ENZYME-ENHANCED MICROBIAL FUEL CELLS

by

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ABSTRACT

Microbial fuel cells utilize the electrons released during the oxidation of organic material by microbes to generate electricity. The electrons may be transferred to an electrode surface by redox mediators or directly by the microbes residing in the fuel source. Wastewater sludge was the source of organic material for the reactors operated during this project. Two phases of experiments were conducted to determine conditions for efficiently operating microbial fuel cells powered by waste biosolids. The first phase focused on finding a combination of supplemental nutrients and enzyme to sustain a useable electric current within the fuel cells. The second phase focused on identifying an enzyme solution dose to enhance fuel cell performance.

In Phase I, after applying a variety of supplemental nutrient solutions to the reactors, it appeared that tryptic soy broth provides a sufficient balance of carbon, nitrogen, and phosphorus to sustain microbial growth. The reactors which received a supplemental nutrient source had more consistent rates of electrical output than the reactor that received only the enzyme treatment. In Phase II, it was shown that the enzyme treatment enhances fuel cell performance. The optimal dose appears to be between 20 and 40 mg daily of EZ 216, which is a commercial mixture of enzymes. The results of both sets of experiments support the hypothesis that microbial fuel cells can be operated efficiently with waste biosolids. In a batch system, the supplemental nutrient source is necessary to sustain microbial growth, which can be further enhanced by the addition of an enzyme.

Chapter 1

INTRODUCTION

One of the consequences of a rapidly expanding global population is the increased generation of human waste. The treatment and safe disposal of wastewater is a growing issue not only from an economic standpoint, but from a health and environmental standpoint. While there are a few "next generation" treatment facilities being built around the world, the majority of the wastewater generated by municipalities and industries continues to be treated by outdated and inefficient techniques, which are high in monetary and energy costs. In the United States, approximately 1.5% of electricity used goes to wastewater treatment. The annual cost for wastewater treatment is approximately $25 billion, and it is expected that this number will increase significantly over the next twenty years (14).

Recycle and reuse procedures are becoming more common, but many people remain hesitant to utilize treated wastewater for more diverse uses such as agricultural irrigation. A new technology, however, presents a reuse option for wastewater and wastewater sludge that may become more widely accepted. Digested sludge contains many microorganisms that have the capability of contributing to energy conservation practices. In a system called a *microbial fuel cell*, microbes generate electricity from the sludge in which they reside.

1.1 Definition of the Microbial Fuel Cell

Fuel cells are generally thought to be more efficient than combustion engines because of their capacity to directly oxidize and reduce compounds at electrode surfaces. A common example is the hydrogen fuel cell. Hydrogen is oxidized at the anode surface and the electrons are transferred to the cathode, where they are used to reduce molecular oxygen. This highly efficient process is used to generate electricity (2). Microbial fuel cells (MFCs) are defined as devices which use bacterial respiratory processes to generate an electrical current from an organic substrate. Microorganisms in the fuel cell oxidize organic substrates and transfer electrons to an electrode surface (6). Before further details about electricity generation within the fuel cell can be given, it is important that the basic microbiological principles associated with microbial metabolism are understood.

When organisms metabolize organic material to gain energy, they are essentially collecting the energy released during oxidation of organic substances, such as carbohydrates. The basic reaction for aerobic respiration using organic electron donors is:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad [1]$$

Equation 1 is the net reaction of many enzymatic reaction steps, where $C_6H_{12}O_6$ represents a typical organic substrate. In typical microbial catabolism, an organic substrate is oxidized when its electrons are released by these enzymatic reactions. The electrons are first stored in chemical intermediates which are then oxidized themselves to provide fuel for additional reactions, ultimately providing energy for maintenance and growth of the living cells. Using glucose again as an example, the basic reactions for this process are shown by equations 2 and 3, which yield the same products as equation 1 when combined.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad [2]$$

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \quad [3]$$

The main three components of an MFC are the anode, cathode, and, if included, the membrane (14). In the anodic compartment, microorganisms oxidize organic substances, releasing electrons and protons (the H^+ ion). The electrons are transported to an electrode, which becomes negatively charged, and functions as the *anode*. An electric potential develops when the electrons are transferred to the electrode. The protons and electrons are then transferred to the cathodic compartment, the protons through the membrane barrier and the electrons via an external circuit. At the cathode, which functions as a terminal electron acceptor, the protons and electrons react with oxygen to yield water. This process also causes the development of an electric potential. The difference in potential between the anodic and cathodic zones together with the flow of electrons through the system generates electricity within the fuel cell (8, 13, 15, 16). Figure 1.1 shows a schematic of the components of a typical MFC and the flow of electrons within the system that are necessary to generate a current.

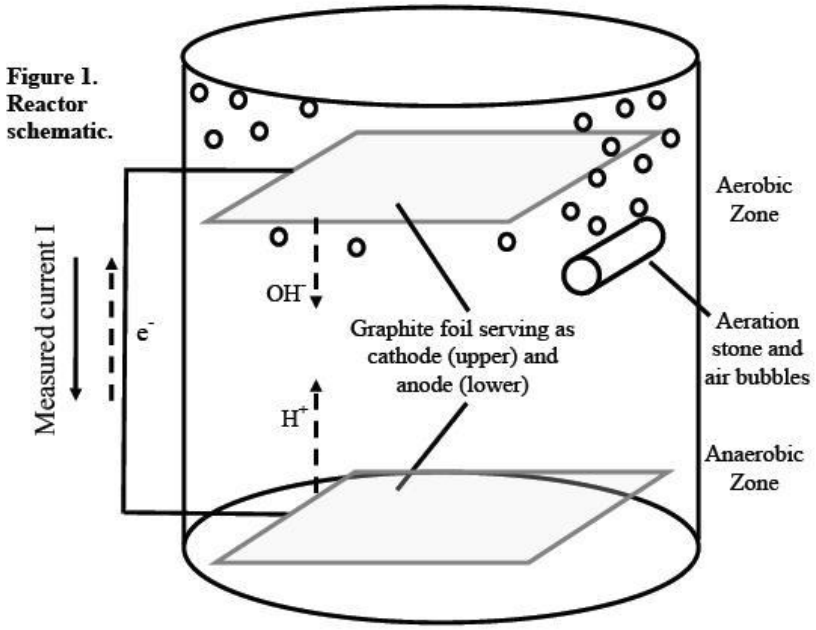

Figure 1.1: Schematic of typical MFC components. This figure was originally published in "Direct Generation of Electricity from Sludges and Other Liquid Wastes," by Dentel, Strogen, et al., 2003 (4).

When the supply of organic substrate is not replenished in the system, the microbes will eventually run out of material that they can oxidize to gain energy and the flow of electrons will stop. In this sense, MFCs can be thought of as simple galvanic cells, or batteries. However, current can be restored and maintained with a continuous supply of substrate, making the system "rechargeable." This is closer to the true definition of a fuel cell because the device is capable of producing electricity for long periods of time (1). Rabaey et al. (2003) describe MFCs as "mimics" of biological systems where bacteria do not transfer their acquired electrons directly to a typical electron acceptor. Instead, the transport process includes an anode, an applied

resistance, and a cathode, all of which together allow for the bacterial energy to be converted into electricity (19).

1.2 History of Microbial Fuel Cells

Due to the potential for energy conservation, there has been in increasing interest in this field over the last decade (6). However, the seminal research conducted to study microbial fuel cells was completed well before the start of the twenty-first century. The first recorded instance of MFC use was in 1910 by Potter, who used *Escherichia coli* and *Saccharomyces* to generate electrical energy with platinum electrodes (5). Cohen used this research in 1931 to support information about the enzymatic processes bacteria use to oxidize organic material (7).

In 1967, a patent was approved by the United States Patent Office for a Biochemical Fuel Cell. The patent was awarded to John B. Davis and Henry F. Yarbrough of the Mobil Oil Corporation, and considers the generation of electrical power by microorganisms and organic fuel. The main objective of the research was to introduce a sustainable method for use of MFCs. Scientists also looked to operate fuel cells using microbial species and organic material that normally do not generate electricity outside of the fuel cell setting (22).

Renewed interest in MFCs sparked in the 1980s when it was discovered that current density output could be greatly improved by the addition of an electron mediator (5). Another patent was awarded in November 1999 to Kim and co-workers for their invention of a biofuel cell that can react with an electrode without a mediator. They were able to show that certain microorganisms can consume electrons generated through metabolism directly, thereby reducing sludge production and increasing the efficiency of the digestion of organic materials (23).

Historically, marine sediments have been commonly used as the fuel source in microbial fuel cells. An electrical potential exists between the sediment and the water above it due to the different redox environments. The marine sediments are reduced, while the water column is oxidized (4). The first practical application of MFCs was reported in 2008. Buoys built to measure air temperature, pressure, relative humidity, and water temperature and transfer the data immediately by radio frequency were powered solely by benthic MFCs. The system does not require any added microorganisms or mediators because of the naturally occurring microorganisms in the sediment. There are many other proposed methods for the use and enhancement of MFCs, but limiting factors such as economic cost and energy efficiency must be improved before this technology can be implemented for broader purposes (6).

1.3 Theories on Microbial Fuel Cell Construction

When selecting materials and the architecture of an MFC, it may be useful to consider the construction of other biofilm bioreactors, such as trickling filters (TF). The biofilm in an MFC grows at the electrode surface, not at the biofilm-water interface as in a TF, so issues like clogging may not be as significant. However, the bacteria must be able to grow and make new cells, so electrode materials with small pore sizes may not be appropriate for MFCs (14). Some of the requirements for the anode include high conductivity, minimal corrosivity, high specific surface area, high porosity, and low vulnerability to fouling. It is also important that the material be inexpensive and easily made to be scaled to larger sizes. It is common to use carbon-based electrodes in the form of paper, cloth, or foam because of their high conductivity and acceptable properties for bacterial growth (14).

In hydrogen fuel cells, membranes are a necessary component of the system because they separate the H_2 and O_2 and provide a means for conducting protons between the gases. In other two-chambered MFCs, the membrane is used as a way to separate the anode and cathode liquids. If substrate is leaked from the anodic zone into the cathodic zone, inactivation of the cathode catalyst may occur. However, membranes are not always necessary in MFCs, as water conducts protons. Some research has shown that MFCs without membranes produce more power than MFCs with membranes bonded to the cathode (14).

The same materials that are used to construct the anode can be used to construct the cathode. Carbon-based materials are the most common. It is important that the design of the cathode allow for the tri-phase reaction (solid catalyst, air, and water) to occur. The electrons, protons, and oxygen must all react at the catalyst, which must be on a conductive surface and exposed to both water and air (14). There are many different configurations that are acceptable for MFC construction, as can be seen in other existing literature. It is important to consider the long-term stability of the materials selected, as MFC experiments are run for periods of time ranging from a few days to several years.

1.4 Microbial Activity within the Fuel Cell

There are several mechanisms for the production of electric current in an MFC. Bacteria living in the sediment will oxidize the organic material and use the electrode as an electron acceptor. The first mechanism is that the electrode functions as an electron "drop-off station" for reducible ions, a process which would energetically favor the bacteria. Reduced inorganic species such as Fe(II) or reduced quinones and other structurally-similar organics can release electrons to the anode through oxidative

half-reactions. These species may then function as redox mediators and shuttle electrons between the microbes and the anode. Second, certain bacterial species are capable of physically attaching to the electrode surface and can transfer electrons directly without use of a mediator. Direct deposition of electrons on the electrode by bacteria would indicate that the electrode couples the bacteria's metabolism with the reduction of oxygen to achieve a more energetically favorable process (4, 21).

Regardless of the method, the fact that current is detected within MFCs indicates that electron flow within the cell is energetically favorable (4, 21). Coulombic efficiency is a common measure of MFC efficiency. The measured number of coulombs measured as electrical current is compared to the theoretical maximum number of coulombs obtainable from the organic substrate in the system. Coulombic efficiency is partly dependent on the microorganisms in the system, as different microorganisms metabolize organic material at different rates and by different pathways (6).

1.4.1 Redox Mediators

When oxygen is not available in the system, electrons can be released with the help of an oxidation-reduction, or *redox*, mediator (1). A redox mediator works to accelerate the transfer of electrons from the microbe to the electrode. Most microbes have a non-conductive membrane which mediators have to move across to capture and release the electrons to the anode (5). The mediator enters the outer cell membrane, is reduced, and exits the cell in the reduced state (1). The reduced mediator diffuses to the electrode surface, where it is electrocatalytically oxidized. The oxidized mediator is now free to return to the cell and repeat the cycle (7). Du et al. (2008) have classified "good" mediators as those which are able to diffuse across the cell

membrane easily, can capture electrons from the electron transport chains within the cell, and have a high electrode reaction rate (5).

Another important feature of mediators is how efficiently they can be reduced by the cells they enter. Park et al. state that electron mediators used in MFCs should have a high negative $E°$ in order to maximize electricity generation (17, 18). A mediator with the lowest redox potential, theoretically providing the largest redox potential difference between the anode and cathode, may not necessarily be the most efficient at extracting electrons from the cell. A mediator with a higher redox potential will yield a higher overall electrical output rate than a mediator with the lowest redox potential (5, 7). It is important to note that the electron exchange and high current densities observed in MFCs are artificial and will not occur naturally (6, 7). One type of mediator that has been proposed for use in MFCs is Neutral Red. It was reported that Neutral Red (NR) can increase electricity production by up to ten times as compared to other electron mediators used in MFCs (17, 18). The study by Park et al. (2000) showed that electricity generation efficiency is proportional to the NR concentration in the system, bacterial density, and contact rates of the bacteria to the electrode surface (17).

Many redox proteins are electrochemically active, but Kim et al. (1999) state that direct electron transfer between the proteins and an electrode is not always possible because of the folded structure of the molecules. Special positioning of the proteins on the electrode surface is necessary to make sure the electrochemical reaction occurs. They have also indicated that bacterial cells containing these proteins attached to the electrode surface will be electrochemically inactive because of the non-

conductive cell wall that surrounds the cell. The mediators are used to carry the electrons between the cells and the electrode (9, 11).

1.4.2 Theory of Mediator-less MFCs

Lee et al. (2003) define a mediator-less microbial fuel cell as "a device that converts chemical energy to electrical energy with the aid of the catalytic reaction of electrochemically active microorganisms." Electron mediators are not necessary because the microorganisms are capable of transferring the electrons to the electrode directly (12). More specifically, some iron-reducing species have respiratory enzymes spanning their outer membranes that allow for the direct transfer of electrons to external metals such as Fe(III) and Mn(IV). When these species are used in MFCs and attached to the electrode surfaces, the electron transfer is directly to the anode. The measured equilibrium potentials are comparable to those of purified cytochrome samples used by the respiratory chains of these bacteria, indicating that the enzymes are used to complete the electron transfer to the electrode (2, 13).

An example of such a species is *Shewanella putrefaciens*, which is a Fe(III)-reducing bacterium. Park and Zeikus (2000) measured a maximum power density of 10.2 mW/m^2 using a manganese-graphite electrode with lactate as a substrate (10, 12, 14). The direct transfer of electrons by this species is believed to be possible because of the electron carriers, which may be cytochromes, on the outer membrane of the cells. Kim et al. (2002) found that the current generation by the MFC was dependent on the concentration of bacteria within the system and on the surface area of the anode (11).

Another example of a microbe that conserves energy by completely oxidizing organic compounds through direct transfer of the electrons to the MFC anode is

Geobacter sulfurreducens. This species, along with *S. putrefaciens*, is classified as an *exoelectrogen*, "exo-" meaning exocellular and "electrogen" implying that the organism is capable of directly transferring electrons to a chemical or material that is not the immediate electron acceptor (14). Exoelectrogens also have high coulombic efficiency and long-term stability attributed to the conservation of energy for maintenance and growth purposes. The direct transfer of electrons to the anode surface by the bacteria eliminates the need for mediators (6). Acetate is very common is anaerobic environments and can be used as an electron donor by anaerobic respiratory bacteria. While methanogens and sulfidogens are the more recognizable acetate-oxidizing anaerobes, metal reducers in the *Geobacter* genus can also use acetate effectively (12). Bond and Lovely have shown that *G. sulfurreducens* can produce a significant power density (49 mW/m^2) from acetate degradation in a two-chamber MFC (14). In this instance, power density is the time rate of energy transfer per unit surface area of the anode. They have also reported the formation of a monolayer biofilm on the electrode surface (2, 7).

Several factors have been identified as limiting for mediator-less MFCs. The oxidation of organic material and electron transfer rates by the microbes to the anode, as well as the resistance of the circuit, proton transfer rates, and oxygen reduction at the cathode are all factors which affect the efficiency of an MFC (8). There are very few exoelectrogens that have been isolated from MFCs. Currently, an isolate has not been identified that is capable of producing power densities equal to those produced by mixed cultures under the same conditions. However, the internal resistance of the MFCs used by many researchers when studying pure cultures may not reflect the true power generation potential of the bacterial strain. Therefore, it is uncertain whether

isolates indeed have the capacity to generate high levels of power or whether the cells must be grown in the presence of other bacteria to achieve high power density (14).

When examining MFC biofilms at the microscopic level, it does not appear that any one particular species dominates the others that develop on the anode surface. One likely explanation is that several different species of bacteria have been used to generate electricity. There are other varying factors such as operating conditions, system architectures, and electron donors and acceptors that may contribute to the species diversity observed. It is also possible that certain parts of the community are sustained by metabolic processes that do not generate electricity, such as fermentation and methanogenesis (16). However, the information available about the composition of bacterial communities that can be sustained in MFCs is sparse. The effects of MFC architecture and more complete data characterizing electrochemically active bacteria are just a few areas of knowledge that need to be expanded to understand how these communities contribute to power generation (14).

1.5 Research Objectives

In the United States, aerobic treatment technologies are the primary type of wastewater treatment. Anaerobic treatment technologies are being used more often, but the diluteness of the wastewaters often makes these techniques economically impractical (15). Anaerobic digestion of primary wastewater sludge involves converting the organic material contained in the solids to methane and carbon dioxide. Roman et al. (2006) have reported that enzyme additives, specifically cellulase and pronase E, enhance digester performance in terms of COD degradation, reduction in solids, and pH stability (20). Microbial fuel cells are a new approach to wastewater

treatment because energy can be captured during the process, instead of being a total drain on electrical energy (14).

Liu et al. (2004) reported some of the first research conducted to examine electricity generation using domestic wastewater in MFCs. Prior to that, MFC research was primarily focused on small batch-fed systems with very specific substrates and MFCs used to collect energy from marine sediments. Liu et al. found that electricity can be generated in a single-chamber MFC using wastewater as the substrate. However, in terms of the treatment of the wastewater, they found that the majority of the organic material in the wastewater was removed by processes that did not generate electricity (13).

The microbial fuel cell can be considered an anaerobic treatment method for wastewater because of the microbial activity that occurs to generate electricity in the absence of oxygen. However, because oxygen is used in the cathodic zone, MFCs must also be considered aerobic, though microbial respiration does not require oxygen. When oxygen is leaked into the anodic zone, the bacteria will consume it quickly to maintain a low redox potential (15).

Using MFCs in wastewater treatment is desirable because of the potential for energy recovery and reduction of excess sludge production. It is predicted that MFCs generate less excess sludge than typical activated sludge processes because the majority of energy available in the system is either converted to electricity, with a much smaller proportion used for microbial growth (8). Currently, the primary goal of MFC research is to develop a scalable technology for the treatment of various types of wastewater. The energy that could be captured by a full-scale system may be large enough to power a treatment facility, potentially leading to energy sustainability of the

water infrastructure. Energy savings may also result in decreased operating requirements for aeration and solids handling systems (14).

To date, MFC research has focused on use of wastewater as the source of organics to oxidize. MFCs utilize anaerobic biodegradation, leaving problematic ammonia, sulfides, and other reduced substances in the wastewater, which could be considered counter-productive in terms of the effectiveness of treatment. Therefore, biosolids may be a more suitable nutrient source for MFCs, because electricity will be generated. The purpose of this project was to study the effects of an enzyme treatment on the generation of electricity due to bacterial activity in wastewater sludge. It is hypothesized that MFCs can be equally effective for treating waste biosolids as for wastewater. It is also hypothesized that the enzyme treatment will improve bacterial growth within the MFCs, resulting in higher electrical generation rates. This hypothesis is proposed because in sludge treatment, the initial step of breaking down, or *hydrolyzing*, complex organics can be rate limiting. The enzyme may accelerate the rate limiting step during the breakdown of organic molecules by the microbes, resulting in higher electrical output. Experiments first focused on operating an MFC using biosolids from an anaerobic digester, monitoring performance as the experimental control. Experiments were then conducted to observe the effects of applying an enzyme treatment to additional MFCs, comparing performance to that of the control, or no-enzyme, unit. In the following chapters, the methods used to conduct two phases of experiments will be detailed. The data obtained during these experiments will be provided, along with an interpretation of the results and conclusions regarding the hypotheses made.

Chapter 2

METHODS

The experiments conducted for this study were divided into two phases. Phase I was conducted in two parts, the major part of study occurring during the first 72-day period of reactor operation. In total, the Phase I reactors were operated for 108 days, in January through April 2011. Phase II was conducted over a 64-day period in September through November 2011. It is important to note that the methods for reactor assembly were identical in Phase I and Phase II. Variations in other parts of the methodology will be given, as appropriate. The following sections discuss the physical setup for experimentation, the methods used for preparation and application of nutrient and enzyme solutions, and the procedure for daily data collection. Details have also been provided about SEM imaging that was performed on the anodes used during Phase II of this project.

2.1 Reactor Assembly

The reactor vessels for these experiments were 2-gallon aquaria. Four reactors in total were used, each identical in size, shape, and material. The tanks were hexagonal in shape, and approximately 16 inches tall. They were made from a somewhat flexible, clear plastic, very strong in structure. Each reactor was thoroughly cleaned with a mild soap and warm water before assembly to remove any dust and dirt lingering from previous use.

The anodes and cathodes for each tank were cut from a roll of soft graphite. The anodes were each 100 cm^2, 10 by 10 cm. The cathodes were 50 cm^2, 5 by 10 cm. The decision to make the cathodes exactly half the size of the anodes was supported by Strogen's research. He stated that this size ratio would ensure that the cathode area would not limit the current production in the reactors (4, 21). Single-strand copper wires were threaded through each electrode to provide multiple contact points. Two pin-sized holes, spaced approximately 1 cm apart, were made to thread the wire through. The wires were then epoxied into place on the graphite electrodes to prevent damage or separation during experimentation. The wires were measured and cut so that the ends used for current measurement would rest outside of the reactors to limit liquid-induced corrosion and be easily accessible.

Plastic sample tubes (1.5 mL) were used as supports for the anodes. The tubes were adhered to a Petri dish in a triangle pattern. They were cut so that the capped ends could be glued directly to the dish and the cut open ends would provide a flat surface to support the graphite electrodes. Waterproof silicone glue, similar to that which might be used for weather-proofing purposes on a house, was used to attach the tubes to the dish, and then the anodes to the supports. The entire structure was then firmly attached to the bottom of the tank with the same silicone glue. The tubes were cut so that the anode would be elevated approximately one inch above the bottom of the tank. The wires attached to the anodes were brought up and out of the tanks to be accessible for current measurement. For the cathodes, two thin, non-conductive plastic rods were used to support the cathodes across the width of each tank. To determine the height at which each cathode was placed, the tanks were filled with 5 L of water and the supports were placed exactly one inch below the water surface level.

Because the dimensions of the reactors were constant, this measurement confirmed that each cathode would be placed at the same elevation in each reactor. The silicone glue was also used to attach the rods to the reactor walls. The cathodes were mounted to the rods, parallel to the span, with the silicone glue. Mounting the cathodes and anodes as described not only provided a stable configuration for experimentation, but maximized the electrode surface area exposed to and used by the microbes for electron transfer and electricity generation. Figures 2.1 and 2.2 show the completed reactor assemblies before the addition of wastewater sludge and water.

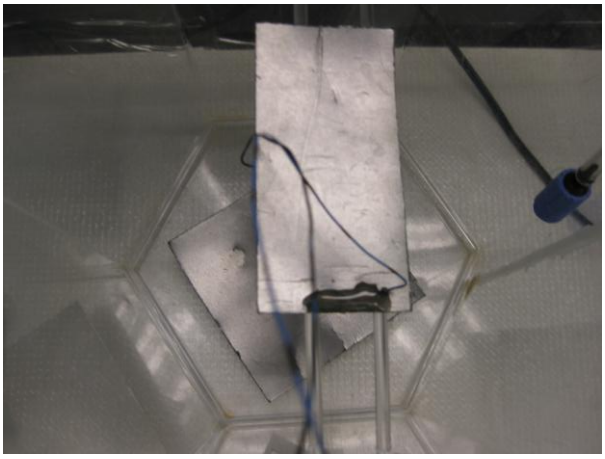

Figure 2.1: Aerial view of cathode and anode mounts.

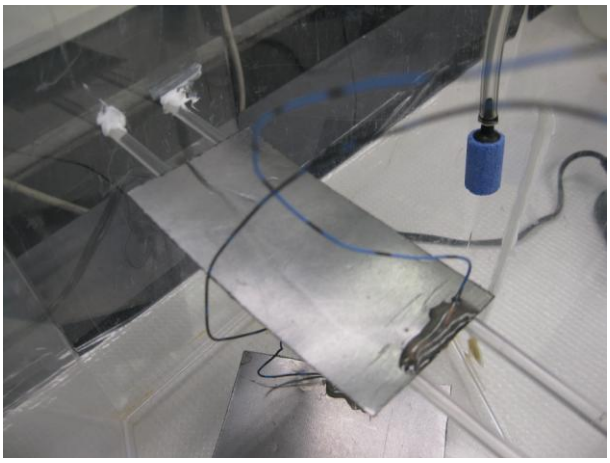

Figure 2.2: Side view of cathode and anode mounts and wire attachment. The air stone is also visible.

A 48-hour period was allotted between mounting the electrodes and filling the tanks to allow all glue to dry completely. Digested wastewater sludge was collected from the Wilmington Wastewater Treatment Plant to act as the source of organic material for the MFCs. The sludge was stored in the refrigerator in the time between collection and filling the reactors to prevent degradation of the organic material in the sludge and preserve the microbial populations residing within. It can be assumed that the solids in the sludge naturally settled during this time. Before filling the reactors, the sludge was stirred vigorously to ensure that the solids content of the sludge in the storage container was constant. This was also the best way to control the solids content of the sludge in each of the four reactors, making it as constant and unbiased as possible. A 1.5 L volume of the sludge was added to each reactor, completely submerging the anodes. Next, 3.5 L of deionized water was then added to the reactor, bringing the total volume to 5 L. 5 grams of 99% pure NaCl were dissolved in the last liter of deionized water before it was added to the tank. It has been shown in previous

experiments that a 1 g/L salt concentration optimizes conductivity within the reactor (4, 21). The cathodes were now completely submerged also. The water addition was done as slowly as possible to minimize mixing between the water and sludge layers. Total separation of the layers cannot be achieved by these methods, so it was necessary to allow the layers to settle overnight before experimentation with the reactors could begin.

After the sludge settled, small air pumps were assembled to diffuse air into the cathodic zones of the reactors. Plastic tubing was connected to the pump and a small air stone was inserted into the open end. The air stone was lowered into the tank just far enough to be completely submerged in the aerobic water zone, but not disturb the anoxic sludge layer. The tubing was secured to the side of the reactor with tape to ensure the air stone would stay in place throughout experimentation. The air pumps ran constantly over the entire period of experimentation, diffusing oxygen through the cathodic zone to sustain the electrochemical reactions necessary to generate a current. Figure 2.3 shows a filled reactor at the start of operation.

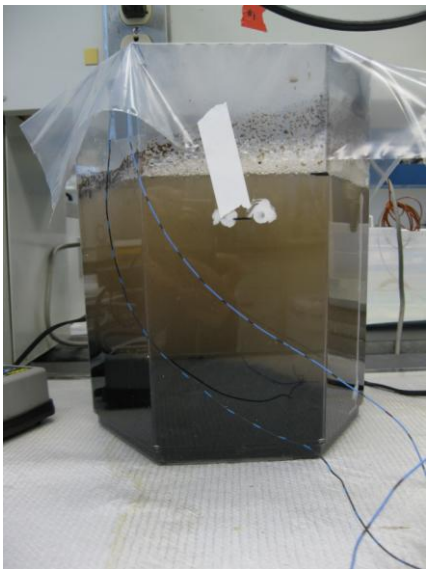

Figure 2.3: Filled reactor at the start of experimentation.

2.2 Supplemental Nutrient and Enzyme Preparation

The experiments conducted during Phase I focused mainly on finding a combination of nutrient and enzyme solutions to maximize electricity production in the fuel cells. Phase II focused more on identifying the optimal enzyme dosage, while supplementing the nutrient supply with only one source. The following sections provide explanation for the nutrient and enzyme sources used during Phase I and Phase II, as well as the steps taken to prepare and apply all solutions.

2.2.1 Supplemental Nutrient Preparation Details

All supplemental nutrient solutions were prepared using deionized water from the tap in the laboratory. The beakers used to contain the solutions were rinsed in deionized water prior to preparing the solutions to remove any dust or dirt from the glassware. The dry nutrient source was dissolved in the measured volume of deionized water using a standard magnetic stir plate and small stir bar. Table 2.1

shows the nutrient types and solutions prepared during Phase 1 and Phase 2. The solution strengths used, as given in the table, reflect the recommended preparation standards for culture medium provided by the companies from which these chemicals were obtained. Typically, approximately 200 mL of solution were all that was needed, so the mass of nutrient source in solution was adjusted accordingly.

Table 2.1: Nutrient Solution Preparation

Nutrient Type	Phase Applied	Solution Concentration
Sodium Acetate	Phase I	500 mg/L
Cellulose	Phase I	500 mg/L
Sigma-Cel 20	Phase I	500 mg/L
Tryptic Soy Broth	Phase I	30 g/L
LB Broth	Phase II	25 g/L
Tryptic Soy Broth	Phase II	25 g/L

It is fairly obvious that the nutrient sources used during Phase I mainly targeted the carbon supply in the fuel cells. However, it became clear that the microbes require a better balance of nutrients. Tryptic soy broth, a protein solution, was determined to be more effective at maintaining a supply of carbon, nitrogen, and phosphorus, all of which are important for sustaining anaerobic bacterial growth, within the fuel cells.

As stated before, the experiments conducted during Phase I were mainly concerned with finding a nutrient and enzyme combination to support growth within the MFCs. Each of the nutrient sources named in Table 2.1 was independently applied over a period of a few days. This allowed for observation of the microbial response to each nutrient source. It cannot be determined from these experiments alone how long each substrates lasts in the MFC, but a reasonable estimation can be made based on

increases and decreases in the electrical output of the system after application of the substrate. During Phase I, finding the optimal dosage of nutrient solution was also important. There was one control reactor that did not receive a supplemental nutrient source throughout the majority of Phase I. The other three received varying dosages of the solution. Table 2.2 shows the schedule for feeding used for the fuel cells during Phase I. It provides the volume of solution applied to the reactor, as well as the equivalent mass of nutrient contained in solution.

Table 2.2: Phase I Schedule for Nutrient Application

Nutrient Type	Reactor #	Application Dates	Solution Dosage	Nutrient Dosage
Sodium Acetate	2	Day 6	50 mL	25 mg
	3	Day 6	100 mL	50 mg
	4	Day 6	150 mL	75 mg
Cellulose	2	Days 23-26, 36-54	10 mL	5 mg
	3	Days 23-26, 36-54	20 mL	10 mg
	4	Days 23-26, 36-54	30 mL	15 mg
Sigma-Cel 20	2	Days 55-70, 84-89	10 mL	5 mg
	3	Days 55-70, 84-89	20 mL	10 mg
	4	Days 55-70, 84-89	30 mL	15 mg
Tryptic Soy Broth	1	Days 90-106	1, 3, 10 mL	30, 90, 300 mg
	2	Days 90-106	1, 3, 10 mL	30, 90, 300 mg
	3	Days 90-106	1, 3, 10 mL	30, 90, 300 mg
	4	Days 90-106	1, 3, 10 mL	30, 90, 300 mg

The sodium acetate was applied only once in a relatively large dosage, functioning as a sort of “spike”. As will be seen in the Results section, the effect was immediate, but could not be sustained. It was decided that applying the cellulose daily, in smaller doses, would better control the nutrient supply within the reactors. Cellulose has a

much larger molecular structure, as can be seen in Figure 2.4, and requires extracellular enzymes to initiate biodegradation. Sigma-Cel 20 is highly-purified cellulose, made by controlled evaporation into particles of a uniform 20 micron particle size. These substrates were used to provide a more stable and complex nutrient to the microbes, with the expectation that a more stable current generation rate would result.

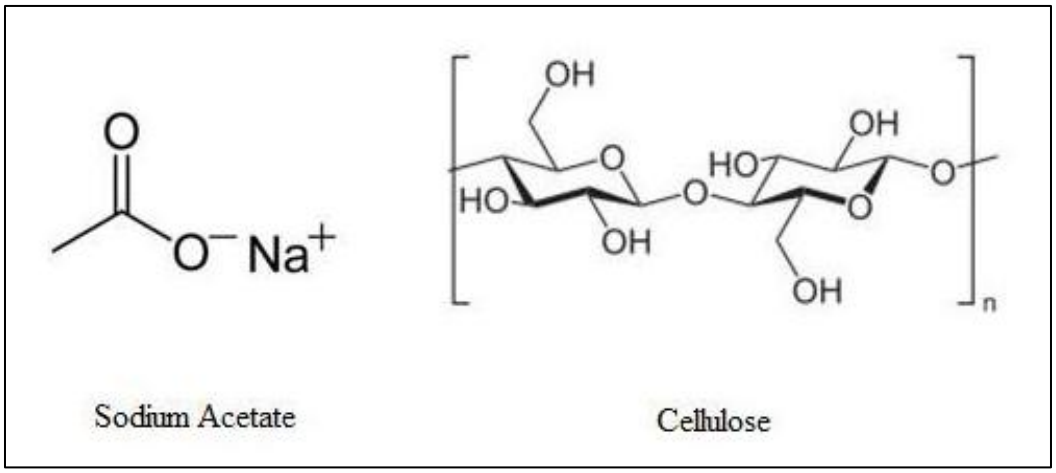

Figure 2.4: Molecular structures of sodium acetate and cellulose.

A rest period was enforced after Day 70, during which no supplemental nutrient source was provided except for a small amount of glucose solution. This decision was made because of the overall decline in electrical activity seen in the four reactors. It was thought that the microbes may be exhausted and the sludge zone saturated with undigested material. After this period, which lasted for 12 days, the Sigma-Cel 20 was again tried, but the reactors continued to respond poorly.

All four reactors began to receive tryptic soy broth on Day 90 in an attempt to revive the microbial populations. It was determined that TSB is a much better balance of nutrients than any of the other nutrient sources used during Phase I. It contains sources of carbon, nitrogen, and phosphorus, which is generally recommended for microbial growth in other wastewater treatment processes. In the Results section, it will be shown that the electrical output was more consistent during the period of TSB application as compared to the periods with carbon-only nutrient applications. The dosage was uniform for the four reactors, and was increased every three days, following the pattern shown in Table 2.2. However, the microbial population was believed to be too exhausted to recover fully by the time these measures were taken, ending Phase I.

It was determined at the start of Phase II that experimentation would not last as long as Phase I to avoid complete exhaustion of the microbes. Phase II was limited to 64 days, in an attempt to monitor the microbial activity at peak strength. Finding the optimal enzyme solution dosage was the focus of study during Phase II, and the nutrient dosage was controlled for the four reactors. Table 2.3 shows the feeding schedule for the four reactors during Phase II.

Table 2.3: Phase II Schedule for Nutrient Application

Nutrient Source	Application Dates	Solution Dosage	Nutrient Dosage
LB Broth	Days 1-11	50 mL	1.25 g
Tryptic Soy Broth	Days 12-64	50 mL	1.25 g

Tryptic soy broth was not available in the laboratory at the start of Phase II, so LB Broth was used as a substitute until the tryptic soy medium could be obtained. The

nutrient content of both sources is beneficial for microbial growth, so there was no risk of impairing the growth or function of the microbes in the sludge.

All supplemental nutrient solutions were applied to the fuel cells during Phase I and Phase II using the same technique. After the nutrient source was dissolved in solution, a syringe was used to extract the precise dosage from the beaker. Because the microbes living in the sludge require the nutrients, the nutrient solutions were applied directly to the anaerobic sludge zone, as shown in Figure 2.5. This involved submerging the syringe into the sludge and releasing the contents within the zone. Care was taken not to disturb either electrode in the process.

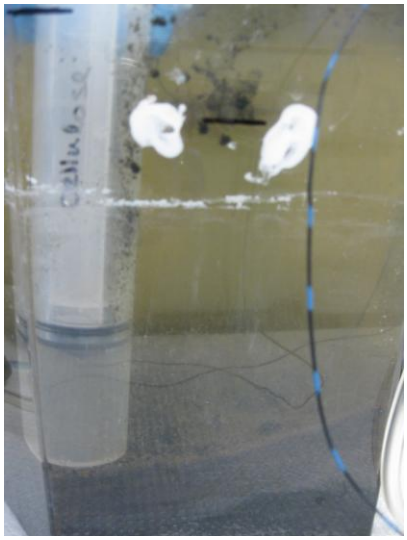

Figure 2.5: Method of supplemental nutrient solution application.

It is assumed that diffusion distributes the nutrient solution evenly throughout sludge zone. However, two application points, around the anode in each reactor, were used to

ensure that the contents of the solution were distributed as evenly as possible. Half of the solution was injected on one side of the zone, and the other half was injected on the opposite side. Any air bubbles that escaped into the anaerobic zone were small and insignificant, as the oxygen was either consumed rapidly by the microbes or the bubble floated to the aerobic zone immediately.

2.2.2 Enzyme Preparation Details

The enzyme solutions used during experimentation were prepared in a similar manner to the nutrient solutions. During Phase I, several different enzyme types were applied to the reactors to determine the best nutrient and enzyme combination for encouraging microbial growth and productivity. The dry enzyme source was dissolved in deionized water, using a magnetic stir plate and stir rod. Some of the enzymes used do not completely dissolve in water, but they were put in solution to maintain consistent application methods. The enzymes that remained in the solid state were assumed to solubilize soon after addition. Because the enzyme type was variable throughout Phase I, the dosage of enzyme solution applied to the four reactors was kept constant. During Phase II, the enzyme type was kept constant, but the dosage was varied in the four reactors. Reactor 1 did not receive the enzyme source at all, and Reactors 2, 3, and 4 received incremental doses of solution daily. Table 2.4 shows the enzyme sources used during Phase I and Phase II, as well as the concentrations at which they were prepared.

Table 2.4: Enzyme Solution Preparation

Enzyme Type	Phase Applied	Solution Concentration
EZ 216	Phase I	1 g/L
Cellulase	Phase I	1 g/L
EZ 216	Phase II	1 g/L

EZ 216 is a mixture of enzymes used commercially for septic tank treatment. It was used for these experiments because of its success in aiding microbes to break down the organics found in wastewater. Cellulase was used in an attempt to better target the cellulose solutions being injected into the reactors. It was thought that providing a compound-specific enzyme may enhance the microbial metabolic processes, thereby increasing the electrical output generation rates. However, when significant improvement in electrical output was not observed after the change, it was decided to continue with EZ 216 for future experiments, as it is the more economical choice.

During Phase I, all four reactors received 30 mL of solution, or 30 mg of enzyme, daily. Syringes were also used for enzyme solution application to precisely measure the volume of solution applied and affirm that the solution was applied directly to the microbes in the sludge zone. One of the objectives of Phase II was finding the optimal enzyme solution dosage. Table 2.5 shows the dates over which each enzyme solution was used during Phases I and II, as well as the dosage applied to each reactor.

Table 2.5: Schedule for Enzyme Application

Enzyme Type	Reactor #	Dates Applied	Solution Dosage	Enzyme Dosage
EZ 216 (I)	ALL	Days 36-65	30 mL	30 mg
Cellulase (I)	ALL	Days 66-71, 84-106	30 mL	30 mg
EZ 216 (II)	2	Days 31-64	20 mL	20 mg
	3	Days 31-64	40 mL	40 mg
	4	Days 31-64	80 mL	80 mg

During both phases, the initial application of enzyme did not occur until about one month after the startup of the reactors. This was to allow time for the microbes to fully adjust to their new environment before adding another source of variation. When applying the nutrient supplements to the reactors, the nutrient solutions were always applied first, followed by the enzyme solution. It is assumed that natural diffusion processes distributed both of the solutions evenly throughout the anoxic sludge zone.

2.3 Data Collection

Data collection methods were identical during Phases I and II. A standard voltmeter was used to measure the electrical output of the four reactors. The wires attached to the anodes and cathodes were connected to leads from the voltmeter via standard alligator clips. To get an accurate measurement of the current being generated by the reactors, a certain resistance is required. A series of resistors was used when making measurements to estimate the actual electrical output. Because these systems operate very much like a battery, it was necessary to apply the resistances in descending order so that the electrical current would not be drained after using the first resistor. The voltmeter automatically measures voltage in mV until the

voltage reaches 400 mV, and then it measures voltage in V. All voltage measurements shown in the Results section will be reported in mV.

Before any resistance was applied, the open circuit voltage measured initially by the voltmeter was recorded. The following resistances were then applied, with a one-minute rest period between each application: 11.5 kΩ, 4.61 kΩ, 458 Ω, 46.8 Ω. The resistors were spliced into the circuit by connecting each end to one of the leads from the voltmeter. The connection was made for a very short time to avoid completely "draining" the system of power. Similarly, when measurements were not being made, the wires from the anodes and cathodes were disconnected from the voltmeter. The final experimental setup is shown in Figure 2.6.

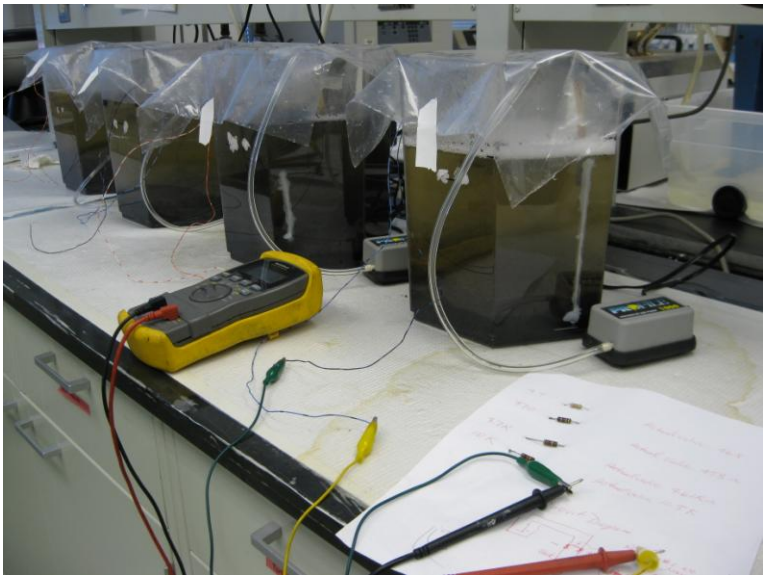

Figure 2.6: Complete experimental setup, including voltmeter used to collect data.

When measuring electrical output, it is much more useful to report data in terms of current output. Therefore, Equation 4 was used to convert the voltage measurements to current after each applied resistance:

$$I = V/R \quad [4]$$

where voltage V is in mV, resistance R is in ohms, and current I is in mA. In the Results section, current measurements will be reported in terms of current density, which is computed by dividing the current measurements by the surface area of the anode. Current density is expressed in units mV/m^2. On the voltmeter used for these experiments, there was a setting to directly measure current. After measuring voltage with the application of resistance, the current was measured directly as a form of comparison.

2.4 Preparation of Samples for Microscope Imaging

The scanning electron microscope (SEM) at the Delaware Biotechnology Institute was used to examine the surfaces of the anodes for biofilm development at the end of Phase II. Small samples of the anodes were carefully pulled out of the four reactors. To transport the samples to DBI, approximately 20 mL of the wastewater sludge from the reactor was extracted and placed into the Petri dishes holding the anode samples. This was thought to be the best way to maintain a semi-consistent environment for the bacteria possibly residing on the anode surfaces during transport to the facility.

A critical point drying (CPD) procedure was performed on each sample to preserve any biofilm before using the SEM. Table 2.6 provides information about the steps taken and chemicals used during fixation and dehydration.

Table 2.6: Fixation and Dehydration Procedure

Process	Procedure Step	Time Applied
Fixation and Post-Fixation	Fixation with 2% Gluteraldehyde	1 hour
	Wash Buffer	3 times
	OsO_4	1 hour
	Rinse with DI H_2O	3 times
Dehydration	25% Ethanol	10 min
	50% Ethanol	10 min
	75% Ethanol	10 min
	95% Ethanol	10 min
	100% Ethanol	10 min
	100% Ethanol	10 min

The percentages given in the table above reflect the volume of 200 proof ethanol in the solutions used during the dehydration process. After the samples were fixed and dehydrated, they were put into the critical point dryer for 1.5 hours. Rectangular plastic cartridges were used to hold the samples from Reactors 1, 2, and 3 in the machine. A small, round metal dish was used to hold the sample from Reactor 4 because of its smaller size. The critical point drying chamber was filled with ethanol first, and then purged with liquid carbon dioxide to cool and dry the samples and inner chamber.

When the samples were removed from the critical point dryer, they were mounted to aluminum stubs using a silver conductive paint. This step was performed here because the samples were not very flat, which had the potential to affect the quality of the images from the SEM. The paint was allowed to dry overnight before any further preparation was made. The last preparation step involved coating the samples with a gold and palladium mixture under argon gas. The coating itself was

very fine, in terms of particulate size. It had an appearance similar to a somewhat reflective golden "snow." All SEM images shown in this report were captured by Debbie Powell at DBI.

Chapter 3

RESULTS

The results from Phase I and Phase II will be provided in the following sections. In both phases, variation in the electrical output of the four reactors could be correlated to differences in the applied nutrient and enzyme dosage. It will be shown that a supplemental nutrient source is necessary to sustain electricity generation for extended periods of time in these batch systems. The enzyme treatment also appears to have a positive effect on the electrical output, with some exceptions. These findings will be detailed and displayed in figures to follow. Comparative statistics have also been reported in these sections, to form the basis for the conclusions to be made in the Discussion section. Finally, a brief summary of the findings from the imaging performed with the scanning electron microscope at the Delaware Biotechnology Institute will be reported.

3.1 Phase I Results

Phase I was conducted over a 108-day period in January through April 2011. The reactors were operated with a continuous supply of oxygen to the cathodic zone. The primary research objective for Phase I was to identify a combination of nutrient and enzyme sources to maximize microbial growth and performance, as measured by electrical output from the fuel cells. Tables 2.2 and 2.5 in the previous chapter list the dates of application and the nutrient and enzyme solution dosages provided to the reactors during Phase I. Enzyme dosages were uniform for the four reactors, while

supplemental nutrient dosage was varied. The results of Phase I seem to indicate that the type of enzyme used was less important than the source of nutrients, but that a supplemental nutrient supply is necessary for consistent electrical output.

3.1.1 Sodium Acetate Spike

Sodium acetate solution was applied to Reactors 2, 3, and 4 on Day 6 of experimentation. The objective was to measure the microbial response to an easily digestible nutrient source in terms of electrical output. There was a dramatic rise in the voltage in the days following this addition. The current density also began to increase. The electrical output of Reactor 1 also began to increase, but was not as stable and did not follow the same trends as the experimental reactors. The decline in current density observed in the experimental reactors starting around Day 17 (see Figure 3.2) prompted plans to use a more complex and stable nutrient source, in terms of molecular structure, and a daily dosage application plan.

3.1.2 Daily Cellulose Addition

Cellulose solution was applied to the experimental reactors during two separate periods during Phase I. The first period of application occurred daily between Days 23 and 26. Voltage in the experimental reactors decreased suddenly, as shown in Figure 3.1. The addition of the cellulose solution was stopped temporarily to determine if the cellulose was inhibiting microbial growth. Once it was concluded that the cellulose was not the cause of the decline in electrical output, daily applications resumed. Cellulose solution continued to be applied daily to the experimental reactors between Days 36 and 64. Voltage levels in both the control and experimental reactors peaked during this period. Current density continued to increase

as well, but the generation rates of the experimental reactors were much more consistent than the current density output of the control reactor, which fluctuated often.

3.1.3 Daily Sigma-Cel 20 Addition

The decision to replace the cellulose solution with Sigma-Cel 20 was made in an effort to control the purity of the cellulose solutions. The experimental reactors received Sigma-Cel 20 solution dosages between days 55 and 70. Voltage output began to decline, and current density began to fluctuate on a daily basis. A rest period was enforced between Days 71 and 83. Small doses of glucose solution were applied to all four reactors twice during this rest period to maintain an influx of nutrient, but avoid exceeding the microbial population's assimilation capacity. On Day 84, application of the Sigma-Cel 20 solution resumed, but a decline in the electrical output of all four reactors continued. These trends can be seen in both Figure 3.1 and 3.2.

3.1.4 Tryptic Soy Broth Addition

On Day 90, in a last attempt to revive the microbial populations, tryptic soy broth was applied to all four reactors. Uniform dosages were applied to the reactors, and increased consistently. Around Day 95, it seemed as though the additional nutrients were having a positive effect on the electrical generation rates, as there was a significant increase in current density and voltage levels were constant in all four reactors. However, this effect could not be sustained. It was assumed that the microbial populations were exhausted at this point, and Phase I was concluded.

3.1.5 Enzyme Application

As discussed previously, the enzyme dosage was held constant in all four reactors during Phase I. The first type of enzyme used was EZ 216, applied to each reactor starting on Day 36 and continuing daily until Day 65. Microbial response did not appear inhibited by the addition of the enzyme. As can be seen in Figures 3.1 and 3.2, the voltage and current density measured in all four reactors increased to peak values within the first two weeks of the enzyme addition, corresponding to the second period of cellulose addition. On Day 66, the EZ 216 solution was replaced with cellulase solution in an attempt to better target the microbial digestion of the Sigma-Cel 20. However, voltage and current density did not seem significantly affected by this change. The trends observed seem better correlated to the changes in nutrient source. The rest period enforced between Days 71 and 83 was also targeted for the enzyme application. Cellulase application resumed on Day 84 and continued daily until the end of Phase I.

3.1.6 Summary of Phase I Data

Figures 3.1 and 3.2 show the complete set of data collected for the four reactors operated during Phase I, reported as open circuit voltage and current density. Moving 3-day averages were computed to eliminate some of the "noise" associated with the raw data, allowing for the general trends in the data to be observed more clearly. Voltage measurements are reported in mV, to 4 significant digits. This level of precision was chosen because of the level of precision of the voltmeter used to collect this data. Current density is reported in mA/m^2, also to 4 significant digits.

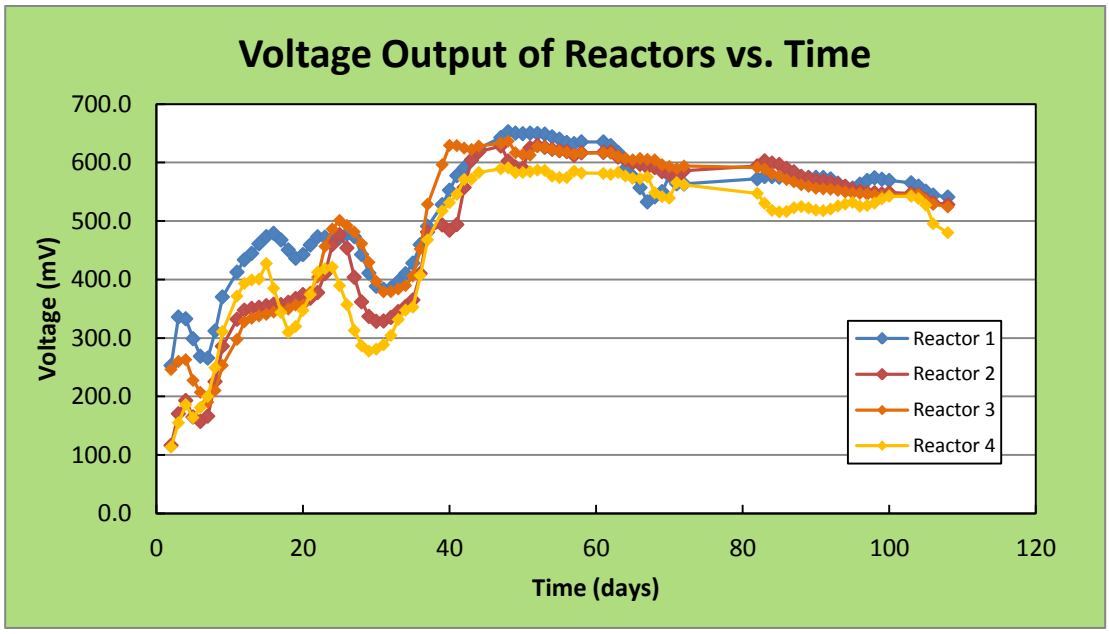

Figure 3.1: Phase I open circuit voltage data, reported as moving average values.

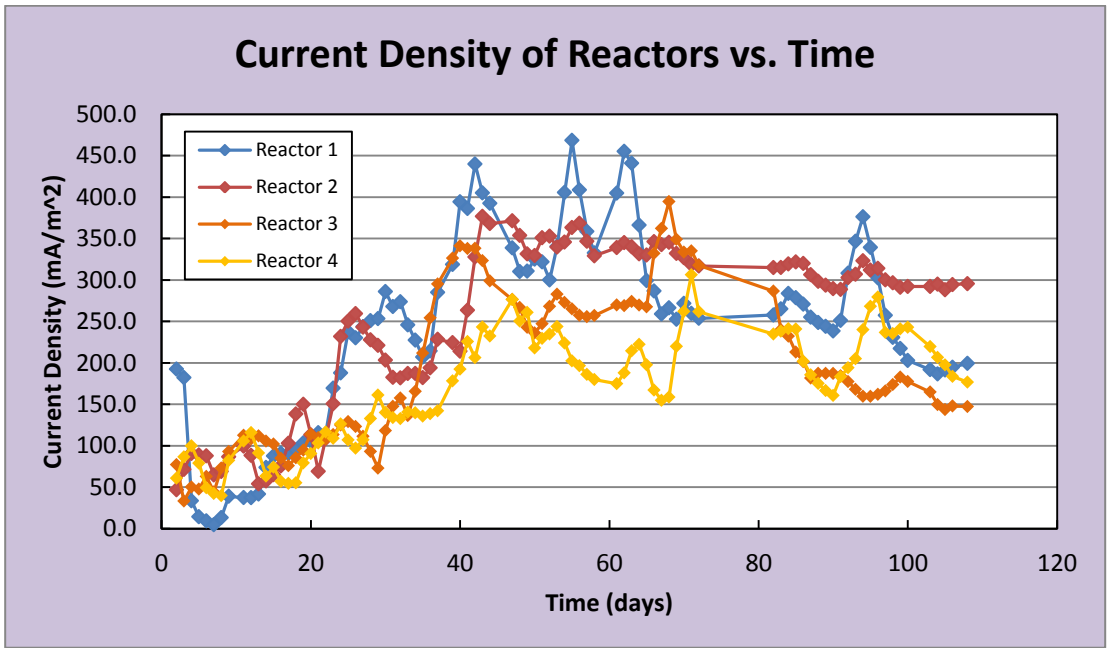

Figure 3.2: Phase I current density data, measured using a 46.8 Ω resistor, reported as moving average values.

Average values for the overall voltage and current density output were also calculated for each reactor. However, these values may not be reflective of the specific effects of the different supplemental nutrient solution applications, so the data from three, ten-day periods throughout Phase I were also averaged. The first period corresponds to the second period of cellulose addition (Days 36-45). The second period corresponds to the first period of Sigma-Cel 20 addition (Days 55-64). The third period corresponds to the final ten days of Phase I (Days 99-108), when tryptic soy broth was the nutrient source. Table 3.1 shows these averaged values for voltage and Table 3.2 shows the averaged values for current density. Together, a reasonable comparison of the electrical output of the four reactors can be made.

Table 3.1: Average Voltage (in mV) measured during Phase I

Reactor #	**Period 1**	**Period 2**	**Period 3**	**Overall V**
1	552.8	628.9	557.3	515.0
2	517.1	615.0	540.4	477.9
3	589.4	614.8	538.1	495.8
4	523.2	578.8	522.3	456.1

Table 3.2: Average Current Density (in mA/m^2) measured during Phase I

Reactor #	**Period 1**	**Period 2**	**Period 3**	**Overall I**
1	356.9	406.1	196.8	241.8
2	273.1	346.8	293.0	245.9
3	314.4	263.8	160.3	192.5
4	198.9	199.0	212.2	168.8

3.2 Phase II Results

Phase II was completed over a 64-day period in September to November of 2011. The four reactors were operated with methods identical to those used during Phase I. The objective of these experiments, however, was to identify the enzyme dosage needed to optimize electrical output. Nutrient source and dosage were kept constant for all of the reactors, while enzyme dosage was varied. The results of Phase I seem to indicate that the supplemental nutrient source is more important for sustaining microbial growth than enzyme type. Therefore, the EZ 216 solution was the only solution used during Phase II. It is more economical and targets multiple nutrient sources for the microbes. The following sections summarize the results of these experiments and describe the images captured from samples taken from the reactors with the scanning electron microscope at the Delaware Biotechnology Institute.

3.2.1 Summary of Phase II Data

Daily supplemental nutrient solution applications began immediately after assembly of the reactors. Voltage output from each reactor increased greatly in the first five days of operation. Current density, however, was quite low for several weeks, and fluctuated on a daily basis. Replacing the LB broth solution with the tryptic soy broth on Day 11 did not affect the electrical output of the reactors. Enzyme addition did not begin until Day 31, when current density began to stabilize. To be able to clearly observe any effects, positive or negative, of the enzyme application, it was necessary to begin with relatively consistent current density values for the four reactors. Voltage levels remained relatively constant after enzyme addition began until the end of Phase II, but the current density levels varied significantly. In

Reactors 2 and 3, dramatic increases were observed starting around Day 45 and were sustained until the end of experimentation. Current density in Reactor 4 increased slightly, while current density in Reactor 1 actually decreased towards the end of experimentation.

Figures 3.3 and 3.4 show the complete set of data collected for the four reactors operated during Phase II, reported as open circuit voltage and current density. Like in Phase I, moving averages were computed for these values to eliminate some of the "noise" associated with the raw data. Again, voltage and current density measurements are reported in mV to 4 significant digits and mA/m^2 to 4 significant digits, respectively.

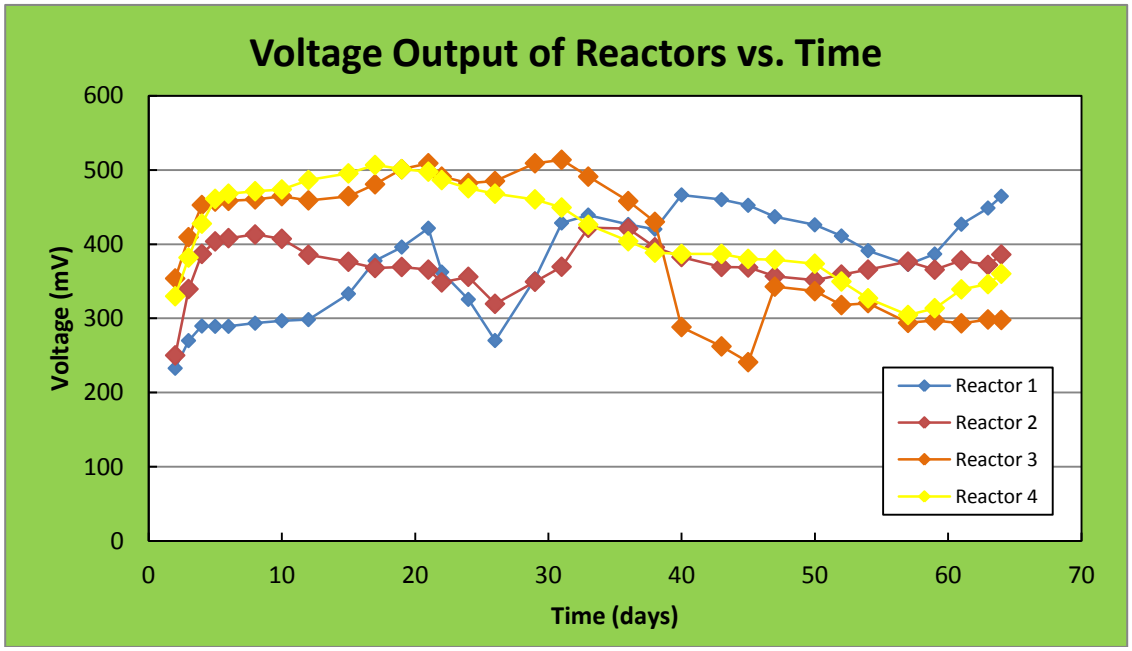

Figure 3.3: Phase II open circuit voltage data, reported as moving average values.

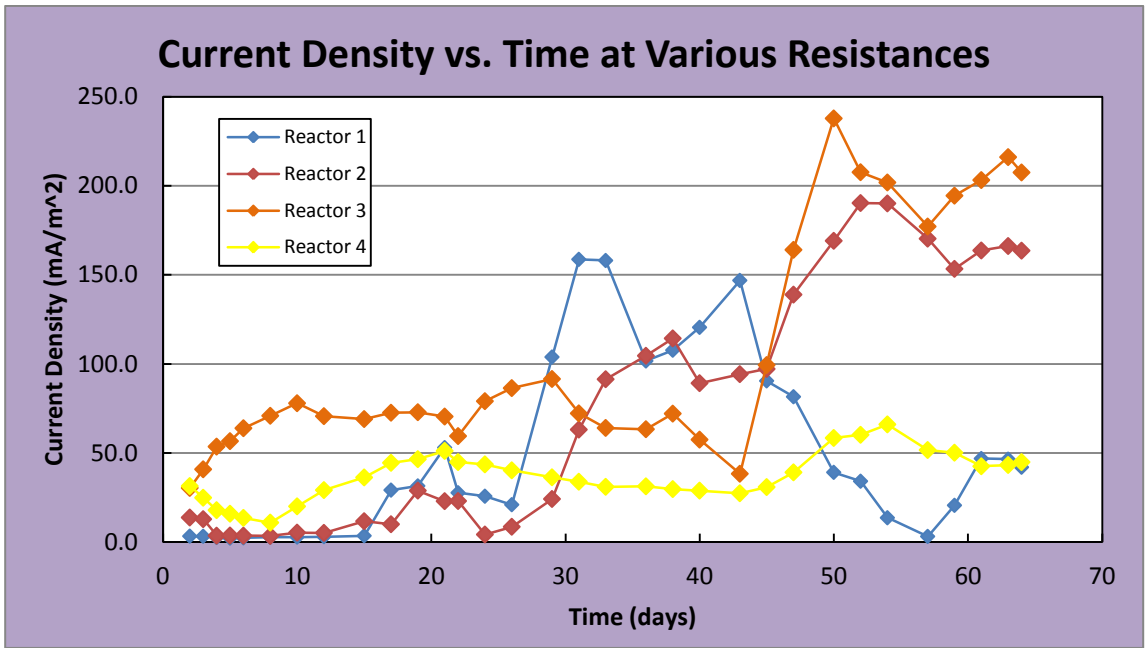

Figure 3.4: Phase II current density data, measured using a 46.8 Ω resistor, reported as moving average values.

Similarly to Phase I, average values for the overall voltage and current density output were also calculated for each reactor. Data from three, ten-day periods were also averaged to examine the trends observed throughout Phase II more closely. The first period was designated as Days 1 through 10. The second period corresponds to Days 31 to 40. The third period corresponds to the final ten days of Phase II (Days 54-64). Table 3.3 shows the averaged values for voltage and Table 3.4 shows the averaged values for current density.

Table 3.3: Average Voltage (in mV) measured during Phase II

Reactor #	Period 1	Period 2	Period 3	Overall V
1	268.2	447.7	410.7	367.8
2	348.1	406.1	374.2	365.2
3	419.7	464.0	296.2	400.5
4	414.7	409.5	325.3	412.1

Table 3.4: Average Current Density (in mA/m^2) measured during Phase II

Reactor #	Period 1	Period 2	Period 3	Overall I
1	2.8	148.9	24.8	46.0
2	7.4	102.9	168.2	71.3
3	52.2	69.8	196.6	101.9
4	20.5	30.8	47.4	36.8

3.2.2 SEM Images

Samples of the anodes from each reactor were extracted at the end of data collection and examined for microbial populations using the scanning electron microscope (SEM) at the Delaware Biotechnology Institute. The preparation procedure for these samples is detailed in section 2.4 of the Methods chapter. The objective of this portion of the project was to determine whether a biofilm formed on the surface of the anodes during experimentation. It was also important to see if a variety of species were able to survive in the system. Evidence of microbes attaching directly to the anode surfaces was present on the samples from all four reactors. Figure 3.5 shows examples of images captured for each sample. More details about the results of the imaging will be given in the Discussion chapter.

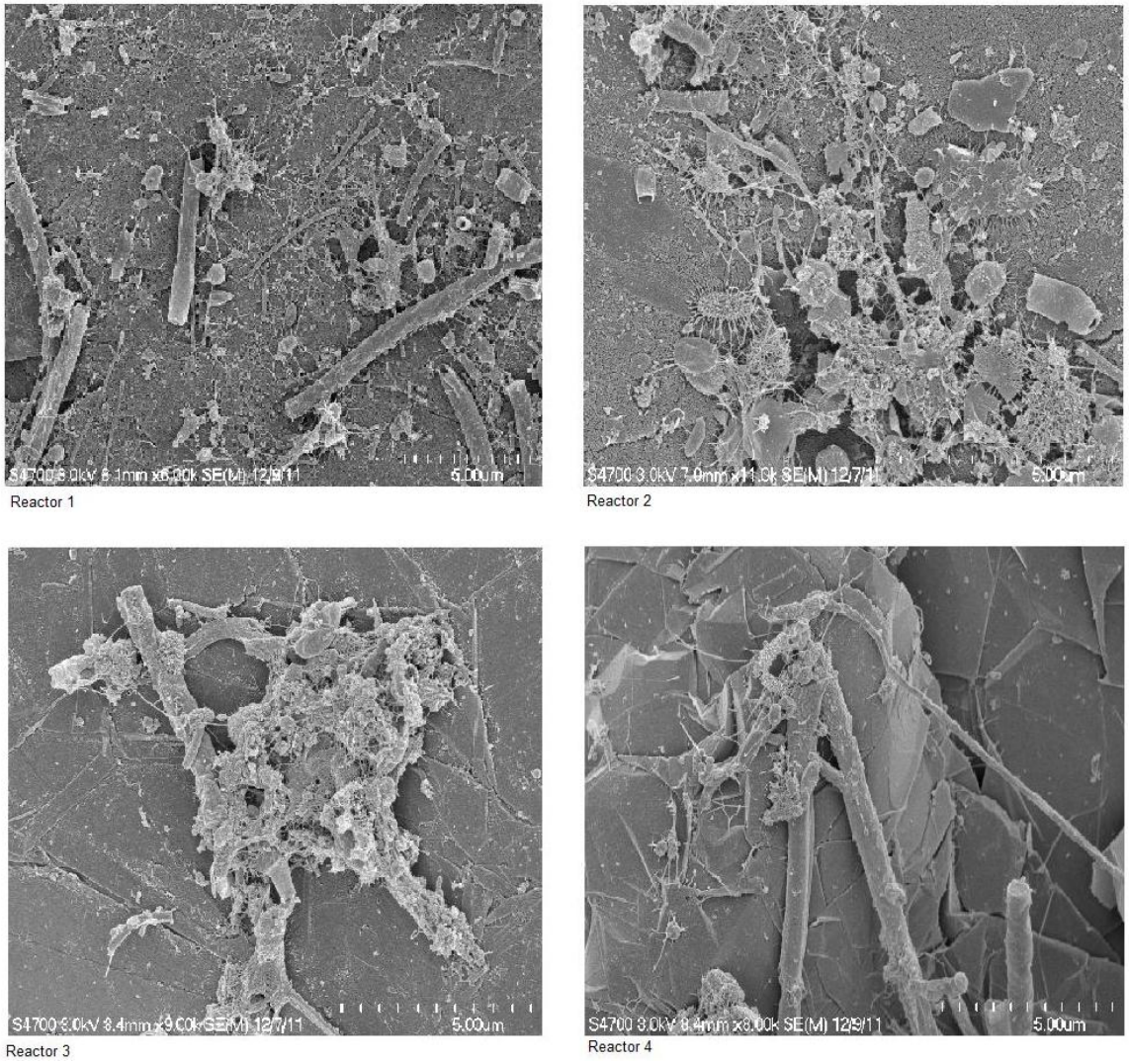

Figure 3.5: Sample images for each reactor taken using SEM at DBI.

Chapter 4

DISCUSSION

Several variables were examined during the Phase I and Phase II experiments. It became immediately evident that microbial fuel cells can be operated effectively using biosolids as the fuel source. To examine the second major hypothesis for this project, it was necessary to explore the effects of different nutrient and enzyme solutions on electrical generation rates. The following sections provide an interpretation of the results reported in the previous chapter, as well as a detailed discussion of the findings of the SEM imaging study performed at the end of Phase II. Finally, the conclusions of this research project will be summarized at the end of this chapter.

4.1 Effects of Supplemental Nutrients

The results of the Phase I experiments strongly support the application of a nutrient solution to the reactors on a regular basis. For shorter periods of time, carbon sources, such as sodium acetate and cellulose, are adequate for supplementing the nutrient supply in the sludge zone. This was seen with the sudden increase in electrical output in the experimental reactors upon acetate application, as well as the electrical generation rates observed during cellulose addition. It became obvious during experimentation that the current level could not be sustained at a usable level for longer periods of time with only the application of a carbon source. A more balanced supply of carbon, nitrogen, and phosphorus, such as that found in tryptic soy broth, is necessary to optimize microbial performance. During Phase II, the tryptic soy broth stabilized and sustained the microbial activity levels within the fuel cells and

allowed the effects of the enzyme to be clearly identified. A daily dose of 1.25 g appears to be beneficial for the system. A different "optimal" dosage may exist, but more experimentation is needed to identify this dosage.

In systems that have a continuous fuel supply into the anodic zone, the nutrient concentrations are fairly constant. The setup for these experiments essentially created batch systems, as the reactors were inoculated only once at the start of experimentation. Any changes in the microbial populations during experimentation resulted either from changes in or depletion of nutrient sources within the sludge or competition with other microbial species present. In future work, it may be beneficial to identify the microbial species present at the beginning of experimentation and compare those present at the conclusion of experimentation.

4.2 Effects of Enzyme

Enzyme solution was applied in equivalent volumes to all four reactors during Phase I. Nutrient source was the independent variable for this set of experiments. Therefore, it is difficult to quantify the effects of the enzyme on electricity generation. It was determined, however, that there was no difference in electrical output when using EZ 216 solution versus the cellulase solution. The cellulase was originally intended to target the cellulose solution and make it easier for the microbes to digest the molecules. When significant differences in current levels were not observed during Phase I as a result of the change in enzyme type, the decision to use only EZ 216 during the Phase II experiments was made to reduce the economic cost of using an enzyme.

The effects of the enzyme treatment were seen more clearly during Phase II. Reactor 3, which received a daily enzyme dose of 40 mg, had the highest sustained

electrical generation rate. Reactor 2, which received a daily enzyme dose of 20 mg, also had a high electrical generation rate, as compared with Reactors 1 and 4. Therefore, it can be reasonably concluded that the optimal daily enzyme dosage for this system is between 20 and 40 mg. The performance of these reactors with the enzyme treatment was significantly better than the control reactor. This indicates that an enzyme treatment is effective at enhancing microbial activity.

It can be reasonably ascertained that the 80 mg dose used for Reactor 4 was too high, as its overall current production was relatively similar to that of Reactor 1. This indicates that the optimal dosage estimated by the output from Reactors 2 and 3 is reasonable and that it is possible to "over-dose" the system. One explanation might be that if the supply exceeds the demand, the system may become saturated to a point where the microbes consume the enzyme instead of their typical nutrient source. This would reduce the effectiveness of the enzyme on the hydrolysis process.

Based on the experiments conducted for this project, it seems logical that a full-scale treatment operation involving enzyme-enhanced MFCs could be achieved. One component that could be altered to optimize the system would be the supply of organic material, or system inoculation frequency. For these experiments, it was reasonable to use batch systems because the microbial species present in the wastewater sludge were not altered by unnatural means. However, a continuously-fed system, meaning that biosolids would constantly flow through the anodic zone, would eliminate the need for supplemental nutrient application. The supply of organic material available to the microbes would be replenished automatically. The flow of biosolids through the reactor would have to be slow enough to allow for a residence time high enough to achieve optimal digestion and, therefore, optimal electrical

generation rates. The enzyme would be effective at enhancing the electrical output of the reactor, but, as shown in these experiments, sufficient time is needed for this effect to take place.

4.3 Implications of SEM Images

One of the debated hypotheses about microbial activity in fuel cells is how electrons are transferred to the electrode surface. In Chapter 1, a discussion of the mechanism involving exoelectrogens, as well as the mechanism involving redox mediators, was given. In the experiments completed for this project, a synthetic redox mediator was not added to the MFCs. This does not mean that redox mediators were not naturally present in the system, but the addition of a known redox mediator would have required another set of experiments to assess the effects on the control system and systems receiving the enzyme treatment. Time constraints did not allow for these experiments to be conducted; however, in the future, it may be interesting to explore the use of a synthetic redox mediator because of successes reported in literature.

The scanning electron microscope images captured using the Phase II anodes give clear evidence of microbes attaching directly to the anode surfaces. The observed formation of biofilm may support the idea that exoelectrogens are present and can function in a MFC using wastewater sludge as the organic material source. Figure 4.1 shows a grouping of microbes, possibly two different species, captured during imaging of the anode from Reactor 2. One of these microbes has distinct pili-like structures protruding from the cell body. This was the first image captured during the study, prompting a thorough investigation of all of the samples extracted from the MFCs.

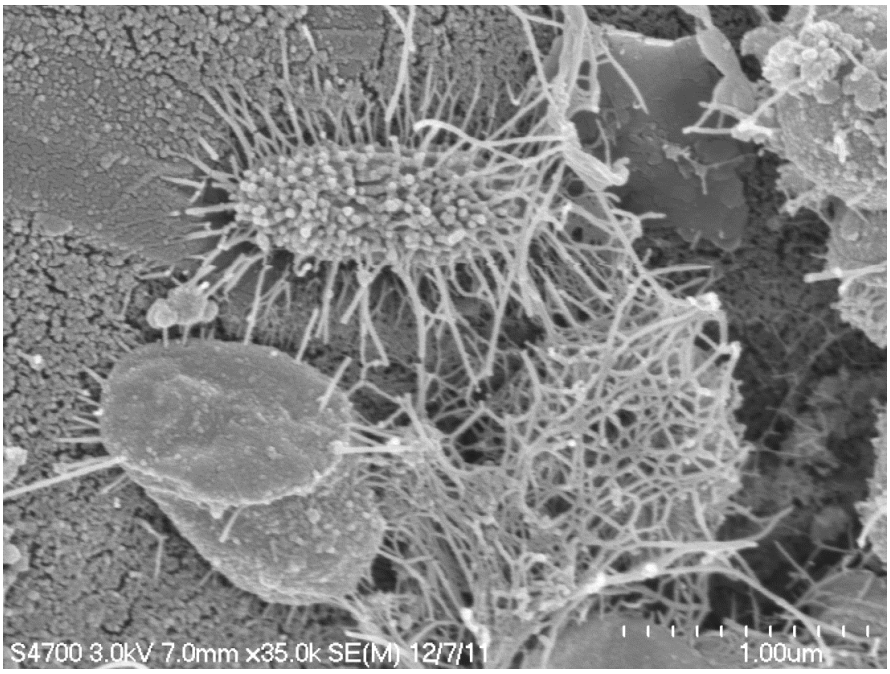

Figure 4.1: Group of microbes present on the surface of the anode from Reactor 2.

Figure 4.2 shows the same region on the sample from Reactor 2 as Figure 4.1, but at magnification 5 times less. It seems like there may be a variety of species on the anode surface, which would support the idea that mixed cultures may be able to sustain high power density better than pure cultures.

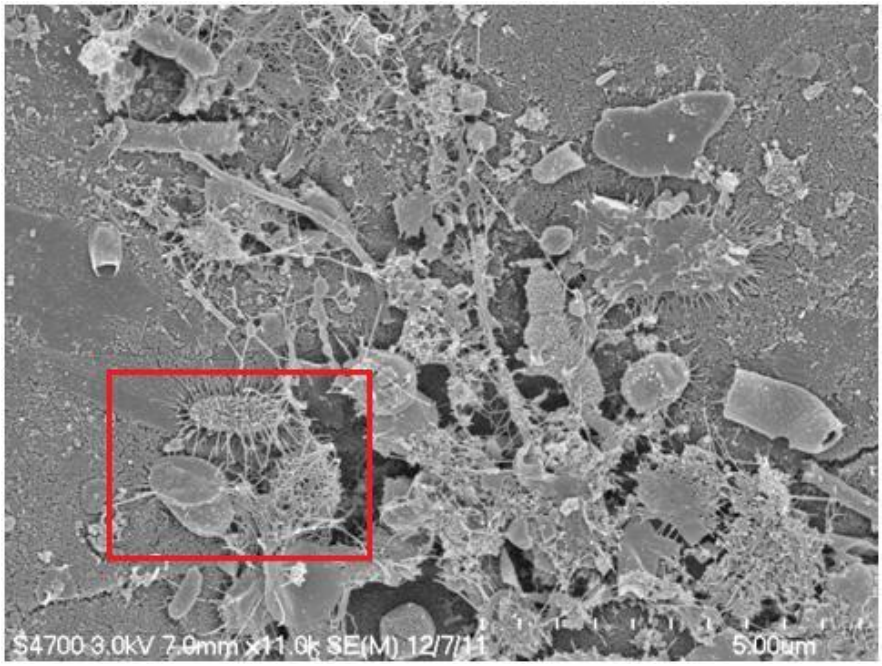

Figure 4.2: Biofilm on Reactor 2 anode surface. Red box indicates region on anode shown at 5 times the magnification in Figure 4.1.

In all four reactors, microbes of various sizes and shapes were observed, and seemed to be grouped randomly across the surface of the anodes. Figure 4.3 shows examples of microbes observed in Reactor 4. The magnification level can be determined using the scale printed on each image. It would be impossible to determine, based on these images alone, if species competition occurs among the microbes present in the MFCs.

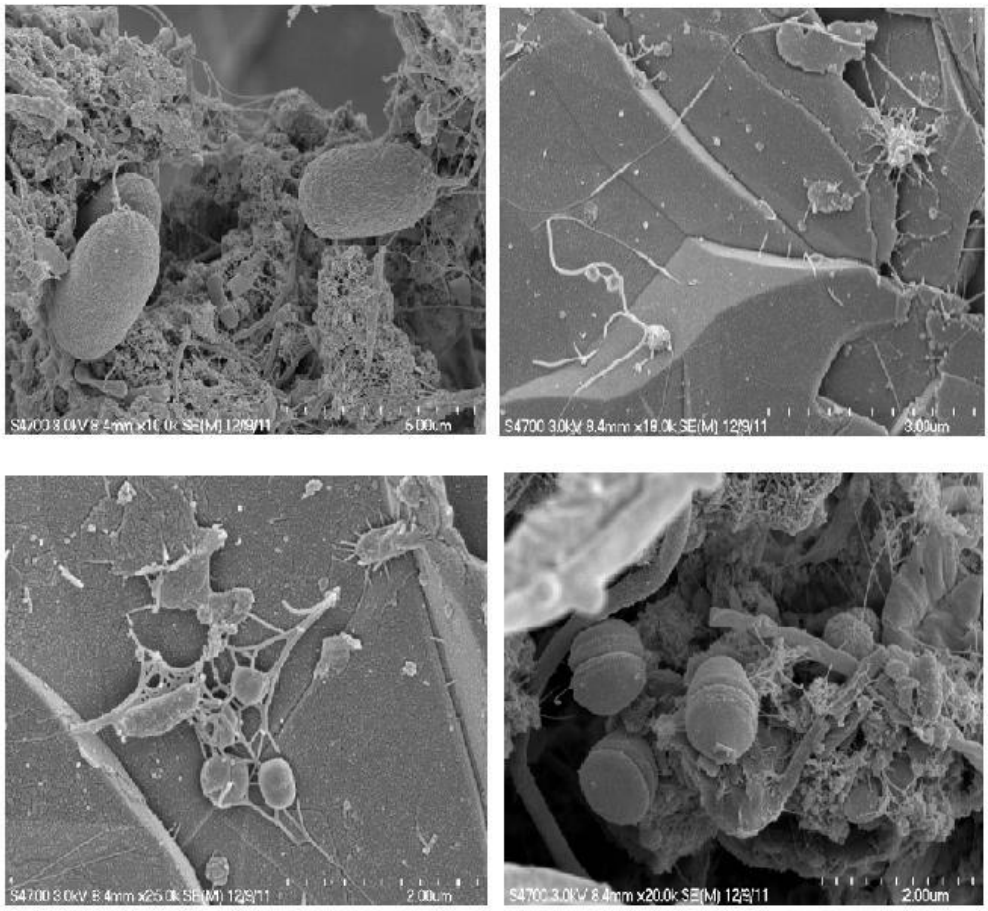

Figure 4.3: Species observed in Reactor 4.

It would also be difficult to identify the species that are actually contributing to electricity generation. The microbes that are "embedded" in the graphite electrodes, such as those shown in Figure 4.1, could be exoelectrogens. However, a definitive identification as an exoelectrogen cannot be made without first identifying each microbial species and then performing experiments with pure cultures. There are many reasons that microbes attach to surfaces, and this particular microbe may not be an exoelectrogen. For wastewater treatment purposes, operating with pure cultures

would be very impractical, as wastewaters naturally contain many species of microbes. Because of the seemingly high number of species observed after several weeks of experimentation, it can be reasonably concluded that the microbes are able to coexist. Figure 4.4 shows another diverse grouping of microbes, captured on the sample from Reactor 2. The pilus-like structures are visible for some of the cells, but others appear to simply be resting on the surface of the electrode.

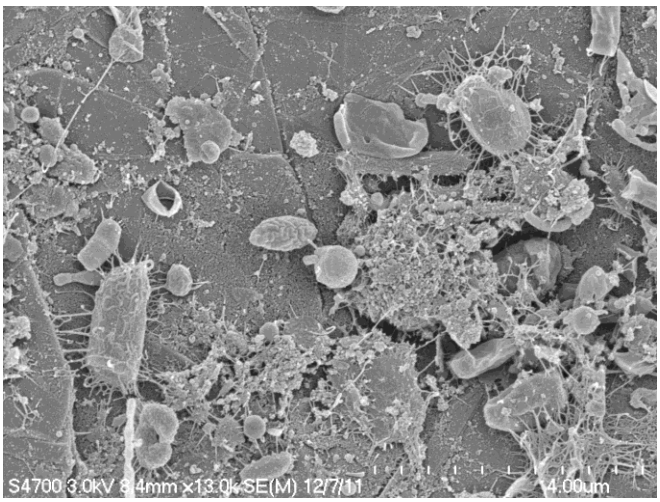

Figure 4.4: Evidence of "attached" microbes and microbes without pilus-like structures. This again demonstrates the diversity of the populations existing in the MFCs.

On a larger scale, there is no distinct distribution pattern, as the attached "clumps" of biofilm are rather scattered. During experimentation, it is possible that a more consistent biofilm formed on the surface of the anode. If it existed, it could have been disturbed during extraction of the anode samples, which would explain the

random patches of biofilm observed on all four samples. Figure 4.5 shows examples of biofilm "clumps" observed on each sample.

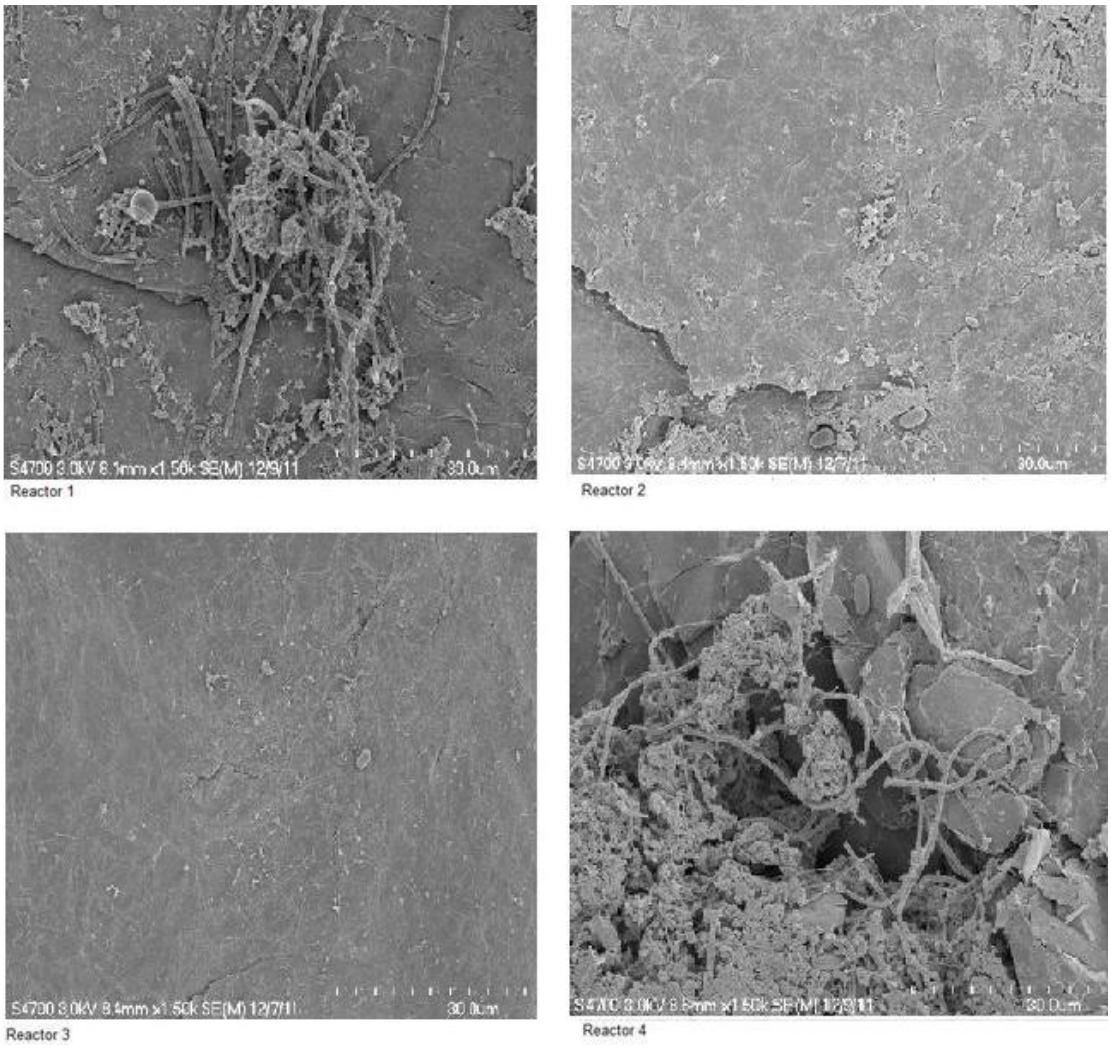

Figure 4.5: Small and large clumps of biofilm observed on each reactor, shown at a 30 μm scale.

The images shown above for Reactors 2 and 3 seem to indicate the presence of rather small clumps of biofilm. However, it is important to recognize that each image shows only a fraction of the entire sample. These images were selected because of the diversity of "clump" size represented. Figure 4.6 shows a much larger biofilm clump located on the sample from Reactor 2, at the same scale as the images in Figure 4.5.

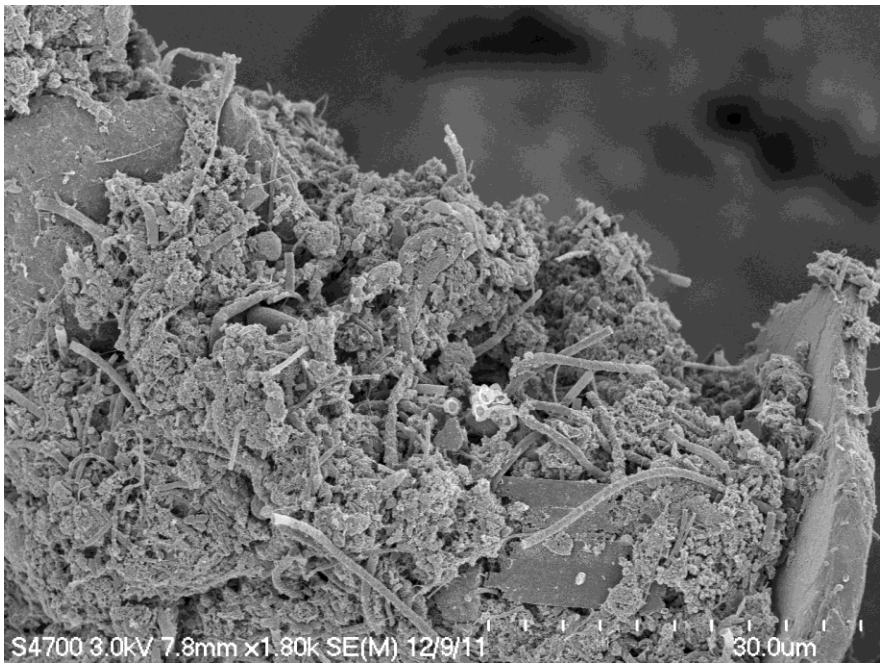

Figure 4.6: Large biofilm formation on the Reactor 2 sample.

It is possible that multiple species of fungi are also present on these samples. The long, tube-like structures observed in many of the figures are characteristic of fungi. Figure 4.7 shows an example of these structures, as well as a few other unknown organisms observed. In future experiments, it may be interesting to do a

more detailed study on the different types of bacteria, fungi, and other microorganisms present in MFCs powered by wastewater sludge, as the range is obviously very broad.

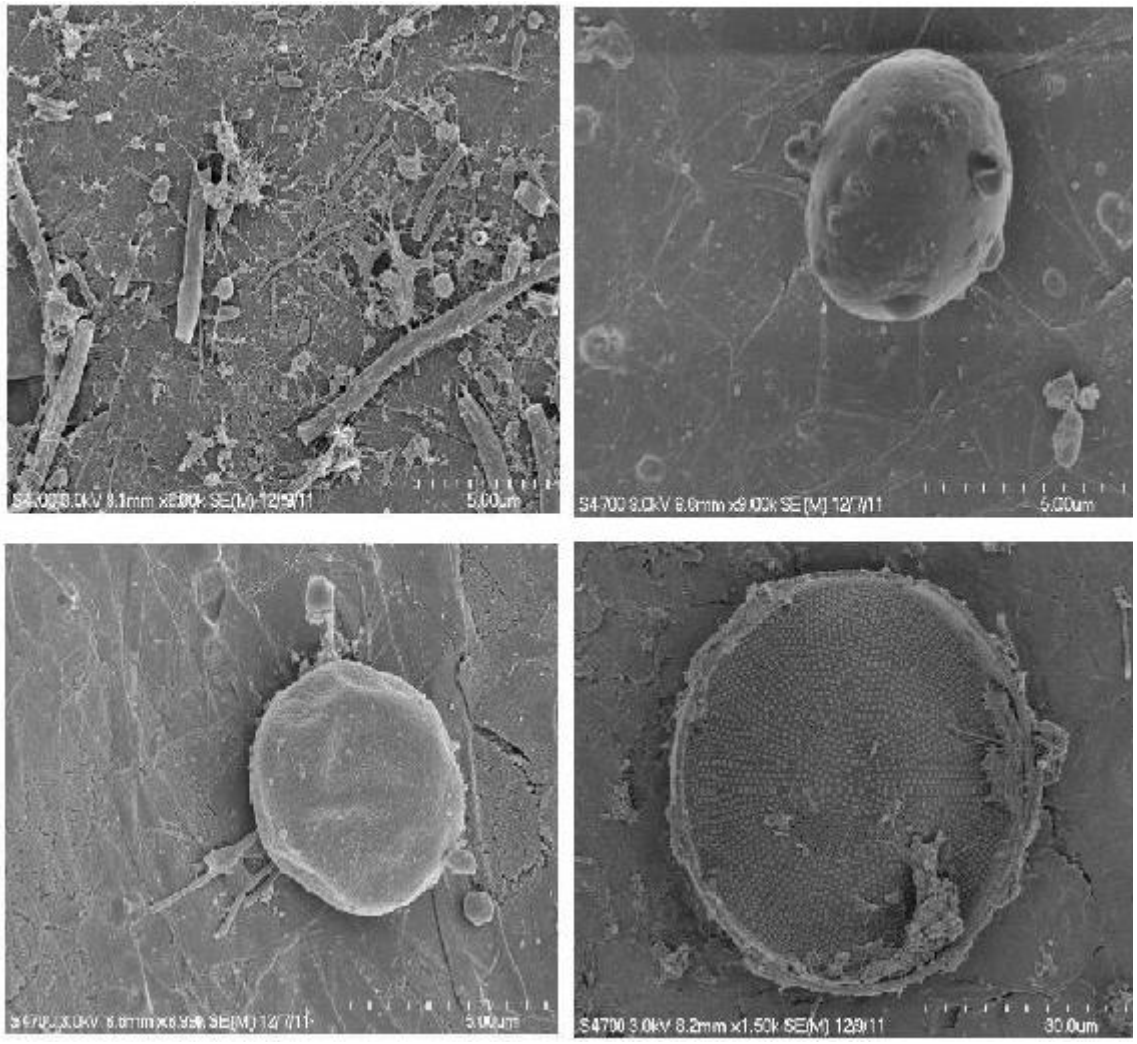

Figure 4.7: Unknown species observed during imaging.

The scope of this project was not large enough to include an experimental comparison of redox mediators and exoelectrogens in wastewater-powered MFCs.

However, the SEM imaging study gives reason to believe that certain microbes residing in the wastewater sludge may be capable of directly transferring electrons to the electrode. A denser and more robust biofilm may form on the surface of the anode, but future experiments are also needed to examine this theory more closely.

4.4 Conclusions

The first hypothesis made for this project proposed that biosolids can substitute for wastewater as an effective source of organic material for microbial fuel cells. This hypothesis was proven at the conclusion of Phase I, and confirmed by Phase II. A sustainable, usable current was generated within each fuel cell, regardless of the supplemental nutrient source or enzyme application. The energy released by the microbial digestion of organic material in the sludge was converted to a measurable amount of electricity, instead of methane gas. A balanced nutrient source is required to optimize results in this type of system. Without this supplemental source, the microbes quickly consume all available nutrients within the system and cannot sustain their growth and activity levels.

It is possible that the nutrient source also stabilizes the microbes so that they are more able to benefit from the applied enzyme effects. The second hypothesis made for this project proposed that an enzyme treatment can be used to enhance microbial growth, boosting electrical generation rates. This hypothesis was also proven, by the experiments performed during Phase II. The optimal daily enzyme dosage is estimated to be between 20 and 40 mg, when using digested wastewater sludge. The electrical output measured for reactors receiving an enzyme dose in this range performed significantly better than reactors that did not receive an enzyme treatment and reactors that received a higher enzyme dosage. The last part of that

statement indicates that it is possible to apply too much enzyme, making the treatment ineffective. It is important to have the correct balance of nutrients and enzyme to achieve high electrical generation rates. The implications of this research will not be fully understood until a full-scale reactor can be operated under the conditions used to conduct these experiments. These systems have the potential to significantly reduce the economic and energy costs associated with modern wastewater treatment and the handling and disposal of biosolids.

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