DESIGN, IMPROVEMENT IN THE CONSTRUCTION AND ANALYSIS OF THE ELECTRICAL EFFICIENCY OF A MICROBIAL FUEL CELL

by

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Abstract

The scientific community is in urgent need to develop instrumentation capable of using alternate energy sources which help to solve the petroleum crisis that world is encountering today. Among the proposed technologies, microbial fuel cells (MFCs) can be a viable alternative to alleviate this problem. MFCs are devices that allow the conversion of organic matter into electricity through the metabolic activity of microorganisms.

The MFC design used in our investigation consisted of two glass cylindrical compartments arrange one inside the other and partition with a Nafion proton exchange membrane. The larger cylinder composes the anode compartment with a volume capacity of 250mL of microbial growth media, while the smaller cylinder consist of the cathode compartment which holds a volume of 50mL of 0.1M potassium ferricyanide. Using this setup, the use of bacteria obtained from contaminated soil was investigated including the addition of glucose to the microbial growth media (tryptone and yeast extract media) and the addition of PBS in the cathode compartment. The design was tested with different resistors to evaluate the compliance with Ohm's Law.

It was found that the mixed bacterial culture obtained in the contaminated soil belong to the gram negative species with "coccus" and "bacillus" shape, and capable of producing an electric current between 0.35 - 0.36mA and cell voltage 166 - 170 mV using a 470ohm resistor. A 432mC increase in the coulombic output was obtained with the addition of glucose to the protein media and PBS addition in the cathode compartment did not improved MFC performance.

Resumen

La comunidad científica está en urgente necesidad de desarrollar alternativas capaces de utilizar fuentes alternas de energía que ayuden a resolver la crisis del petróleo que el mundo está enfrentando hoy en día. Entre las tecnologías propuestas, las células de combustible microbianas (MFC), constituyen una alternativa viable para aliviar este problema. MFC son dispositivos que permiten la conversión de la materia orgánica en electricidad a través de la actividad metabólica de los microrganismos.

El diseño de MFC utilizado en nuestra investigación consistió en dos compartimentos de vidrio cilíndricos insertado uno dentro del otro y la partición con una membrana de Nafion de intercambio de protones. El cilindro más grande compone el compartimiento del ánodo con una capacidad de volumen de 250 ml de medio de crecimiento microbiano, mientras que el cilindro más pequeño consiste en el compartimiento catódico que contiene un volumen de 50 ml de ferricianuro de potasio 0.10 M. Utilizando esta configuración, el uso de bacterias obtenido a partir de suelo contaminado fue investigado incluyendo la adición de glucosa al medio de crecimiento microbiano (triptona extracto de levadura y medios) y la adición de PBS en el compartimiento catódico. El diseño ha sido probado con resistencias diferentes para evaluar el cumplimiento de la Ley de Ohm.

Se encontró que el cultivo mixto bacteriano obtenido en el suelo pertenecen a las especies gram negativas con formas de "cocos" y "bacilos", capaces de producir una corriente eléctrica entre 0.35 - 0.36mA y voltajes de 166 - 170 mV utilizando una resistencia de 470Ω. Un aumento en la salida 432mC culómbica se obtuvo con la adición de glucosa a los medios de proteína, y la adición de PBS en el compartimiento del cátodo no mejoró el rendimiento de MFC.

Acknowledgements

During the period in which I have led the research detailed here I have received the support of many individuals, who I desire to acknowledge and thank.

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1. Introduction

Fossil fuels have become the central economic growth of several countries during the past century. It was estimated that in 2011 alone 19.0 PetaWatts hours were consumed worldwide of which 85.5% (petroleum 33.1%, coal 30.3% and natural gas 22.1%) comes from fossil fuels (1). The increase in energy and fossil fuel consumption throughout the years has prompted the search for alternative energy routes that help decrease the dependence on fossil fuels for energy generation. Among the proposed alternate routes, MFC technology can be a viable alternative to alleviate this problem (2).

Microbial fuel cells (MFCs) are devices that allow the conversion of organic matter into electricity through the metabolic activity of microorganisms. These devices mainly consist of an anode, a cathode and an electrolyte (usually in membrane form). Microorganisms found in the anodic compartment oxidize the substrate used as fuel in the MFC and protons and electrons are generated in the process. The generated electrons are deposited into the surface of the anode electrode and are transported through an external circuit to the cathode electrode. Protons pass through the proton exchange membrane to the cathode compartment where they combine with electrons from the circuit and oxygen to form water. The amount of electrons and protons produced depends upon the substrate use by the microorganisms. Figure 1 demonstrates a common schematic of a two compartment MFC.

Most common reactions that take place in MFCs are shown below, using glucose as an example substrate:

Anodic reaction: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$

Cathodic reaction: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

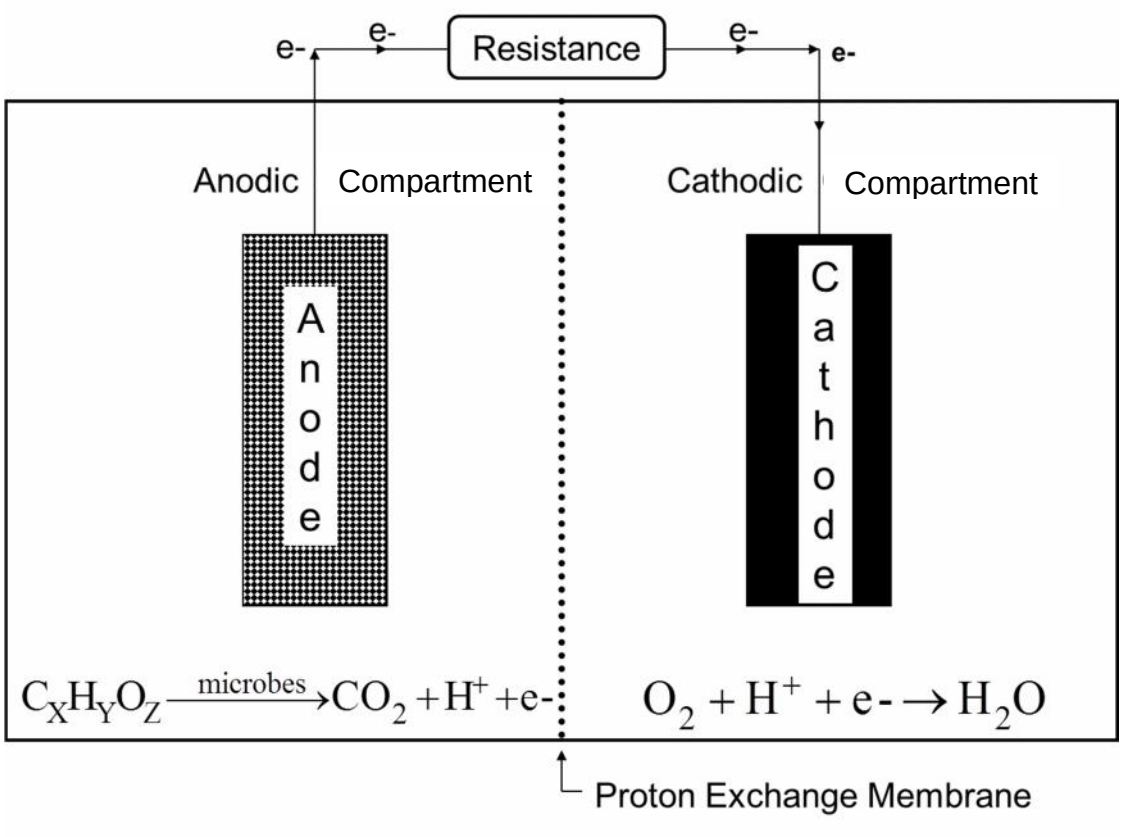

Figure 1: Two compartment MFC diagram. The amount of carbon dioxide, protons and electrons from the fuel degradation depends on the substrate use.

In the anode compartment for each mole of glucose ($C_6H_{12}O_6$) degraded by microorganisms: 24 moles of H^+, 6 moles of carbon dioxide (CO_2) and 24 electrons are produced. While in the cathode compartment, the electrons from the circuit and the protons that migrated through the membrane are combined with oxygen from the environment to form water. For every mole of oxygen (O_2) consumed 4 moles of H^+ and 4 electrons are required to complete the reaction.

The use of O_2 in the cathode compartment has become a limiting factor in the performance of an MFC; therefore, it has been enhanced by electron acceptor agents, such as potassium ferricyanide, whose use has shown to improve the MFC performance by 50% to 80 % (3). When

potassium ferricyanide is utilize instead of O_2 alone the following reactions take place on the surface of the cathode electrode:

Cathodic reactions: $[Fe(CN)_6]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-}$

$$O_2 + 4[Fe(CN)_6]^{4-} + 4H^+ \rightarrow [Fe(CN)_6]^{3-} + 2H_2O$$

The first reaction is the reduction of ferric ion (Fe^{+3}) present in the ferricyanide complex ion ($[Fe(CN)_6]^{3-}$) to the ferrous state (Fe^{+2}). A total of 4 moles of ferrocyanide ion ($[Fe(CN)_6]^{4-}$) reacts with 4 moles of H^+ and 1 mole of O_2 to complete the formation of water and return to their original ferricyanide ion.

Microorganisms are a major factor in the building of a MFC. Usage of pure cultures requires the addition of specific substrates (e.g. glucose for *Escherichia Coli* and acetate for *Geobacter* species) thus limiting the type of substrate used in MFCs (4) (5) . However, the utilization of mixed cultures instead of pure cultures have overcome this limitation and allowed the use of more complex substrates such as wastewater in the operation of MFCs. Substrates used in MFC vary from simple carbohydrates to complex proteins (3) (6). The substrates most common and widely studied are glucose, acetate and wastewater (7).

As mentioned above MFCs are partitioned by proton exchange membranes. The most commonly uses membranes are the Nafion membranes because of their specific conductivity and their extended lifetime (104-105 hrs). Nafion membranes are mechanically stable at temperatures below 100, chemical resistant and durable (8).

The main focus of this research is the design and construction of a MFC for the electrical analysis of microorganisms present in landfills, and the effect of glucose addition to microbial growth media and the effect of buffer addition to the potassium ferricyanide in the cathode compartment. The design consists of two glass cylindrical compartments arranged one inside the

other. The inner cylinder constitutes the cathode compartment with a diameter 1.5 inches and a height of approximately 6.0 inches. The outer cylinder composes the anode compartment with a diameter 2.8 inches and a height 5.7 inches. Both the anode and the cathode electrodes were made with single carbon cloth which was gripped by Platinum (Pt) plates of one square centimeter. These Pt plates were in turn fused to a copper wire insulated with polymers to facilitate connection between the MFC and the instrument.

1.1 Objectives

The objective of this research is to analyze the electricity generated using a design MFC containing microorganisms obtained from a polluted soil.

The specific objectives from this investigation are:

1. Design and construct a new microbial fuel cell with two compartments.
2. Separate microorganisms found in soil contaminated with domestic waste.
3. Determine suitable external resistance for the operation of the MFC.
4. Compare voltage and current measurements obtained with and without bacteria present inside the MFC.
5. Evaluate the effect of adding glucose to a bacterial growth media high in protein.
6. Evaluate the effect of buffer addition to the cathode compartment.

2. Previous Works

Michael C. Potter (6), from the University of Durham, discovered that bacterial culture has the ability to generate electricity. In his experiment, a platinum electrode was placed into *Escherichia coli* (*E.* coli) cultures and a difference in potential was observed.

Barnett Cohen (8), from Johns Hopkins Medical School, demonstrated that microbial fuel cells connected in series were capable of producing higher voltages, creating a bacterial battery. This bacterial battery was able to produce 2 milliamps of current.

Tanaka et al. (4) studied the performances of bio-fuel cells containing *E. coli*, glucose and a series of ferric chelates. Measurements in coulombic outputs indicate that most of the compounds worked effectively as electron-transfer mediators in the fuel cells. These outputs were compared with measured rate constants for the reduction of ferric chelates by *E. coli* and the electrochemical reactions of these compounds at a carbon electrode. Their results suggested that a good mediator for a microbial fuel cell is one which shows fast reduction by *E. coli*, together with a fast electrode reaction. In regenerative fuel cells which were run for five days, coulombic yields over 70% were obtained on the basis of complete oxidation of added glucose.

Tanaka et al. (9) studied the effects of mixed mediator systems consisting of a series of ferric chelate compounds and thionine in bio-fuel cells containing *E. coli*. Significant increases in the coulombic output from the bio-fuel cells containing mixed mediators were observed when compared with those obtained from the fuel cells containing single mediators. A correlation was observed between the cell potential of a bio-fuel cell containing Fe(III)EDTA and thionine and the concentration of Fe(III)EDTA in the anode compartment, indicating that the concentration of the reduced form of ferric chelate was dominant maintaining the cell potential. The reduction of thionine by Escherichia coli in the presence of Fe(III)EDTA, and Fe(III)EDTA by Escherichia

coli in the presence of thionine were measured. Their results support a proposed mechanism involving a redox coupling reaction between Fe(III)EDTA and reduced thionine.

Vega and Fernández (10) obtained potentiometric measurements in MFCs containing *Lactobacillus plantarum, Streptococcus lactis* or *Erwinia dissolvens* as the anodic reducing agent and glucose as the oxidizable substrate. The catalytic effect of five ferric chelate compounds was studied under different conditions. The coulombic yields of the MFC containing the microorganism *Erwinia dissolvens* indicated larger degree of energy conversion in the systems studied, and were larger than those reported in the literature for *E. coli*. The authors also investigated the use of ferric chelate mediators such as Fe(III)TTHA, Fe(III)CyDTA, Fe(III)EDTA, Fe(III)DTPA, and Fe(III)EDADPA. Fe(III)CyDTA, The mediators Fe(III)DTPA, and Fe(III)TTHA produced approximately the same total electric charge when present in a concentration of 0.01 molal. A maximum power output of 0.22mW was obtained in 12h with Fe(III)CyDTA using an external resistance of 560Ω. In regenerative fuel cells which were run for five consecutive days, coulombic yields with an average of 80% efficiency were obtained on the basis of complete oxidation of glucose added.

Pham et al. (11) in a study utilizing a mediatorless MFC attempted to enhance current generation by decreasing the oxygen diffusion present in cation-specific membranes using ferricyanide as mediator and a platinum-coated graphite electrode in the cathode compartment. The authors observed that ferricyanide behaves as an oxidant agent rather than a mediator, and by coating the surface of the graphite electrode with a platinum paste, current generation was 3 to 4 times higher in comparison with the non-coated graphite electrode. They also found that graphite electrodes require more dissolved oxygen concentrations than the platinum-coated graphite electrodes (concentrations of 6.6 mg/L and 2.0 mg/L, respectively). These results

demonstrated that inexpensive electrode materials, such as graphite are adequate for the construction of a MFC.

Min et al. (5) compared the power output in a MFC containing a proton exchange membrane using a *Geobacter metallireducens (G. metallireducens)* pure culture vs. a mixed inoculum from wastewater. Power output with each of the inoculum was essentially the same, 40 ± 1mW/m^2 for *G. metallireducens* and 38 ± 1mW/m^2 for wastewater inoculum. They also examined the power output in a MFC by substituting the membrane with a salt bridge. Power output by the salt bridge MFC, inoculated with *G. metallireducens*, was 2.2mW/m^2. The authors directly attributed the low power output to the higher internal resistance of the salt bridge system (19920 ± 50Ω) in comparison with the membrane system (1286 ± 1Ω), which were measured using electrochemical impedance spectroscopy. In both systems, it was detected that oxygen diffusion from the cathode compartment into the anode compartment was a factor in coulombic efficiency. Sparging nitrogen gas limited the effect of gas diffusion into the anode compartment and increased Coulombic efficiency (47% to 55%) when compared to that obtained without gas sparging (19%). The authors demonstrated that increasing power densities in MFCs will require a reduction the internal resistance of the systems, and control the dissolved oxygen flux in the anode compartment in order to increase overall Coulombic efficiency.

Heilmann and Logan (12) examined the electricity generation from proteins and a protein-rich wastewater using a single chamber MFC. Maximum power densities of 354 ± 10 mW/m^2 using bovine serum albumin (BSA) and 269 ± 14mW/m^2 using peptone (1100 mg/L BSA and 300mg/L peptone) were observed. They found that the recovery of organic matter as electricity, defined as the Coulombic efficiencies (CE), was comparable to that obtained with other substrates with CE=20.6% for BSA and CE=6.0% for peptone. A meat packing wastewater

(MPW) produced a power density of 80 ± 1mW/m^2, and power was increased by 33% when 300mg/L of sodium chloride (NaCl) where added to increase solution conductivity. A wastewater inoculum generated 33% less power than the MPW inoculum. The authors concluded that an MFC is an effective method of wastewater treatment and electricity generation.

Mohan et al. (13) studied the effect of different electron mediators, mediator concentration, ionic strength (salt concentration) of the medium, and the surface area of the salt bridge between the anode (*Enterobacter cloacae* IIT-BT 08 in MYG medium) and cathode compartments of MFCs. In the case of Methyl Viologen (MV) (0.1mM) as the electron mediator, the voltage generation was 0.4 V but not current was detected. Different concentrations of Methylene Blue (MB) were also studied as a mediator. A maximum voltage of 0.37V was seen at 0.05mM MB with a maximum current and power of 56.7μA and 19.2μW. However a voltage of 0.34V was observed in 0.03mM MB with a current density of 9.3mW/m^2 and 27.6mA/m^2. When the surface area of the salt bridge between the anode and cathode compartments was increased, an improvement in the power output from 19.2 to 708μW was detected. The maximum power density and current density observed were 236mW/m^2 and 666.7mA/m^2 respectively.

Daniel et al. (14) developed a MFC composed of *Pseudomonas species*, mediator and potassium ferricyanide as the oxidizing agent for electricity generation using wastewater (obtained from a wastewater treatment) as substrate. Cells were connected in series with the anodic and cathodic solution being introduced in batch and continuous modes. A maximum open-circuit potential of 2.2V was obtained with the anode in batch-fed and cathode in continuous operation mode. Methylene Blue, used as mediator was found to produce a higher output from the cell when compared to Neutral Red. The maximum power output and current density obtained were 979μW/m^2 and 1.15mA/m^2.

Mohan and Das (2) examined the effect of ionic strength, cation exchanger, and inoculum age on the power generation in a mediator MFC with methylene blue as electron mediator using *Enterobacter cloacae (E. cloacae) IIT-BT08*. The effect of ionic strength was studied using NaCl in the anode compartment from two compartment salt-bridge MFC at concentrations of 5mM, 10mM and 15mM. Maximum power density of 12.8mW/m^2 and current density of 35.5mA/m^2 was noticed when 10mM NaCl was used. They observed a 3-fold increase in the power density when the salt-bridge was replaced by a proton exchange membrane. Power density and current density of 37.8 mW/m^2 and 110.3 mA/m^2 were detected. The influence of pre-inoculum on the MFC was studied using *E. cloacae IIT-BT08* grown for 12, 14, 16 and 18 h. It was observed that 16 h grown culture inoculated in the anode compartment gave maximum power output. Power density and current density of 68 mW/m^2 and 268 mA/m^2 were obtained. Results demonstrated that biological parameters need to be optimized in order to improve power generation in MFCs.

Di Lorenzo et al. (15) evaluated the performance of a single compartment microbial fuel cell (SCMFC) using packed beds of irregular graphite granules as anode material and microorganism culture from wastewater in batch and continuous operation mode. The current output was found to be higher with the increase in the thickness of the anode bed and with the approximate anode area. The best performance was obtained using a 3 cm anode bed depth SCMFC. When operated in batch mode, Coulombic efficiencies vary from 30% to 74% depending upon glucose feed. In continuous operation mode, Coulombic efficiency was 68% with glucose feed of 50 ppm, and a flow rate of 0.0028 cm^3/min. Power performance was also reasonable with a volumetric power density of 1.3 W/m^3, with respect to the net anodic volume (12.5 cm^3). Comparable performance was achieved with wastewater.

Nam et al. (16) investigated the effect of several common buffers (phosphate, MES, HEPES and PIPES) on the power production in single compartment MFCs compared to a non-buffered control. At the same concentrations, the buffers produced different solution conductivities which resulted in different ohmic resistances and power densities. Increasing the solution conductivities to the same values using NaCl produced comparable power densities for all buffers. Very large increases in conductivity resulted in a rapid voltage drop at high current densities. They suggest that solution conductivity at a specific pH for each buffer is more important in MFC studies than the buffer itself given relatively constant pH conditions. Based on the their analysis of the internal resistance and a set of neutral pH, phosphate and PIPES are the most useful buffers of those examined because pH was maintained close to the pK_a of the buffer, maximizing the ability of the buffer to contribute to increase current generation at high power densities.

3. Materials and methodology

3.1 Materials

Table 1: Materials use in the MFC construction and operation

Glass bottle with plastic cap
Glass tubes
Glass pasteur pipet
Silicone
Polyvinyl chloride caps
Leaded 60/40, rosin core wire solder
B-1 Designation B Carbon cloth, no-wet proofing (Clean Fuel Cell Energy, LLC)
Nafion 115 membrane (ElectroChem, Inc.)
Multipurpose nylon binding posts
Copper wire
Platinum film
Resistors: 100Ω, 220Ω, 330Ω, 470Ω, 560Ω and 680Ω

Table 2: Reagents List

2X YT Microbial Medium (Sigma-Aldrich)
Glycerol, 99.5+%, A.C.S reagent (Sigma-Aldrich)
Hydrogen Peroxide 30% solution, H_2O_2 (Fisher Scientific)
Sulfuric acid, H_2SO_4 (VWR)
Ethanol 98% (Sigma Aldrich)
Hydrochloric acid, HCl (VWR)
Potassium Ferricyanide Certified A.C.S., $K_3Fe(CN)_6$ (Fisher Scientific)
D-(+)-Glucose, minimum 99.5 % (Sigma- Aldrich)
Sodium Phosphate Dibasic, minimum 99.0%, SigmaUltra, Na_2HPO_4 (Sigma-Aldrich)
Sodium Phosphate Monobasic Monohydrate, A.C.S. Reagent, $NaH_2PO_4.H_2O$ (Sigma-Aldrich)
Crystal Violet, Fisher Scientific
Iodine Gram Solution, 0.25N, Fisher Scientific
Safranin O, Fisher Scientific
N_2 gas, Industrial grade non certificate (Linde gas)
Calibration Buffer Solution, Certified 4.00 pH, Fisher Scientific
Calibration Buffer Solution, Certified 7.00 pH, Fisher Scientific

Table 3: Equipment

Acculab L-Series Analytical Balance
Glass tube cutter
Market Forge Sterilmatic Autoclave
Precision Reciprocal Shaking Bath
BK Precision model 5492 5 ½ Digit Multimeter
Revco Ultra-low Temperature Upright Freezer
Fisher Scientific Accumet Basic pH meter model AB15
Fisher Scientific Advanced Compound Microscope
Oxford Benchmate Micropipette 100 - 1000µL

Table 4: Materials List

Amber volumetric flask, 50.00mL
Volumetric flask, 1.00L and 2.00L
Weighing dishes, small and medium, Fisher Scientific
Glass microscope slides, 75mm x 25mm, Fisher Scientific
Micropipette tips, VWR, 1000µL
Microcentrifuge tubes**,** 1.50mL
Alcohol Burner

3.2 Methodology

A summary list of materials used for construction of the MFC is shown in Table 1 and discussed in the following section. Table 2 details the reagents used during the course of the experiment. Their purity and vendor are also included. The equipment employ throughout the research is mention in Table 3. Table 4 summarizes glassware and other materials use for solution preparation.

3.2.1 MFC design

The MFC design consists of two cylindrical compartments, anode and cathode, partitioned with a proton exchange membrane. Electrodes made of non-wet proof carbon cloth were employed as reaction sites in both compartments. An outline of the cell design is shown in Figure 2.

The anodic compartment contained microbes isolated from a soil sample taken from an old municipal domestic landfill (no longer in used) located in the town of Rincón, Puerto Rico (Figure 3). Microbes were used as biocatalysts for substrate degradation. The cathodic compartment contained $K_3Fe(CN)_6$ solution as an electron acceptor.

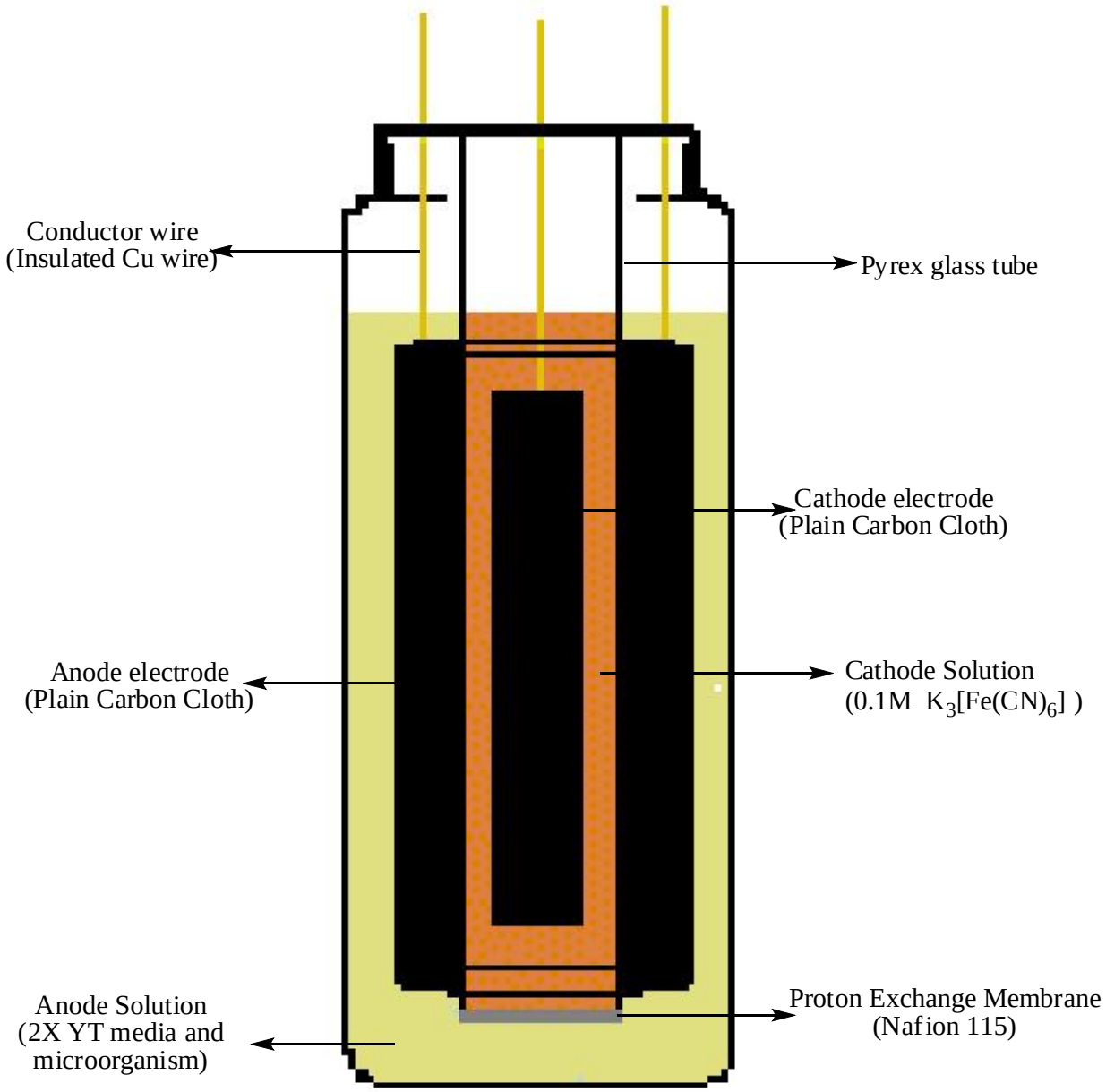

Figure 2: MFC design outline

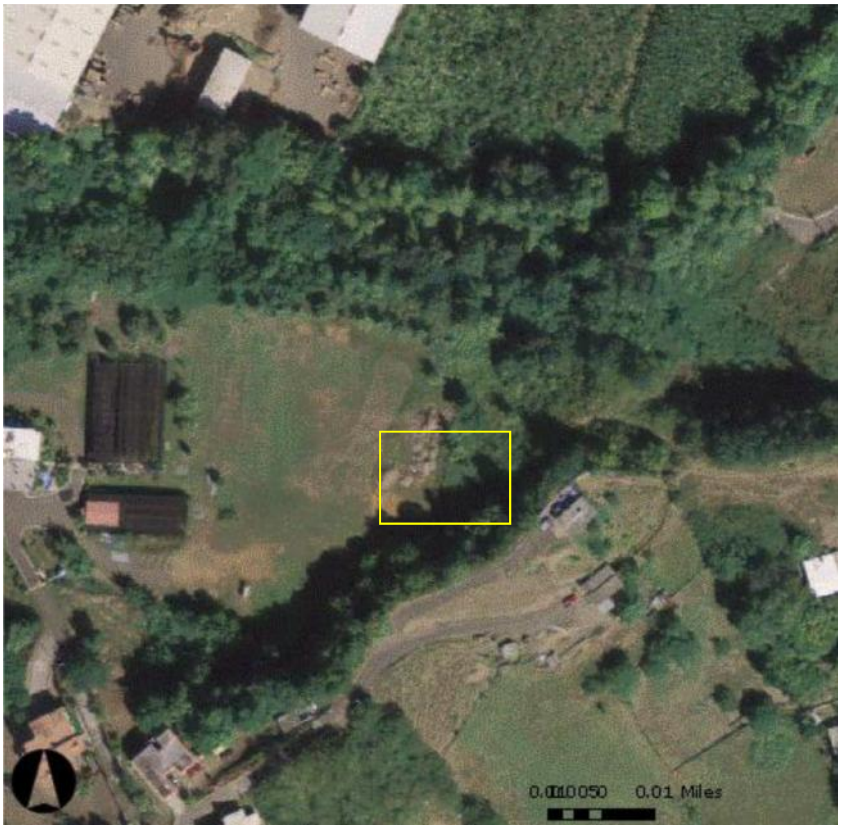

Figure 3: Satellite image of Rincón's old domestic landfill. The yellow square indicates the approximate location where the soil sample was taken.

3.2.2. MFC Construction

3.2.2.1 Anodic compartment

A glass bottle and a plastic cap were utilized for the anode comparment as shown in Figure 4A. A total of five holes were drilled to the plastic cap for the placement of different components (Figure 4B).

Two holes were drilled on one side of the plastic cap; a small Pasteur pipet was inserted in one hole to introduce the nitrogen gas into the anode chamber and a small glass tube that allows the gases in the anode compartment to escape was inserted in the other one. The glass tube and the Pasteur pipet were sealed with silicone in the correspondent holes (Figure 4B). This process requires a 24 hour of curing period prior to be use, and ensures anaerobic conditions within the cell.

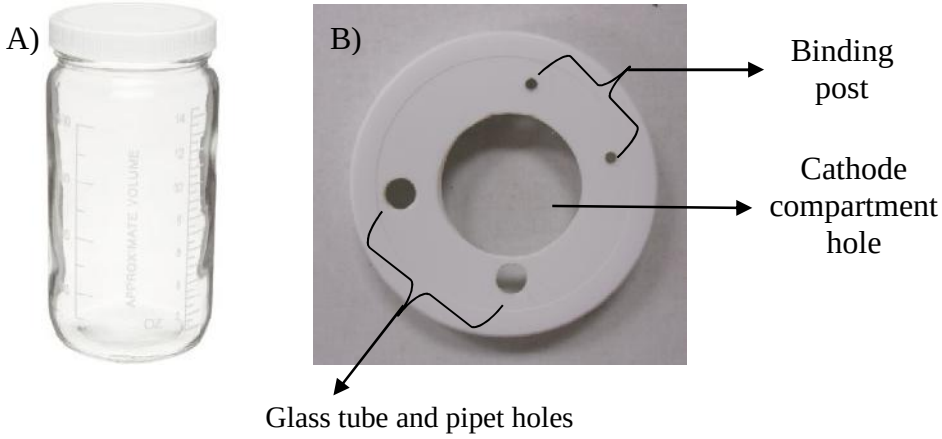

Figure 4: (A) Glass bottle and cap used for the MFC setup. (B) Holes arrangement on the cap of the glass bottle. The holes allowed the placement of different components such as binding posts, cathode compartment and glass tubes.

3.2.2.2 Cathode compartment

A glass tube of approximately 1.5 inches in diameter and 3 feet long was cut into pieces of 5 to 6 inches long. These glass tubes functioned as the cathode compartment.

Figure 5: 2" PVC trap adapter

A polyvinyl chloride (PVC) trap adapter (Figure 5) was secured and sealed, with silicone to one end of the glass piece and allowed to rest for 24 hours for the curing process to be completed. The function of the adapter is to hold the membrane in place, and to avoid the mixing of solutions between compartments.

To ensure that silicone had sealed properly, a test was conducted by placing the glass tube piece in distilled water for a period of 12 hours. If water was visible between the glass tube and PVC adapter after being removed from the water, the silicone had not sealed properly and the seal was redone. This process was repeated until no water was found in between the glass tube and the adapter.

A hole of approximately 3.3 mm in diameter was drilled right in the center of the plastic cap for the placement of the cathode compartment and sealed with silicone to avoid the leak of air in or out of the compartment around the glass tube. The silicone seal was let curing for 24 hours.

3.2.2.3 Membrane

The PEM (Nafion115) were cut in round pieces with a diameter of approximately 3.7 mm.

The Nafion membranes were boiled in H_2O_2 30% v/v for one hour, and later boiled in deionized water for another hour. After this process, the membranes were submerged in 0.50M of H_2SO_4 solution for one hour and washed in deionized water for another hour. Pretreated membranes were stored in deionized water to avoid their swelling during the analysis (17).

3.2.2.4 Electrodes

Two electrodes, one for each comartment, were made of non-treated plain carbon cloth (Clean Fuel Cell Energy, LLC) and cut according to the desired size.

The carbon cloth electrode for the cathode compartment was cut in two inches long by one inch wide, while the anode compartment carbon cloth electrode was cut into a square piece of three by three inches. The electrodes dimensions were held constant throughout all the experiments.

New electrodes were submerged in 98%v/v ethanol for thirty minutes, and later in 1.00M HCl for one hour. Pretreated electrodes were stored in distilled water until their use (17).

3.2.2.4.1 Electrode connections

The electrodes connections were made with a small Platinum (Pt) sheet and insulated Copper (Cu) wire.

Since the carbon cloth is a conductive material, it was only necessary to hold the electrodes from the top with a small Pt sheet. The Pt piece was welded to a piece of Cu wire

using electronic welding material (leaded 60/40, rosin core wire solder) (Figure 6). The exposed weld was sealed with silicone to avoid corrosion and unwanted reactions. The connection was let curing for a period of 24 hours prior to be use.

Figure 6: Electrode connection. A piece of Pt sheet was welded to insulated Cu wire.

In order to connect the electrodes and make the circuit, two small holes were also drilled to one side of the plastic cap, where binding posts were placed to facilitate wiring connection to the multimeter (Figure 7).

Figure 7: Nylon binding posts. The posts facilitated the connection between electrodes, resistor and multimeter alligator clips. The red post was used for the cathode electrode and the black post for the anode electrode

Components

3.3.1 Microorganismss

3.3.1.1 Media Preparation

Growth medium (EZ Mix 2X YT Microbial Medium) was prepared by dissolving 15.8g in 500 mL of deonized water. The medium was divided in five Erlenmeyer flasks and labeled: A, B, C, D and E. These flasks contained volumes of 100 mL, 99 mL, 99 mL, 99 mL and 99 mL respectively. All the flasks containing the medium were sterilized in an autoclave at 121ºC for 15 minutes.

3.3.1.2 Microorganism Preparation from Soil Sample

A soil sample of 1.0147g was added to Flask A and placed in a reciprocal shaking bath at 30ºC and 120 rpm for a period of 24 hours. Shaker parameters were constant the process.

An aliquot of 1.00mL was taken from Flask A and added to flask B. Flask B was placed in the shaker for a period of 24 hours. This process was repeated for flask C. For flask D an aliquot from flask C was added and placed on the shaker for a period of 2-6 hours or until turbidity was observed inside the flask.

Once this process was finished, 1 to 5 aliquots were utilized as freeze stocks.

An illustration of this process is described in Figure 8.

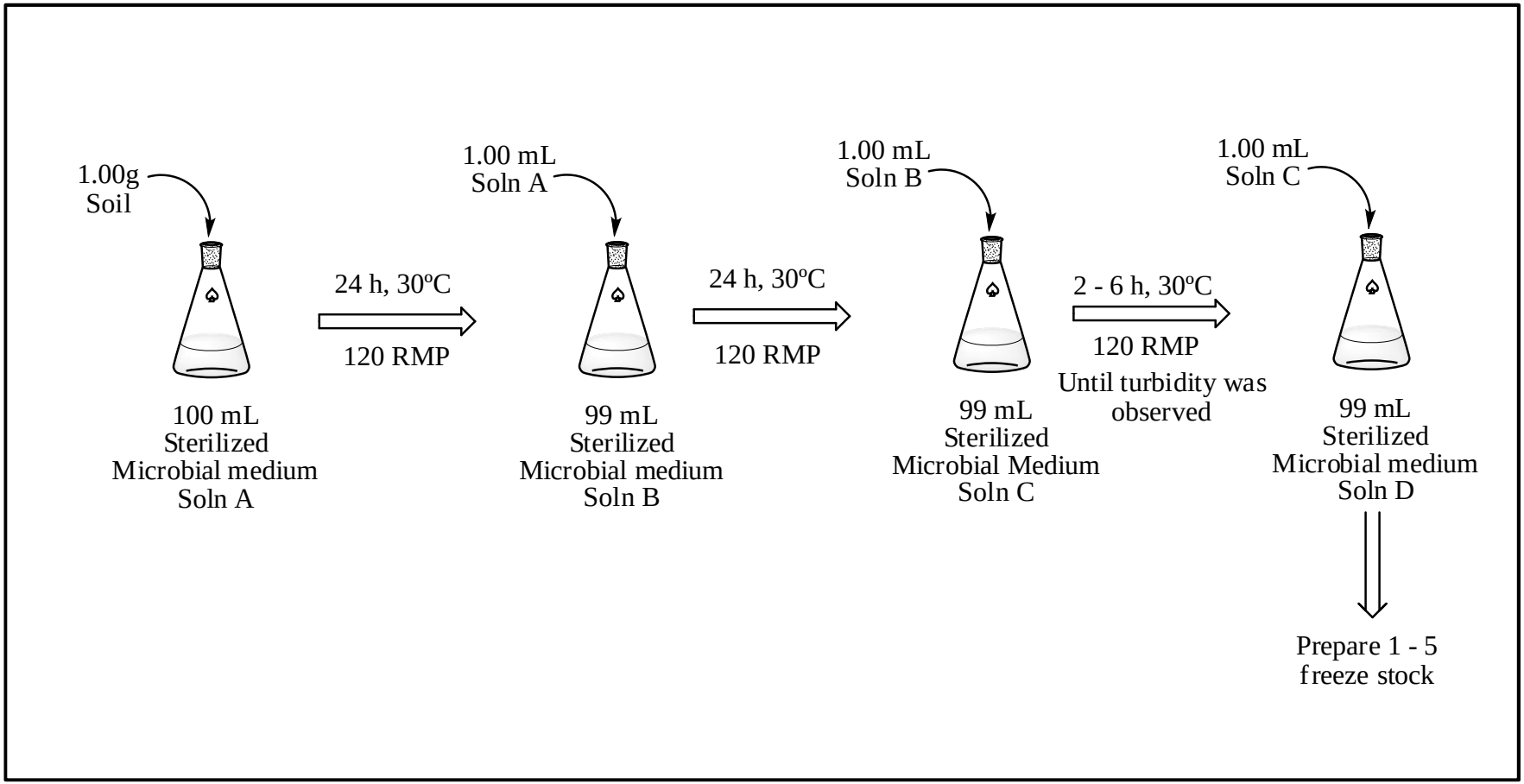

Figure 8: Microbial isolation process from the soil sample.

3.3.1.3 Freeze stocks preparation

A 300µL of glycerol and 700µL of the flask D content were placed together in a microcentrifuge and shaken until a one-phase mix was observed. Samples were stored in a Revco Ultra-low temperature upright freezer at a temperature of approximately -81ºC, for future use.

The addition of glycerol is required to lessen the negative effect caused by the formation of ice crystals when bacteria are stored in freezers.

3.3.1.4 Gram staining procedure

The bacterial sample was placed on a microscope slide. Two to three drops of crystal violet were added, allowed resting on the slide for a period of 1 minute and then washed with distilled water. Then, two to three drops of iodine were added and allowed to stand for a period of 1 minute. Iodine was washed with 98% ethanol for a period of 15 to 30 seconds followed by rinsing with distilled water. Finally, two to three drops of safranin were added to the slide and left standing for 1 minute. The slide was washed with distilled water and dried.

The slide is placed in an advanced compound microscope and observed with a 1000x oil immersion lens.

3.3.2 Electron acceptor agent (cathode solution)

A 0.1M solution of $K_3Fe(CN)_6$ was prepared by placing 1.646g of this salt in an 50.00mL amber color volumetric flask and filled to the mark with distilled water. Solution was done fresh prior to each run.

3.3.3 Anode solution

Growth medium (*EZ Mix 2X YT Microbial Medium*) was prepared by dissolving 36.1g in 1.00L of deionized water. The medium was divided in four bottles of 250mL and sterilized according to the specifications described in section *3.1.1.*

3.4 Final MFC assembly

The MFC was assembly by adding into the glass bottle 250 mL of sterilized medium and one bacterial freeze stock already melted. The anodic electrode was connected to the Pt sheet that was attached to the plastic cap of the bottle which had all the necessary parts for the electric connections. The glass tube and cathode chamber was also connected to the plastic cap.

Once the electrode was connected and the membrane set in the PVC trap, the plastic cap was placed on the bottle and closed. Parafilm wrapping was used to prevent leaking gases. Commercial grade nitrogen gas (Linde Gas Company) was sparged inside the MFC for 5 to 6 minutes to achieve anaerobic conditions. 50.00 mL of 0.10M $K_3Fe(CN)_6$ were added to the cathode chamber, the electrode was placed in the solution and held in place with an alligator clip which contains two pieces of platinum welded to each side. Figure 9 shows final assembly of the MFC.

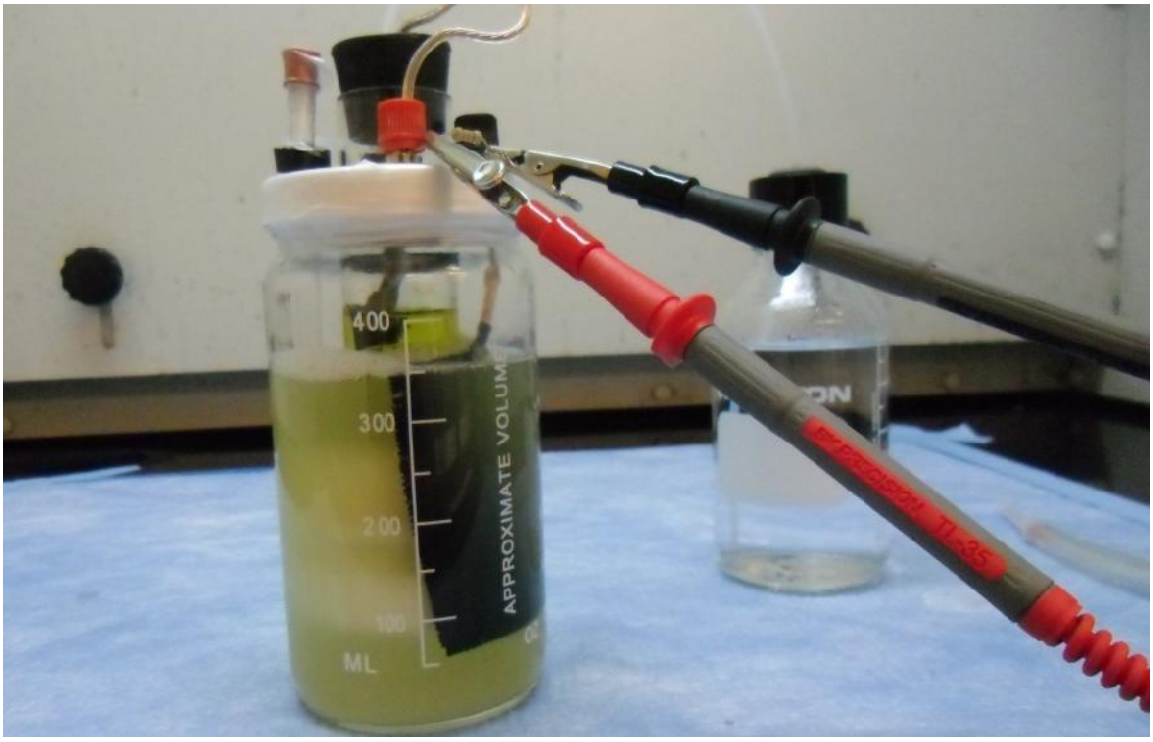

Figure 9: MFC final assembly.

3.5. Voltage measurements

3.5.1 Instrument Setup

Potential measurements were obtained using a BK Precision model 5492 5 ½ Digit Multimeter shown in Figure 10 connected to a PC (Dell Optiblex 380) via RS232 port and USB interface. Operating software used was Bench DMM PC Link downloaded from the manufacturer website. The software setup is described in Table 5.

Figure 10: BK Precision model 5492 5 ½ Digit Multimeter. Instrument use for data collection.

Table 5: Software Parameters

Com port	3
Time setting	Relative
Record Interval	300 seconds
Open Circuit Timer	30 minutes
Closed Circuit Timer	1440 minutes

3.5.2 Evaluation of Different Loads in the MFC Performance

The evaluation of different loads was performed by submitting our MFC to six different resistors (100, 220, 330, 470, 560 and 680 Ω). For each resistor used a MFC was constructed and operated for a period of 24 hours.

3.5.3 Evaluation of glucose addition in the performance of the MFC

Approximately 0.20g of glucose where dissolved in 250.00 mL of microbial medium and sterilized with the specifications mentioned in section *3.1.1*.

The MFC was assembled using this medium instead of the rich medium alone.

3.5.4 Evaluation of buffer addition in the performance of the MFC

A 0.1M phosphate buffer solution (PBS) was selected for the evaluation of buffer addition in the cathode compartment. The PBS was prepared dissolving 9.4796g Na_2HPO_4 and 4.5782g $NaH_2PO_4.H_2O$ in 1.00 L volumetric flask and filled with deionized water.

Since the cathode compartment requires an electron acceptor agent in order to function, the solution was prepared by dissolving 1.646g $K_3Fe(CN)_6$ in 50.00mL of PBS.

4. Results and discussions

4.1 Bacteria

It has been reported that mixed bacterial cultures produce better results than pure cultures of bacteria when used in MFCs, especially if mix community is obtain from wastewater treatment plants or sludge (18). The bacterial cultures added to the MFCs were collected from soil that for many years was used as a domestic landfill.

A Gram's staining was performed to the bacterial sample for preliminary identification. The result of this study is found in Figure 11. In Figure 11A shows a pink color in the slide indicating that the bacterial sample belongs to Gram-negative type. By increasing the magnification of the microscope at 1000x (see Figure 11B) it can be appreciated in detail that the bacteria are shaped like "*coccus*" (represented by the yellow arrow) and "*bacillus*" (shown with white arrow).

It was estimated that the initial concentration of bacteria in the freeze stock used contains an average 895,000,000 colony forming units per mL of sample.

A more detailed analysis should be considered for the identification of bacteria used in these MFCs for the purpose of improving performance.

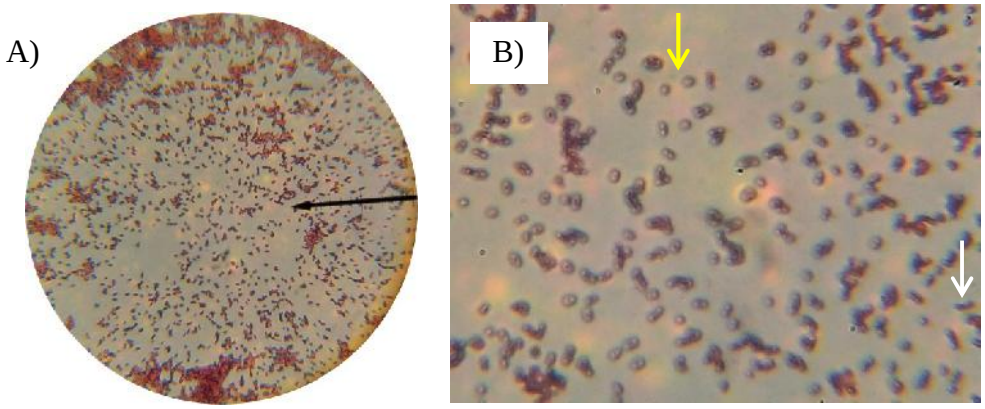

Figure 11: Gram staining results through 100x objective. A) The pink color spots indicate the presences of gram negative bacteria. B)"Coccus" (yellow arrow) and "Bacillus" (white arrow) shape bacteria is observed.

4.2 Effect of the presence of bacteria in a MFC

For MFC to function properly, bacteria in the anode compartment have to deposit electrons directly on the electrode (19). A MFC with the setup shown in Figure 2 and described in section 3.4 was assembled with 100Ω resistor, and voltage data was recorded for a period of 24 hours. Figure 12 represents cell voltage in function of time obtained from this test. A small straight line is observed at the beginning of the graph indicating bacterial culture adaptation to its surroundings followed by a rapid increase in voltage. The increase is attributed to the bacterial growth inside the anode compartment due to medium composition and substrate consumption. After reaching maximum output (36.90mV) a rapid descend in voltage is also observed. At this point it is believed that bacterial death has begun due to lack of substrate in the medium and/or an increase in waste generated by bacteria. This hypothesis could not be confirmed because sampling ports for solution replacement were not integrated in MFC design. Taking this flaw into consideration it was decided that MFC running time should not exceed 24 hours.

The peak in the graph demonstrated the reaction between bacteria and electrode which indicates the presence of electroactive bacteria in the anode compartment.

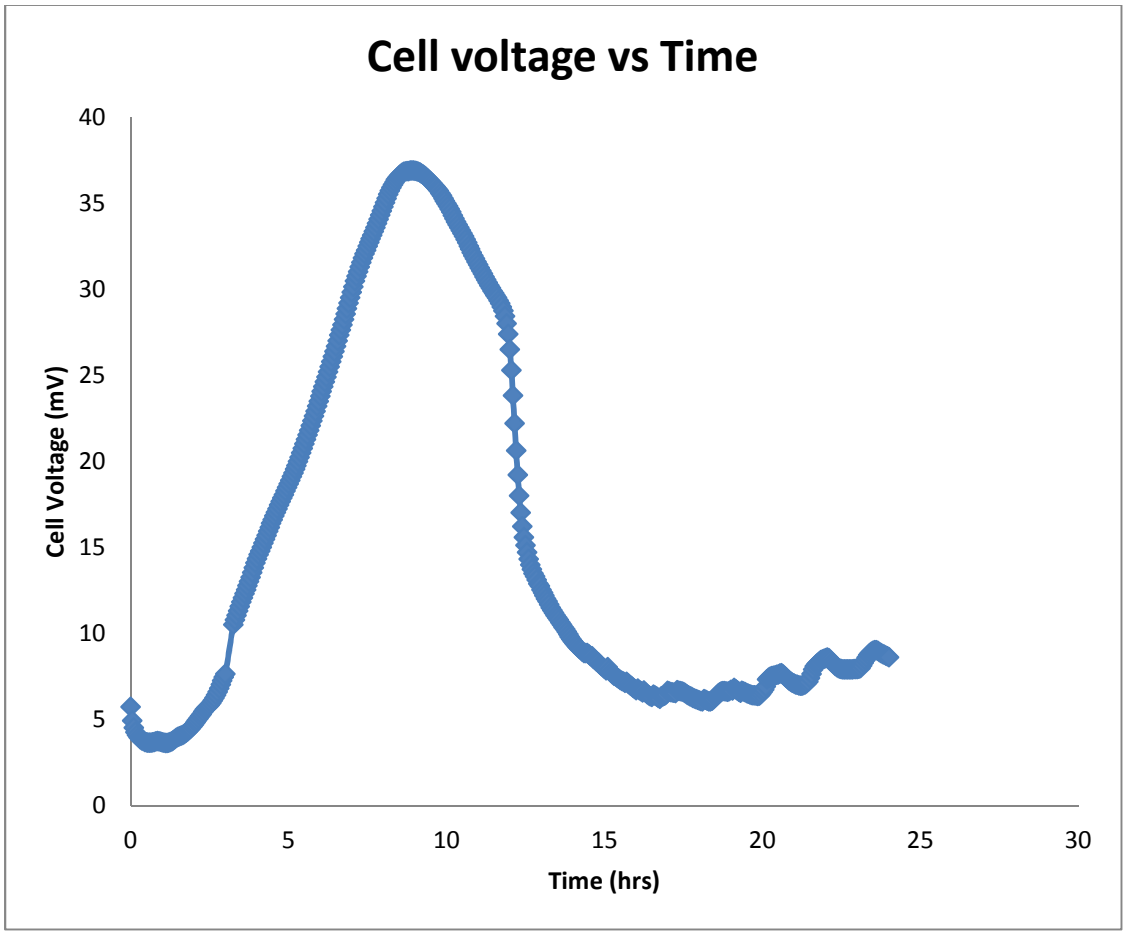

Figure 12: Cell voltage change cause by the addition of bacteria to the MFC. Observed peak demonstrated the presence of electroactive bacteria in the anode chamber.

4.3 Evaluation of Different Resistor in the MFC Performance

The cell voltage (V) in a MFC is a function of the current (I) and the external resistance (R_{ext}), or load on the circuit. It is well known that current production on these devices is rather small (in the order of milliampere to microampere), when MFC are studied this value is calculated from the measured voltage drop against a resistor (20). The following equation is utilized to calculate the current on MFCs which is obtained from the Ohm's Law (Logan, 2006):

$$I = \frac{V}{R_{ext}}$$

All current calculation where done with the formula described above.

The use of the correct R_{ext} in a MFC is very important since a small R_{ext} auses a rapidly discharge and higher R_{ext} favors methanogenic bacteria growth instead of electroactive bacteria necessary for MFC function (20). In this case the optimization of the resistance is carried out by submitting the MFC to six different resistors (100Ω, 220Ω, 330Ω, 470Ω, 560Ω and 680Ω). For each resistor, an MFC was build using the procedure described in section 3.4. Table 6 shows the maximum cell voltage and current values obtained when MFC was submitted to each different resistor.

Ohm's Law states that voltage and current values increase when resistance values decreases (referencia). Therefore, when using a resistor to close the circuit in an MFC, the resistance value remains constant and there is an increase in voltage. Similarly, increasing resistor values also increases voltage output as long as current production does not change. The cell voltage data in Table 6 follow this pattern except cell voltage obtained with a 560Ω resistor. When comparing 560Ω cell voltage (167.46mV) with the data obtained with 470Ω (169.63mV),

a difference of approximately 2.000mV less is observed. This difference is attributed to experimental errors during the operation of the MFC.

After comparing each of the current measurements for the different resistors, it was decided to use the 470Ω resistor as circuit load for future experiments because of its higher current output (0.36mA).

Table 6: Maximum cell voltage and current values obtained with different resistors

Resistance (Ω)	**Cell Voltage (mV)**	***I* (mA)**
100	32.953	0.33
220	76.513	0.35
330	103.39	0.31
470	169.63	0.36
560	167.46	0.30
680	192.89	0.28

4.4 Cell voltage measurements without bacteria present in the MFC (Control)

A total of two MFC were constructed as controls. Controls were assembly using procedure described in section 3.4 without the addition of bacteria in the anode chamber. Figure 13 represents the average cell voltage gather from the MFC during an interval period of 24 hours. A drop in voltage is observed during the initial measurements. This behavior is to be expected since voltage recording began right after the placement of the resistor in the MFC and not after the MFC has reach steady values. No significant voltage change and power generation are detected without the presence of bacteria in the MFC.

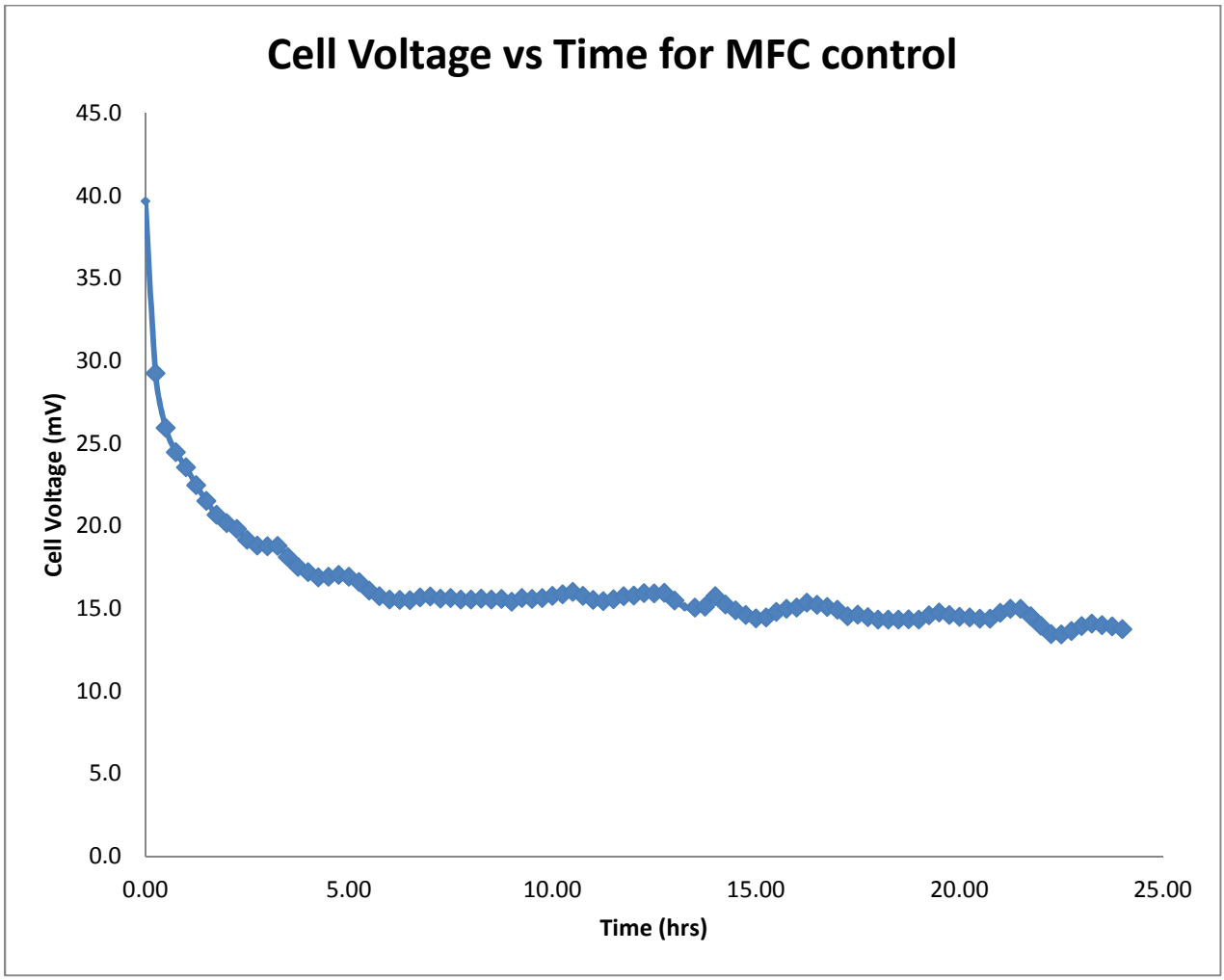

Figure 13: Cell voltage in function of time for MFC Control. The MFC control was run without the presence of bacteria in the anode chamber. No significant voltage change is observed during the 24 hour period. The graphs show the mean data of duplicate experiment.

4.5 Comparison between controls and bacteria addition MFC

A total of three MFC were assembled to observe the effect on the voltage produced by the addition of bacteria to the MFC. Figure 14 shows the voltage obtained from the control and the MFC with bacteria. Maximum voltage was reached after 9.5 hours. Table 7 includes the maximum measurements of voltage, current, power, current density and power density for the control and the MFC with bacteria. Current and power values are calculated using Ohm's law. Current density and power density values are calculated by dividing the current and power measurements between the surface areas of the electrode. Surface area is calculated by multiplying the length by the width of the electrode and then multiplying the result by two, since the electrode is suspended in solution and contains two contact sides (21). The estimated surface area of the electrode used in the anode is $0.0116 m^2$.

When comparing the control voltage with the voltage of the MFC with bacteria there is an increment of about 153mV and an increase in the current of about one order of magnitude. The current density improves from $2.6 mA/m^2$ to $30.9 mA/m^2$ with the addition of bacteria and power density enhances from $44.6 \mu W/m^2$ to $5185.7 \mu W/m^2$. These results evidence that the voltage increase observed in the MFC with bacteria came from the addition of bacteria alone and not from unwanted reactions inside the anode compartment.

Table 7: Voltage, current, power, current density and power density values at 9.5 hours for both control and bacteria MFC.

	Time (hrs)	**Cell Voltage (mV)**	**Current (mA)**	**Power (µW)**	**Current Density (mA/m^2)**	**Power Density ($\mu W/m^2$)**
Control	9.5	15.6 ± 0.7	0.033 ± 0.002	0.52 ± 0.05	2.9 ± 0.1	45 ± 4
Bacteria	9.5	168 ± 2	0.358 ± 0.005	60 ± 1	30.9 ± 0.4	5196 ± 141

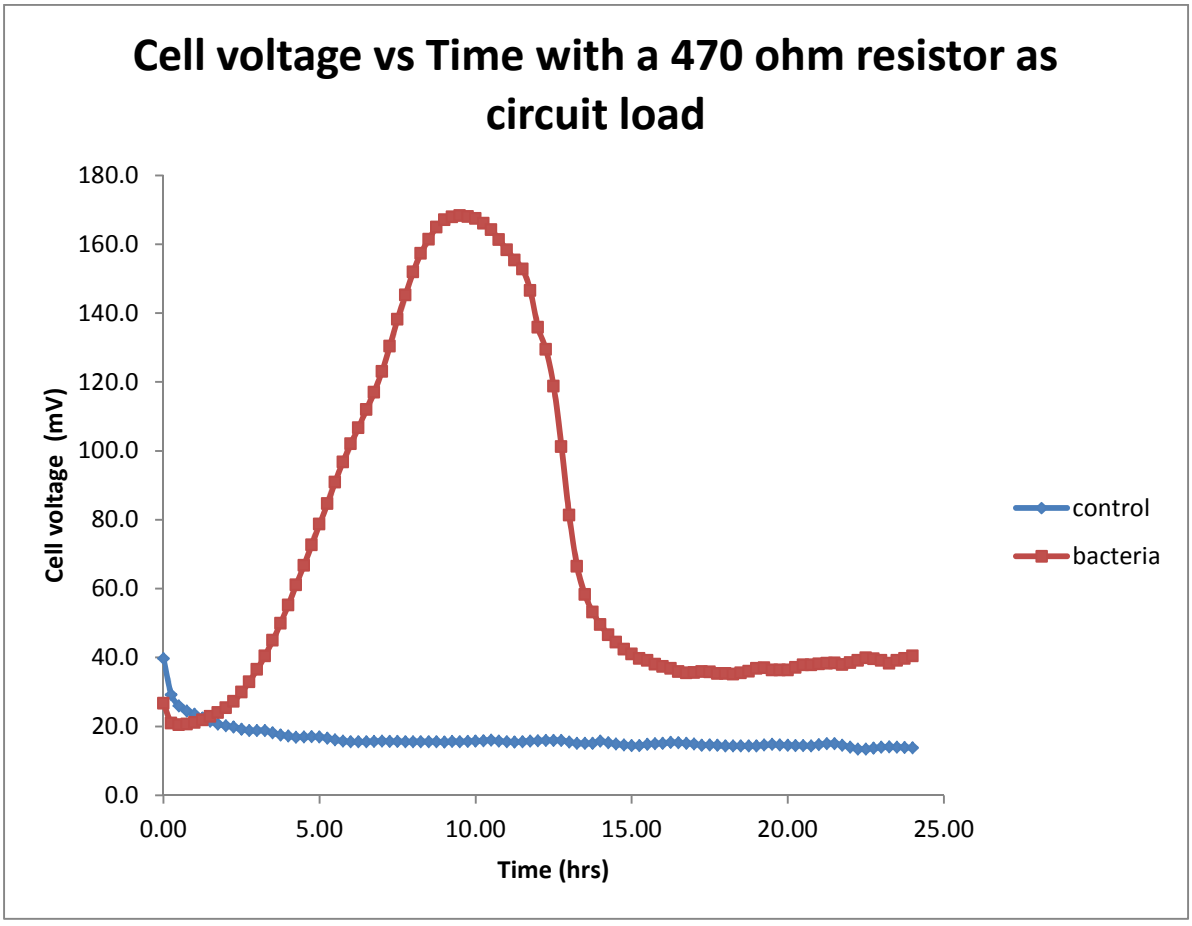

Figure 14: Cell voltage in function of time for MFC using a 470Ω resistor. Significant voltage change is observed when compare to controls MFC. The graph shows average data obtained in both cases.

4.6 Effects of glucose addition to a protein media

It was established in section 4.2 that the addition of bacteria to our MFC setup increased voltages and current output. This is due to the media composition chosen as anode solution which contains high amounts of organic substrates (in the form of yeast extract and tryptone). It is thought that rising substrate concentration in the media would improve the coulombic out and efficiency. To study this effect 1.11 mmoles of glucose were added to the MFC. Results are exhibited in Figure 15 and table 8. Result indicates that the addition of 0.20g of glucose increase the coulombic output of the system by 16%. The graph demonstrates that the addition of glucose to the MFC, lessen time required for achieving maximum readings by one hour and sustain the voltage output at higher levels for two hours. It is recommended that further studies be conducted with higher glucose concentrations.

Table 8: Voltage, current, power and coulombic output with and without the presence of glucose

Anode solution	Time (hrs)	Cell Voltage (mV)	Current (mA)	Power (µW)	Coulombic output (mC)
2X YT	11.5	153 ± 2	0.325 ± 0.003	50 ± 1	1584
	13.5	58 ± 6	0.12 ± 0.1	6 ± 2	
2X YT with 1.11mmoles glucose	11.5	133 ± 5	0.28 ± 0.01	38 ± 3	2016
	13.5	130 ± 6	0.28 ± 0.01	36 ± 4	

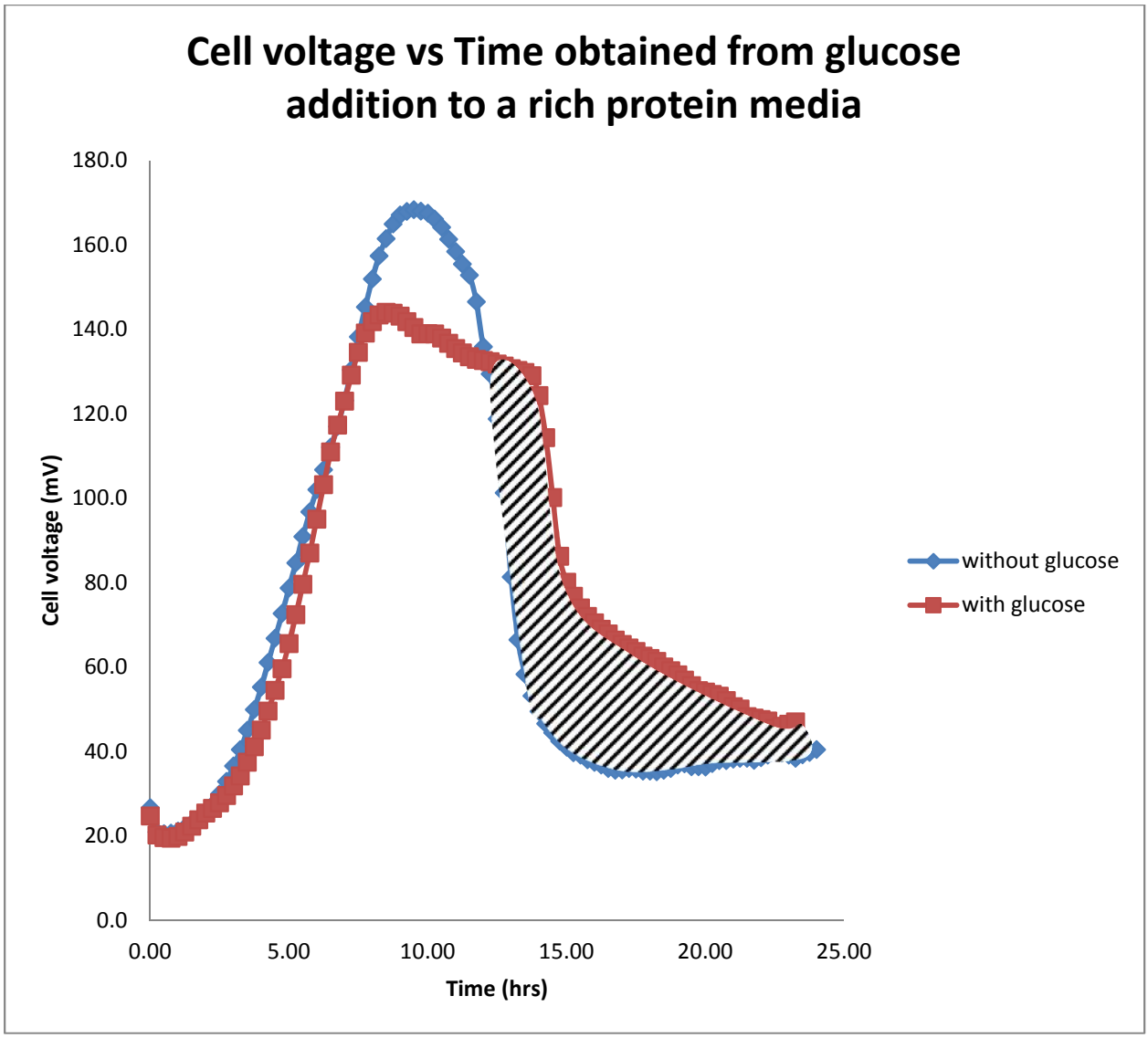

Figure 15: Results obtained when glucose was added to a rich-protein media growth.

4.6 Effect of buffer addition to the cathode solution

Table 9: Concentration and pH measurement of the solution used in the cathode compartment

Solution	***Concentration***	***pH***
PBS	*0.1M*	*7.05*
$K_3Fe(CN)_6$	*0.1M*	*6.29*
$K_3Fe(CN)_6$ in PBS	*0.1M*	*6.89*

The use of a membrane in MFCs allowed the migration of protons from one chamber to another causing a pH imbalance in the cathode chamber of an MFC. It has been suggested that pH variations inside the cathode chamber can limit current generation in MFCs (3). In this case a buffer solution was employed to evaluate the effect of pH in the cathode compartment. PBS was chosen among all the buffers reported in the literature for being affordable and the easiest to prepare. In this assay, the PBS solution measured a total pH of 7.05 but when used to dissolved the $K_3Fe(CN)_6$ final pH decrease to 6.89 as shown in Table 9. This phenomenon is expected since the ferric ions are hydrolyzed with water, increasing proton concentration in the solution (22).

To evaluate the effect of buffer addition to the cathode compartment on the performance, three MFCs were constructed. Voltage measurements obtained with PBS are demonstrated in Figure 16. It is noticed that the addition of PBS to the cathode chamber lessens the time needed to achieve maximum voltage and current output by 30 minutes as shown in **Error! Reference source not found.** and Figure 16 when compared with non-buffer MFCs. In the graph, higher

voltages were achieved with the PBS MFC, but a fall in voltage is observed much faster than the non-buffer MFC. This decrease suggests a change in cathode solution conductivity but further studies are needed to confirm the findings.

The output of the MFC did not improve with the addition of PBS to the cathode compartment.

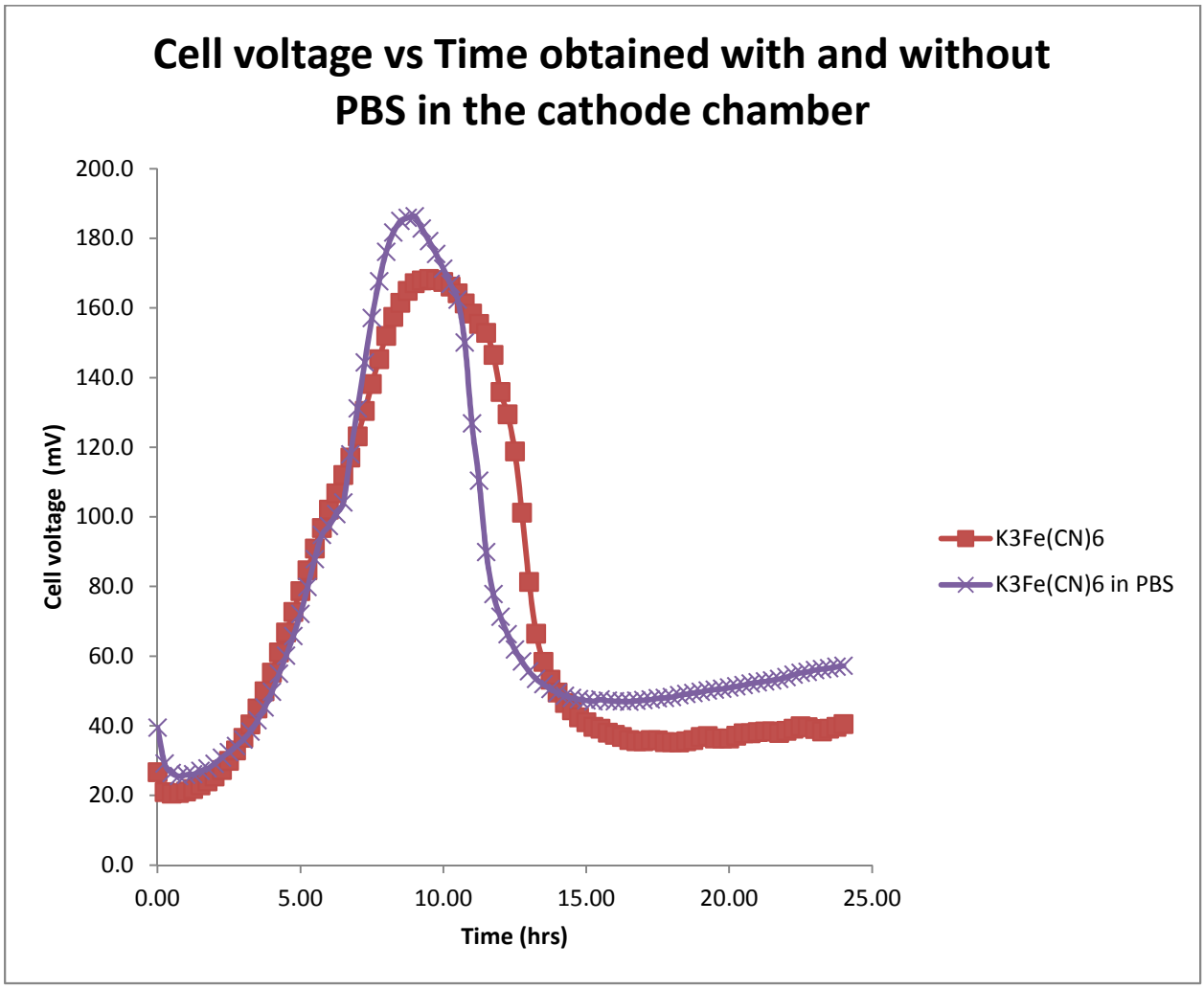

Figure 16: Cell voltage in function of time for MFC with and without PBS in the cathode chamber. The graphs show the average data of triplicate experiment in both cases.

5. Conclusions and recommendations

- Bacteria obtained from polluted soil turn out to be electroactive bacteria capable of producing cell voltage over 150mV and currents over 0.35mA. Characterization of the bacteria employed in the MFC is recommended for voltage and current generation enhancement.
- MFC has been set up to six different resistors demonstrating that our design is behaving according to Ohm's law.
- Results obtained with MFC control (without bacteria) show no voltage change as a function of time. Addition of bacteria to the media produced a voltage change which indicates that the voltage and current using a 470 Ω resistor comes from the flow of electrons derived from the degradation of the substrate by the bacteria.
- Glucose addition to the MFC extended the period of time in which it kept the highest current and voltage. It is recommended to the analysis of higher glucose concentration in the MFC.
- PBS addition to the cathode compartment in the MFC did not improve voltage and current performance.

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