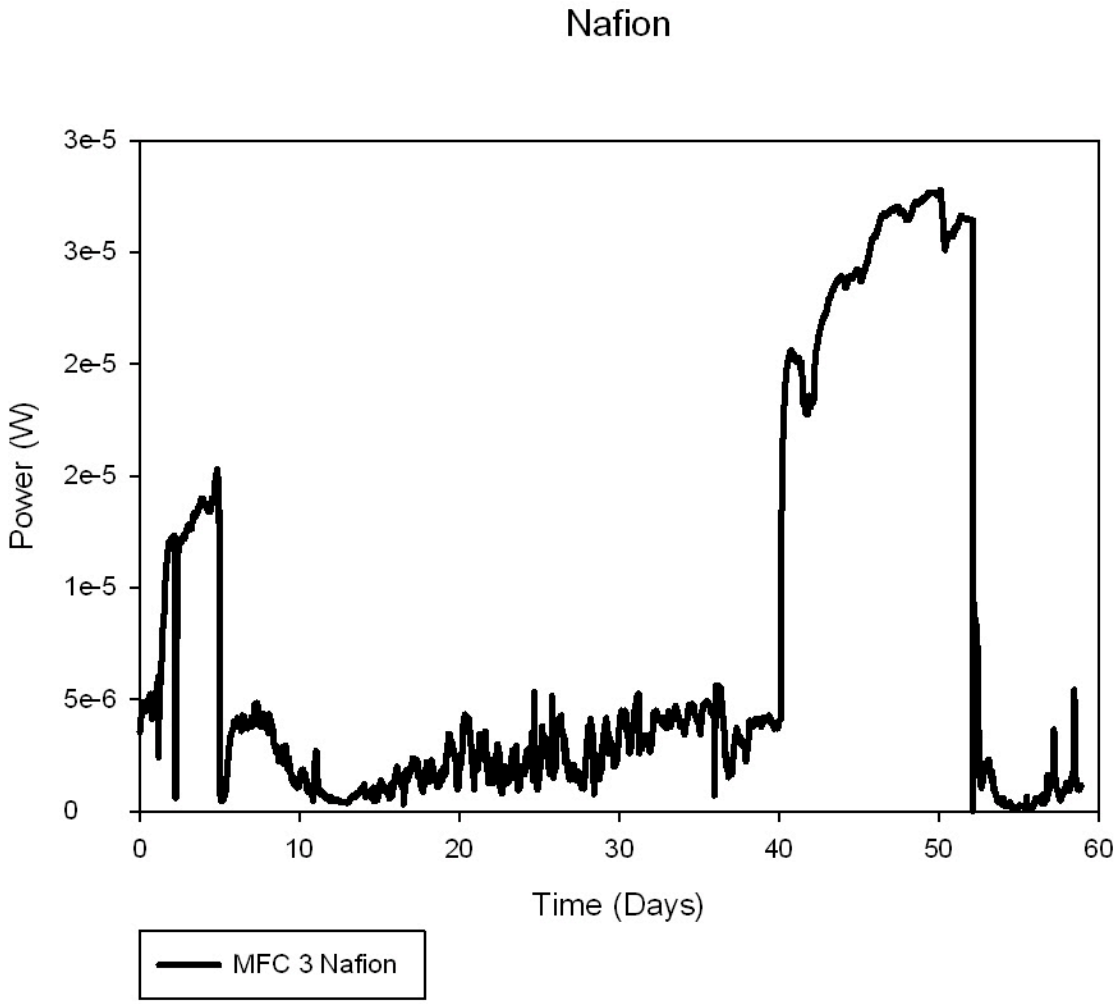

Figure 3.15 Record of voltage production during the Second Generation MFC growth experiment for Nafion membrane.

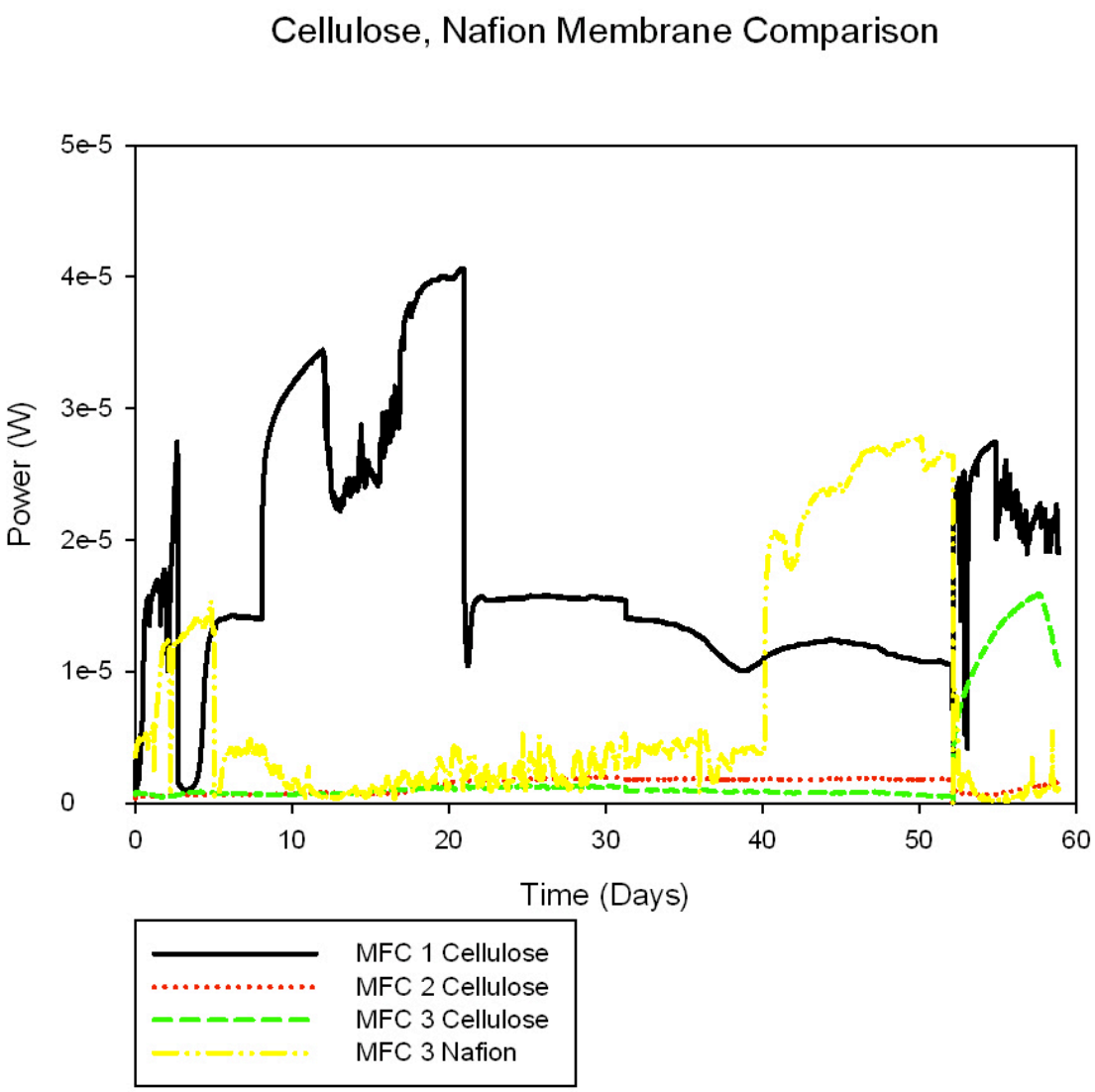

Figure 3.16 Comparison of Cellulose and Nafion membranes. Record of voltage production during the Second Generation MFC growth experiment.

CHAPTER 4

FUTURE METHODS

4.1 DNA Extraction

DNA extraction will be done to establish relative abundance of the microbes in the biological based PEM. The DNA from the biological based PEM will be extracted using the MO BIO Laboratories, Inc. PowerBiofilm™ DNA Isolation Kit. The PowerBiofilm™ DNA Isolation Kit is a unique kit that is designed for isolating high quality DNA from various kinds of biofilm samples. The use of beads in the sample tubes enhance lysis of biofilms. With this enhancement and the removal of proteins, humic substances, polyphenolics and polysaccharides the kit prepares high quality DNA. This is important for future methods that involve PCR and metagenomic analysis.

4.2 Amplification of 16S rRNA Genes

The 16S rRNA is a component of the 30S subunit of prokaryotic ribosomes. The 16S rRNA gene is used for phylogenetic studies, as it is highly conserved between different species of bacterium and archea (Woese et al., 1977). PCR primers are used to amplify the 16S rRNA gene. Weisburg et al. developed the most common universal primer pair, 27F and 1492R. For this study shorter primers will be used to amplify the V1 though V3 region (Weisburg et al., 1991). Those primers are 27F and 534R. These primers will target the conserved region and will amplify the V1 and V3 region for analysis of the microbial community.

4.3 Comparative Microbial Community Analysis

After the amplification of the targeted region of the 16S rRNA gene, the DNA will be sent for sequencing. With the sequencing data, we will use bioinformatics software and databases such as Green Genes to classify what microbes we have in relative abundance on our biological based PEM. It is important to note that with all the libraries of 16S rRNA genes that there is still an incomplete picture all the bacteria in the world and the sequencing may give us an unknown during the classification of the DNA.

CHAPTER 5

EXPECTED RESULTS

Since this work is on going until the end of the semester, a comparative microbial community analysis will be completed on the microbial-based proton exchange membranes. This analysis will inform us what microbes are present in relative abundance using the 16S rRNA gene. Online data bases of microbial 16S rRNA genes will inform what microbes are known and not known in our microbe-based proton exchange membranes. From these results, we can isolate this microbe or microbes and build a working prototype of a microbe-based proton exchange membrane for MFCs. Creating pure culture MFCs with 1) known microbes that breakdown carbon and transfer electrons, such as *Shewanlla* and *Geobacter*, and 2) known microbes used as the proton exchange membrane. These MFCs have the potential for high energy densities with the ability to operate with high proton permeability in the complex ionic media of MFCs. This potential for increasing energy output would change the way MFCs are used and regarded as an alternative energy source. Without the need for Nafion as a PEM, ultimately the price to build a MFC goes down drastically. The ability to create cost effective renewable energy not only helps solve world energy problems but also environmental pollution, since MFCs are carbon neutral.

The future of the MFCs is very bight and hopeful. The current practical applications powered by MFCs are meteorological buoys. These buoys measure water

temperature, relative humidity, pressure and air temperature. These buoys are BMFCs that allow them to operate uninterrupted. This is small scale of the potential for MFCs. The problem with MFCs is scaling up. With the high cost of Nafion and experiments that show slow rates of substrate degradation, scaling up the MFC has not been proven to be a viable alternative energy source as of yet.

Another gap in the advancement of MFCs is the small number of microbes that have been studied on how they donate electrons to electrodes and less is understood how electrons transfer from electrodes to cells. Development in this area will help the progress of scaling up MFCs. Understanding the basic mechanisms of the microbe electron transfer may help with scaling up to alternative energy source.

Overall the technology for MFCs to become a viable alternative energy source is still fifteen to twenty years away. With more funding and researchers studying microbes, electrodes and proton exchange membranes there is more likely a change that MFCs will be apart of the energy discussion in the future.

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US Dept of Energy, Genomic Science Program (Image; Figure 2)

APPENDIX A: ACETATE MEDIA

The following is the recipe used toe make the acetate media used in the sediment removal growth experiments.

Deionized Water	800 ml
Sodium Bicarbonate	2.5 g
Ammonium Chloride	0.25 g
Sodium Phosphate Monobasic	0.6 g
Potassium Chloride	0.1 g
Vitamin Mix	10 ml
Mineral Mix	10 ml
Sea Salts	30 g

APPENDIX B: VITAMIN MIX

The following is the recipe for making the vitamin mix used in the acetate media.

Deionized Water	800 ml
Biotin	0.005 g
Pantothenic Acid	0.0001 g
B-12	0.005 g
P-Aminobenzoic Acid	0.005 g
Thioctic Acid	0.005 g
Nicotinic Acid	0.005 g
Thiamine	0.005 g
Riboflavin	0.005 g
Pyrodoxine HCL	0.01 g
Folic Acid	0.002 g

Dissolve in the above order and bring final volume to 1 liter using deionized water (Lovely et al., 1988).

APPENDIX C: MINERAL MIX

The following is the recipe for making the mineral mix used in the acetate media.

Deionized Water	800 ml
NTA Trisodium Salt	1.5 g
Magnesium Sulfate Anhydrous	3.0 g
Manganese (II) Sulfate Monohydrate	0.5 g
Sodium Chloride	1.0 g
Iron (II) Sulfate Heptahydrate	0.1 g
Calcium Chloride	0.1 g
Cobalt (II) Chloride Heptahydrate	0.1 g
Zinc Chloride	0.13 g
Copper (II) Sulfate Pentahydrate	0.01 g
Aluminum Potassium Sulfate Dodecahyrate	0.01 g
Boric Acid	0.01 g
Sodium Molybdate Dihydrate	0.025 g
Nickel (II) Chloride Hexahydrate	0.024 g
Sodium Tungstate Dihydrate	0.025 g

Dissolve in the above order and bring final volume to 1 liter with deionized water (Lovely et al., 1988).