CONTINUOUS POWER GENERATION AND TREATMENT OF LANDFILL LEACHATE

USING MICROBIAL FUEL CELLS

By

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(Under the Direction of Jenna Jambeck)

ABSTRACT

Microbial fuel cells (MFC) could be a more sustainable approach in treating wastewater since they produce energy by oxidizing organic material present in the wastewater. Landfill leachate is used as the organic substrate in this study with no external inoculation. Alternative cathode catalyst and anode materials were explored. A large semi-continuous MFC was designed, constructed and operated to test an MFC in more scalable conditions. The alternative cathode catalyst reduced chemical oxygen demand (COD) ranging from 62-75% producing a peak voltage of 451 mV and a power density of 0.432 W/m^3. The alternative anode cell (biochar) produced a maximum voltage of 437 mV and had a power density 0.407 W/m^3 while showing 22-38% reductions in COD. The semi-continuous MFC showed 77% reduction in BOD while maintaining stable voltage for a consistent amount of time.

INDEX WORDS: Landfill Leachate, Microbial Fuel Cell, Biochar, Activated Carbon

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CHAPTER 1

INTRODUCTION

Sanitary land filling of Municipal Solid Waste (MSW) is a very important issue in waste management. MSW predominantly consists of household waste and commercial waste collected by the municipality in a given area. Some landfills also collect non-hazardous industrial waste depending upon the size of the landfills and the regulations of the local government. Land filling is the most preferred solid waste management strategy for the disposal of residue waste from separation, recycling and incineration of different types of wastes. In 2008, United States generated about 250 million tons of MSW, of which 83 million tons were recycled and composted (USEPA 2008). Individual waste generation was estimated to 4.5 pounds per person per day, and on average 1.5 pounds of individual waste was recycled and composted.
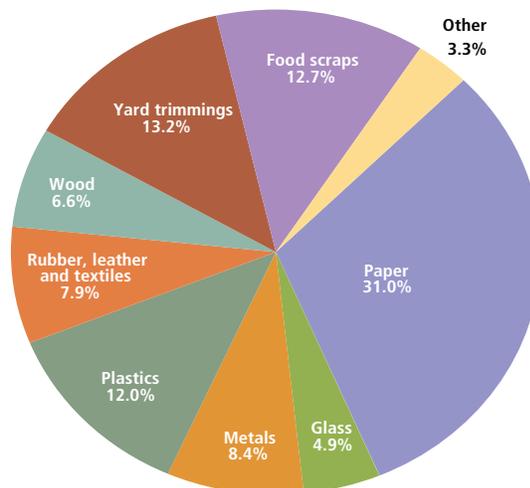
Figure1: Categorical percentage of 250 million tons of MSW generated in the U.S. in 2008

The constant emphasis on minimizing waste and resource conservation has led to an increase in recycling rate over the years. In 2008, the Unites States recovered 66 million tons of waste through recycling and 22.1 millions tons through composting. The EPA estimated that recycling and composting 83 million tons of MSW saved 1.3 quadrillion Btu of energy, equivalent to more than 10.2 billion gallons of gasoline. Although several approaches are being taken to reduce waste and greenhouse gas emissions, there is still a growing need for alternative energy and processes that reduce energy use.

The generation of leachate, a fluid with high organic, salts and heavy metal content is often a difficult problem in managing a landfill. Leachate is produced by the percolation of rain, surface water run-off and moisture generated, through decomposing waste. Modern landfills are designed with an impermeable liner system, as well as leachate collection and monitoring systems. High organic loading and trace constituents in leachate require it to be treated before discharged. There are several approaches to treat leachate from biological methods to physico-chemical techniques. Most of the approaches are energy intensive and expensive. Microbial fuel cells (MFC) can be used as a sustainable approach to treat leachate and produce electricity simultaneously.

Microbial fuel cell is a bioelectrochemical system that uses organic material present in wastewater to produce energy. Bacteria present in an MFC oxidize (degrade) organic substrates to produce electrons and protons as a part of their metabolism. The electrons are captured at the anode and conducted over a resistor to the aerobic cathode, where oxygen is reduced and meets with protons to produce water molecules. While power densities and treatment efficiencies documented in literature are nearing

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requirements for practical application, full-scale implementation of an MFC for wastewater/leachate treatment is complicated because of certain microbiological, architectural and economic challenges that need to be resolved that have not previously been encountered in any other wastewater treatment system.

1.1 Objective and Scope

There are several factors that remain as an obstacle to the practical and large-scale application of MFC's: expensive catalyst, anode/cathode materials and scalable designs. The power produced in an MFC depends on several factors from the electrode material used to various operational parameters. Some of the important factors that affect the power density are: 1. Bacterial community, 2. Electrode material, 3. Electrode catalyst. 4. Loading rate and recirculation rate of the substrate, and 5. pH of the system & ionic strength of the electrodes. This study focuses on bringing MFCs closer to real-world applications by investigating the impact of an alternative cathode and anode on power density and treatment efficiency. The specific objectives were to:

1. Explore an alternative cathode (Experiment 1) and evaluate an alternative anode (Experiment 2).

2. Design, construct and operate a semi-continuous MFC that can provide further data for exploring a scaled up design for leachate treatment (Experiment 3).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter includes a literature review on microbial fuel cells and landfill leachate characteristics. A comprehensive study on the generation of leachate, as well as the microbial metabolism, MFC architecture, voltage and power generation in MFC's is discussed in this chapter.

2.2 Composition and formation of municipal solid waste leachate

The rapid rise in population, industries, affluent increasing lifestyles and urbanization has led to an increase in MSW throughout the world. Landfilling of MSW is one of the most common solid waste disposal method with waste from separation, recycling and incineration also disposed in a landfill. Most of the modern landfills are engineered for the proper disposal of waste and designed to reduce public health hazards and increase safety.

A landfill generally consists of a liner at the bottom, layers of soil covering the daily deposited waste, leachate collection and extraction system and landfill gas collection system. The leachate generated from different sources (moisture, rain, surface water run-off, precipitation) is collected by a well-networked pipeline and stored in a reservoir. Landfill leachate is defined as any fluid that percolates through solid waste inside a landfill or is produced by the waste within the system. Common sources of leachate include rainfall, surface drainage, groundwater and water from underground

springs and the liquid produced from the biological decomposition of wastes. Leachate can be toxic because of its high organic loading, trace metal and other trace organic and inorganic constituents. Leachate, based on its pollutants is categorized into four main groups: 1. Dissolved organic matter, 2. Inorganic macro components, 3. Heavy metals and 4. Xenobiotic compounds. Table 2.1 outlines the compounds found in leachate and their concentrations compiled by *Kjeldsen et al. (2002)*

Leachate composition may vary according to the age/phase of the landfill. Landfills have four main phases: 1. Initial aerobic phase, 2. Anaerobic acid phase, 3. Initial methanogenic phase and 4. Stable methanogenic phase. In the initial aerobic phase, oxygen present from the readily buried waste is rapidly consumed by aerobic biological activity. Oxygen present in the aerobic phase lasts only for a few days, since there is no source for oxygen to enter once the waste is covered. Leachate produced from this phase is largely from the release of moisture during compaction. The depletion of oxygen after few days makes the cell anaerobic and initiates anaerobic fermentation reactions. In the second acid phase, hydrolytic and fermentative bacteria hydrolyze cellulose and hemicellulose compounds, which comprise 45-60% of the dry weight of MSW. The resulting monosaccharaides are then fermented to alcohols and carboxylic acids. Acetogenic bacteria present, convert the alcohols and carboxylic acids to acetate, hydrogen and carbon dioxide. The collective bacterial activity results in the accumulation of carboxylic acids and hence a low pH environment is found within the landfill. Since the pH is very acidic in this phase, it increases the solubility of many compounds. It is also reported that BOD and COD concentrations are highest at this phase. The initial methanogenic phase starts when the pH sets to neutralize. During this

phase, the acids generated are converted to methane and carbon dioxide by methanogenic

bacteria. Elevated methane levels are achieved in the final methanogenic phase.

Table 2.1: Composition of landfill leachate (Kjeldsen et al. (2002)

Parameter	Range
(Values in mg/L unless otherwise noted)	
pH	4.5-9
Specific conductivity (µS/cm)	2500-35000
Total solids	2000-60000
Organic Matter	
Total Organic Carbon	30-29000
Biological Oxygen Demand (BOD_5)	20-57000
Chemical Oxygen Demand (COD)	140-152000
BOD_5/COD ratio	0.02-0.80
Organic Nitrogen	14-2500
Inorganic Macrocomponents	
Total Phosphorous	0.1-23
Chloride	150-4500
Sulphate	8-7750
Hydrogen Bicarbonate	610-7320
Sodium	70-7700
Potassium	50-3700
Ammonium-N	50-2200
Calcium	10-7200
Magnesium	30-15000
Iron	3-5500
Manganese	0.03-1400
Silica	4-70
Heavy Metals	
Arsenic	0.01-1
Cadmium	0.0001-0.40
Chromium	0.02-1.5
Cobalt	0.005-1.50
Copper	0.005-10
Lead	0.001-5
Mercury	0.00005-0.16
Nickel	0.015-13
Zinc	0.03-1000

Methane production rate reaches maximum in this phase and decreases thereafter as the substrate concentration decreases. Earlier studies have reported that the rate of methane production is dependent on the rate of cellulose and hemicellulose hydrolysis. Since the acids and hydrogen gas produced from the acid phase are converted to methane and carbon dioxide, the pH within the landfill rises to more neutral values. Thus the pH of the leachate formed is also neutral, additionally the BOD, COD and conductivity of the leachate is also reduced. In this maturation phase, the rate of methane production diminishes significantly over a period of time. The principal gases evolved from the initial and stable methanogenic phases are predominantly methane and carbon dioxide. During the final maturation phase, leachate is often found to contain significant amounts of humic and fulvic acids.

The duration of each of the phases in a landfill is dependent on various parameters. The time scale of each phase can vary depending upon the distribution of organic constituents in the landfill, the availability of nutrients and microorganisms, moisture contents and also the degree of initial compaction. The carbon/nitrogen ratio also impacts the duration of each phase, as it directly affects the growth and productivity of different microbial species.

2.3 Municipal solid waste leachate management

Efficient management of leachate is necessary to eliminate a landfill's potential to pollute underground aquifers. Historically leachate has been managed in four ways: 1. Leachate recycling, 2. Leachate evaporation, 3. On site treatment followed by disposal and 4. Discharged into a municipal wastewater collection system. Recycling leachate into

the landfill is often considered as an effective method for treating leachate. It is sprayed back onto the working face of or can be injected through an interior piping system.

Recycling the leachate helps in attenuating leachate constituents by biological activity and other physical and chemical reactions inside a landfill. Leachate recirculation also increases the rate of waste biodegradation and the gas production rate. Finally the leachate residue is collected and treated before disposal. Large landfills are often required to have leachate storage facilities.

Leachate evaporation is one of the simplest leachate management strategies. Leachate is discharged into lined ponds and subjected to evaporation. In high rainfall areas, the evaporation pond is often covered with a geomembrane to prevent the entry of rainwater.

In landfill's where leachate recycling or evaporation cannot be performed, the leachate is often pretreated in an on-site treatment facility. Depending upon the leachate characteristics various treatment technologies are used. Since the collected leachate varies widely, different treatment methods are used to target specific groups of contaminants. Often, a treatment method is selected based on the contaminant to be removed and the concentration of other major pollutants. Common leachate characteristics of concern include total dissolved solids (TDS), chemical oxygen demand (COD), sulfate (SO_4^{2-}), heavy metals and other nonspecific toxic constituents. Leachates with high COD value can be treated anaerobically, as aerobic treatment can be very expensive. On the other hand leachate with high sulfate concentration may not be treated with anaerobic methods because of the production of odors from the biological reduction of sulfate. Leachate with high TDS loading is also found to be difficult to treat

biologically. Often, landfills will pre-treat the leachate to remove some major constituents that can cause issues at a wastewater treatment plant (e.g., high COD or BOD). Pretreatment often includes aeration, and the addition of activated carbon.

When a landfill is located near a wastewater treatment facility, a pressure sewer can be used to connect the landfill leachate collection system to a wastewater collection system. In other cases, leachate is stored and then tanker trucks transport it to a wastewater treatment facility. In many circumstances, pretreatment is required to reduce the organic loading before discharging into the wastewater collection system *(Tchobanolous .G et al.,)*

2.4 Microbial fuel cells

A microbial fuel cell is a bioreactor that converts chemical energy in organic compounds (wastewater) to electrical energy. In an MFC microorganisms oxidize organic matter to generate electrons and protons. The electrons are transferred through various methods to the anode (as an electron acceptor). These electrons are then conducted over a resistor to the aerobic cathode where oxygen is reduced and meets with protons to produce water molecules. Microorganisms carry out the conversion under anaerobic conditions. In 1911, M.C Potter was the first person to observe the generation of electric current by bacteria. However only since the early 1990's fuel cells became of more interest and the research on microbial fuel cells intensified. Different designs of MFC's have evolved over time to optimize performances and increase power densities. Most MFC's consist of an anodic chamber, were organic substrates undergo oxidation and a cathodic chamber for the oxygen reduction reaction. The anode should be the terminal electron acceptor (TEA) in a MFC. Since oxygen is ubiquitous, it is important to keep the

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anode chamber from oxygen. If oxygen is present in the anode chamber, it will act as the TEA and accept the electron. This process can account for major voltage loss in the cell. The cathode compartment is kept aerobic (i.e. oxygen is present) as it facilitates oxygen reduction and combines with the protons from the anode, to form water. The overall reaction in an MFC is the breakdown of organic substrate to carbon dioxide and water, with electricity as by-product. Figure 2.1 illustrates a basic microbial fuel design, with an anode and cathode chamber separated by a membrane permeable to protons. Early experiments conducted on MFC's have required the use of chemical mediators or electron shuttles to carry electrons from the inside of the cell to the exogenous electrodes. The biggest breakthrough in MFC's occurred in 1999 when *Kim et al. (1999c & 1999d)* recognized that external mediators need not be added for electron shuttling and certain species of bacteria are capable of shuttling electrons to the electrodes. Currently, real-world operations of MFCs are limited because of the low-level power densities and operating costs of MFCs.

Using acetate as an example of an organic substrate, typical electrode reactions can be comprehended well. One molecule of acetate is oxidized at the anode to generate 7 protons and 8 electrons. These protons and electrons both travel to the cathode chamber where 1 molecule of oxygen is required to create 2 molecules of water. 2 molecules of carbon dioxide are formed at the anode. However, there is no net carbon emission because the carbon dioxide on the renewable biomass originally comes from the atmosphere through the photosynthetic process. Electric current is made attainable by keeping the microorganisms separated from oxygen or any other end terminal electron

acceptor such as nitrate, sulfate and iron that can readily diffuse into the cell and form other products.

Anode reaction:

$$CH_3COO^- + 2H_2O \longrightarrow 2CO_2 + 7H^+ + 8e^-$$

Cathode reaction:

$$O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O$$

Bacterial Metabolism

Bacteria are unicellular microorganisms that are a few micrometers in length and are found in various shapes (spherical, spiral and rod shaped), they also lack a proper nucleus. Bacterial metabolism is classified into nutritional groups based on three criteria i.e. 1. The kind of energy used for growth, 2. The source of carbon and 3. The electron donors used for growth. Microbial fuel cells use anaerobic bacteria to oxidize organic substrates and produce electricity through a complex process. Anaerobic bacteria have evolved over years using various methods to reduce compounds to support their metabolism. A bacterial cell extracts energy from organic compounds and uses the energy to grow and maintain necessary cellular functions. Bacteria often choose a metabolic pathway with the highest energy gain. Study by *Schroder (2007)* have demonstrated that depending upon the terminal electron acceptor (TEA) present, bacteria can either choose a respiratory pathway or a fermentative pathway. The respiratory pathway is a combination of the reduction of a TEA (oxygen) and the oxidation of an organic substrate where the electrons are transported to a TEA through the electron transport chain. However aerobic respiration is the most energetically favorable pathway, although anaerobic respiration occurs when oxygen is not present in the system.

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Electrons are transferred within the bacterial cell by a mechanism called electron transport chain. Electron transport chains are cellular mechanism used for extracting energy from different sources such as sunlight in photosynthesis, and also form redox reactions. It conserves energy by synthesizing adenosine triphosphate (ATP), a compound that stores energy within its phosphate bond and releases the energy back once the bond is broken. Bacteria also use nicotine adenine (NAD), another intermediate compound to store and release energy when required *(Chapelle 2001)*. NAD can be reduced to NADH or oxidized to NAD^+. The main function of an electron transport system is to produce a transmembrane proton electrochemical gradient. Such a proton gradient enables the bacterial to do mechanical work. The created proton gradient increases the potential difference between the electron donor (substrates with low potential) and electron acceptor, this potential difference is processed for the generation of energy.

Bacterial metabolism in microbial fuel cells

The outer layers of most of microbial species are composed of a non-conductive lipid membrane; peptidoglycans and lipopolysaccharides that hinder the direct electron transfer to the anode *(Z Du et al 2007)*. In MFC's, bacteria transfer electrons to the anode by various mechanisms. Microbial fuel cells exploit this extracellular electron transfer of anaerobic bacteria to the anode. Bacteria that transfer electrons outside the cell to a terminal electron acceptor are termed as *exoelectrogens*. The anode chamber in an MFC must not contain oxygen because it is a more energy favorable TEA and can inhibit the transfer of electrons to the anode. Many anaerobes can transfer electrons only to soluble compounds such as nitrate or sulfate that can diffuse across the cell membrane and into

the cell. Exoelectrogens have the ability to directly transfer electrons outside of the cell, which enables them to function in an MFC.

Mechanisms of electron transfer

Bacteria have known to transfer electrons to a surface through two mechanisms: electron shuttling via self-producing mediators and by nanowires. Adding artificial mediators can also achieve electron transfer to the anode. A good mediator is required to posses the following features for better efficiency *(Ieropoulos et al., 2005)*: 1. It should cross the cell membrane easily, 2. Seize electrons efficiently from the electron transport chain, 3. Poses a high electrode reaction rate, 4. It should have good solubility in the anolyte, 5. It should be non-toxic to microbes and non-biodegradable. *Rabaey et al. (2005)* showed that the mediators produced by one type of bacteria could be used by other species and improve the electron transfer of the overall system. Bacteria produced mediators tend to oxidize faster and hence cause fewer over potential loses in the cell and also accelerates the electron transfer to the anode *(David and Higson 2007)*. The mediator moves across the cell membrane and releases the electrons to the anode (TEA) and become oxidized in bulk anode solution. Several synthetic mediators have been used in MFC's to increase the efficiency of electron transfer to the anode. A mediator with higher redox potential tends to give a higher overall power than a mediator with a low redox potential *(Ieropoulos et al., 2005)*. Commonly used synthetic exogenous mediators include dyes and metallorganics such as methylene blue (MB), neutral red (NR), thionine meldola's blue (MelB), 2-hydroxy-1, 4-naphthoquinone (HNQ) and Fe (III) EDTA. The toxicity and instability, even in small concentrations of these synthetic mediators limit their applications in MFC's.

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Certain microbial species use naturally occurring microbial metabolites as mediators. *Lovley (1993)* reported that humic acids, anthraquinone, sulphate and thiosulphate have the ability to transfer electrons from the cell to the anode. Artificial mediators have the potential to remove more electrons than the natural transfer to a TEA would remove. (*Lovley 2006*). *Rabaey et al (2005 & 2004)* have demonstrated the production of pycocyanin, a self-producing mediator by *Pseudomonas aeruginosa* that shuttles electron from the cell to the anode surface. The study also reported that artificial exogenous mediators are not necessary for electron transfer. The true purpose of self-produced mediators are still unclear, if they are generated specifically for exocellular electron transfer or if they assist in other cellular activities. A major advantage of self-produced electron mediator is the long-range interaction between the anode and bacterial biofilms. *G.fermentans* displayed the potential to transfer electrons to an anode, which was not in contact with the bacteria. This study by *Bond and Lovley (2005)* demonstrated the electron transfer through a mediator by producing a thick extracellular matrix to reduce the possibility of losing the mediator to the bulk solution.

Bacteria in MFC's are known to transfer electrons to the anode surface by direct contact through nanowires. In order to transfer electrons directly, the organism requires having a membrane bound electron transport relays, such as c-type cytochromes. This transfer mechanism allows for only one layer of bacteria in direct contact with the electrode to transfer electrons. *Gorby and Beveridge (2005)* reported the occurrence of conductive appendages for *Geobacter and Shewanella* species that transfer electrons directly from the inside of the cell to the anode surface. *Reguera et al. (2005)* observed similar conductive appendages in *G. sulfurreducens* but found that the structure of the

nanowires were different in *S. oneidensis*. Conductive nanowires were thought to exist only in iron-reducing bacteria until they were found in photosynthetic microorganisms. *Gorby and Beveridge (2005)* found conductive appendages in phototrophic and oxygenic cyanobacteria. Further studies of these bacteria in an MFC in carbon dioxide limited environment, showed that they can produce electricity in the presence of light and not in the dark. Several researchers have claimed that there is a potential for interspecies electron transfer. *Gorby et al. (2006)* have found that *Pelotomaculum thermopropionicum* produce thick appendages that resemble pilli. When this species is in a co-culture, the appendages seem to connect these fermentative bacteria with *M. thermautotrophicus* (a methanogen). It has been hypothesized that such a connection could enhance the possibility of interspecies electron transfer.

Apart from the transfer of electrons through conductive nanowires and self-producing mediators like pyocyanin, electron transfer can occur through interspecies hydrogen transfer.

Microbial community analysis

Microbes that are capable of self-mediating electrons to the anode are often chosen for oxidizing organic substrates in microbial fuel cells. Such organisms can be found in marine sediments, soil, freshwater sediments, and wastewater and activated sludges. Power production in the absence of external mediators was first demonstrated by *Kim et al. (1999), Gammaroteobacteria and Shewanella* species performed the exogenous electron transfer in this experiment.

Park and Zeikus (2002) constructed a fuel cell with an air-cathode and Mn^{4+} - graphite electrode. *Shewanella putrefacians* was used to inoculate the cell; they obtained

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a power density of 9.4mW/m^2 when pyruvate was used as the substrate and 1.6 & 1.9 mW/m^2 when acetate and glucose were used as substrate respectively. The power density increased 10 fold when a mediator was incorporated into the graphite electrode. In another study, the same fuel cell was used but it was inoculated with sewage sludge, the power density of *S. putrefacians* was six times lower than sewage sludge *(Park and Zeikus 2003)*.

Studies performed on MFC biofilms communities have revealed that *Geobacter and Shewanella* is the predominantly found bacterial community members *(Holmes et al. 2004 & Logan et al. 2005)*. However the molecular characterization of these biofilms have shown that our understanding of electrochemically active bacteria and their interactions in the biofilms are inadequate. Since most MFC's are being inoculated with activated sludge or wastewaters, the microbial community also depends upon the substrate used in the cell, as it directly affects the growth of certain microbial species. Trends on specific microbial species have not been reported, although the commonly found bacteria include proteobacteria (alpha-, beta-, delta- and gamma-), bacteriodetes and firmicutes (*Logan and Regan 2006 & Logan 2008)*. Occasional cases of alpha-proteobacteria domination in the microbial community were reported and at times, gamma- or beta- domination was seen (*Logan 2008)*.

Geobacter and Shewanella are the commonly found dissimilatory reducing metal reducing exoelectrogens in microbial fuel cells. The dissimilatory metal reducing microorganisms produce biologically useful energy in the form of ATP, by reducing metal oxides under anaerobic conditions. Dissimilatory metal reducing microbes were

considered to be predominant in mediator-less MFC's, although *(Oh and Logan 2006; Park et al., 2001) Clostridium butyricum* was reported as an exception.

Table 2.2: Commonly found bacterial communities from different studies, when oxygen is used at the cathode

Inoculum	Substrate	Community
River sediment *(Phung et al. 2004)*	Glucose + glutamic acid	65% = Alpha-(mainly Actinobacteria),21% =beta-, 3%=Gammaproteobacteria, 8% = Bacteroidetes, 3%=others
River sediment *(Phung et al. 2004)*	River water	11% = Alpha-, 46%= Beta-(related to Leptothrix spp.), 13%=Gamma-, 13%=Deltaproteobacteria, 9%=Bacteroidetes, 8%=others.
Marine sediment *(Logan et al. 2005)*	Cysteine	Gammaproteobacteria (40% Shewanella affinis KMM), then Vibriospp. And Pseudoalteramonas spp.
Wastewater (*Lee et al. 2003*)	Acetate	24%=Alpha-, 7%=Beta-, 21%= Gamma-, 21%= Deltaproteobacteria; 27%=others.
Wastewater *(Kim et al. 2004; Methe et al. 2003)*	Starch	36%= unidentified, 25%= Beta-, and 20%=Alphaproteobacteria, and 19%= Cytophaga+Flexibacter+Bacterioides

Sediment MFC's often have different microbial species dominating the anode when compared to contemporary fuel cells. It can attributed to the total lack of oxygen at anode, even though laboratory scale MFC's are expected to be anaerobic, oxygen leaks across the membrane in open-air cathode MFC's. *Deltaproteobacteria* have been observed to dominate most sediment MFC's, although it is not the case in regular microbial fuel cells. An extensive study on microbial communities in sediment MFC's by *Homes et al (2004)* revealed once again that *Deltaproteobacteria* (54-76%) supremacy was found in sediment microbial fuel cells. The microbial communities tested were obtained from marine, salt marsh and freshwater sediments. Other major species found in

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the study were *Cytophagales* (33%), *Firmicutes* (11.6%) and *Gammaproteobacteria* (9-10%). When an air-cathode MFC was used with river and marine sediments different species were noticed.

A comprehensive study on sediment MFC's by *Reimers et al. (2006)* concluded that microbes in an MFC oxidize more than just organic substrates. The research found that *D. acetoxidans (Deltaproteobacteria)* dominated the anode at a depth of 20-29 cm. This result was found to be consistent with previous studies on sediment MFC's and also found 90% of 346 clones in that specific environment. Such a community development was attributed to the availability of Fe(III) in the surrounding sediments. At depths of 46-55 cm, more diverse but comparable populations of *Epsilonproteobacteria, Desulfocapsa and Syntrophus (23%, 19% and 16% of clones)* were observed. At much deeper depths (70-76 cm) similar clones of *Epsilonproteobacteria* and *Syntrophus* were found. Evidence of elemental sulfur in deeper depths was found at the anode, this was attributed to the oxidation of sulfur resulting in electrocatalytic deposition of elemental sulfur.

2.5 Microbial fuel cells and landfill leachate

Landfill leachate is one of the most difficult effluents to treat due to its high strength and complex composition, including refractory and toxic components such as heavy metals and xenobiotic organic compounds *(Alkalay et al., 1998)*. Several studies have been done on the feasibility of using MFCs for simultaneous leachate treatment and energy generation.

Most of the studies using landfill leachate as substrate in MFCs have been performed on single un-stacked units. A study by *Galvez et al., (2009)* investigated the ability of MFCs to remove pollutants from landfill leachate through a plurality of units

fluidically connected in series and with re-circulation of fluid to increase hydraulic retention time. The MFCs were built with a working volume of 0.9 L, with separate anode and cathode chambers. A cation exchange membrane was utilized to facilitate proton transportation in this system. Diluted activated sludge was used as the inoculant. In this study, three separate MFC columns were connected in series fluidically in one phase of the investigation and the anode surface area was increased in the second phase of investigation by connecting a carbon veil with platinum electrode. In the third phase of the investigation the columns were disconnected from the input bottles and were fluidically joined in a loop. The increase in surface area in the second phase of investigation increased the power output and COD removal efficiencies. The three column system connected in a loop decreased COD by 79 % and BOD by 81 % in 4 days, demonstrating the potential of the system in competing with conventional biological systems in treating landfill leachate.

Landfill leachates may contain high levels of salinity and nitrogen compounds making it a complex fluid to treat using a single treatment method. *Puig S et. al,. (2011)* investigated the feasibility of landfill leachate treatment using microbial fuel cells under these conditions. In this study a tubular air cathode MFC was used with a graphite granule anode and had an empty bed volume of 343 ml. The anode and cathodes were in separate chambers sandwiching a cation exchange membrane. Platinum catalyst on a 30% wet proofing carbon cloth was used as the cathode catalyst. The MFCs were tested in 2 periods: First period, diluted urban leachate was used as the substrate (20 % v/v raw leachate) to minimize the risk of inhibitory effects. In the second period 100 % raw leachate was used to increase concentration of biodegradable organic matter. The HRT

was decreased from 7.4 to 3.2 hours. During period 1, 32 % reduction in COD was observed producing 6.1± 4.2 mW/m^3 of electricity. Even though the substrate used in this MFC was high in nitrogen concentration and salinity, a maximum amount of 8.5 kg COD m^{-3}d^{-1} was removed with a power density of 344 mW/m^{-3}. Ammonia within the system was removed through membrane transfer or through ammonia liquid-gas transfer. A high saline concentration benefited this system by increasing power production due to low internal resistance.

2.6 Microbial fuel cell architecture

The main challenge in designing and constructing a microbial fuel cell is to identify materials and architectures that maximize power generation and minimize construction and operational cost. Several designs have been developed over the years based on the type of application. Three main components of an MFC include a 1. Anode, 2. Cathode and 3. A membrane (PEM) in selected cells. Membranes are not common in MFC's because of their high cost (during scale up) and they also increase the internal resistance within the system thereby affecting power density. The power produced in an MFC depends on several factors from the electrode material used to various operational parameters. Some of the important factors that affect the power density are: 1. Bacterial community, 2. Electrode material, 3. Electrode catalyst, 4. Organic loading rate and recirculation rate of the substrate, and 5. pH of the system & ionic strength of the electrodes. Critical literature review on various types of electrode, cathode catalyst and designs are discussed in this section.

One of the most important challenges to the practical implementation of an MFC is the economic challenge. The catalyst's used for lab scale research so far are expensive and not economically scalable. Practical application of MFC's will require the development of inexpensive and efficient materials, which can be economical for mass production and also produce high power densities. Discussed below are the different types of fuel cell designs evolved over the years for optimal performances.

Two chamber MFC

Two chamber MFC's are commonly used in laboratories to test different materials and substrates. Their simple design and ease of construction make them the preferred design for testing different variables. A two-chamber system consists of an anodic and cathodic chamber, and a proton exchange membrane separating both. A salt bridge or PEM is used to allow the transfer of protons from the anode to cathode and also block the diffusion of oxygen from the cathode to the anode. Proton exchange membranes are often very expensive, *Logan (2008)* demonstrated the use of a salt bridge as a cheap alternative for a PEM. The salt bridge was made from salt and agar. Although such a design can give efficient performances in small-scale cells, the use of a PEM or a single chamber design is recommended for scale-up.

The cathodic chamber is filled with efficient catholytes to increase the cathode reaction and power production. Commonly used chemical catholytes in MFCs are ferricyanide and permanganate. The biggest disadvantage of chemical catholytes is that need to be regenerated chemically or replaced often. Water sparged with air can also be used as a catholyte, however they are not as efficient as their chemical counter-parts. Aeration of the catholyte could cause the leaching of oxygen into the anode chamber.

However *Oh and Logan (2008)* demonstrated that injecting air into the cathode could help the cathode reaction and increase the power production by 15.8%. The largest power density (4310 mW/m^2) reported in a MFC was achieved by using ferricyanide as the catholyte *(Rabaey et al. 2004)*. Although high power densities have been achieved by using chemical catholytes, their cost and lack of sustainability has shifted the focus towards proton exchange membranes.

In two chamber MFC's; using a larger size PEM can increase power production. Even though a large PEM will increase the proton transfer, it also facilitates oxygen diffusion into the anode chamber and oxidizes the substrate. This process decreases the coulombic efficiency of the system *(Oh and Logan 2006)*.

Single chamber MFC

Single chamber MFC's (SCMFC) have a simpler design when compared with two-chamber systems and have the potential for real-world applications. A single chamber fuel cell has an anode chamber coupled with a porous air cathode to facilitate oxygen reduction reactions. *Park and Zeikus (2003)* constructed the first cell with such a design; protons were transferred through the anolyte to the air-cathode. SCMFC's have an anode chamber, were the oxidation of organic substrates occurs on solid electrodes (anode) and a carbon cloth cathode coated with a catalyst to facilitate oxygen reduction reactions. Platinum is the most commonly used cathode catalyst in open-air MFC's.

Liu et al. (2004a) constructed a cylindrical SCMFC with Plexiglas, eight graphite rods (anode) were placed in concentric arrangement surrounding a single cathode. Platinum on carbon was used as the cathode-catalyst; it was fused along with a PEM to a plastic support tube to form the air-porous cathode in the center. *Rabaey et al. (2005)* first

established that using sustainable open-air cathodes was the only way to implement MFC's for practical applications.

Cube reactors, up-flow mode MFC's and tubular packed bed reactors are different variations of single chamber MFC's. Each of them has an anode chamber coupled with an open-air cathode loaded with oxygen reduction catalyst. *Jang et al. (2004)* designed a novel tubular reactor in which the substrate was made to flow from the anode and into the cathode in the same column. Glass beads and glass wool separated the anode and cathode. Since the internal resistance of this system was very high, it had a power density of only 1.3mW/m^2 with a glucose-glutamate based substrate. This design was later improvised by *Moon et al. (2005)*, they used a platinum coated graphite felt cathode and perforated polyacrylic plate was used to separate the anode and cathode. Power density increased to 560mW/m^2. The reactor generated stable power for more than two years. In spite of its stable power generation, this design had a major disadvantage, if organic matter wasn't removed efficiently at the anode it will flow into the cathode chamber and create an oxygen demand or leave the wastewater untreated.

Tubular systems often use graphite granules as the anode, with a PEM or carbon cloth encapsulating the granules. Researches often apply catholytes to improve the power density of the cell. *He et al. (2005 & 2006)* developed two tubular flow reactor designs using porous and electrically conductive material. The first reactor had a total volume of 190 mL and the anode chamber was packed with reticulated vitreous carbon (RVC). The flow in this reactor was directed from the anode towards the cation exchange membrane (CEM), above which the cathode chamber was located containing ferricyanide solution.

This design produced power up to 170 mW/m^2 when sucrose was used as substrate. The second design had a CEM contained in a tube that was placed in a packed bed of granular activated carbon (GAC). The reactor produced 29 W/m^3 with COD removal efficiencies that exceeded more than 90%. The difference in power densities is attributed to the difference in internal resistance in both the systems. The first design reactor had an internal resistance of 84 Ω, whereas the latter had a lower resistance of 17.13 Ω. Although high power was produced from the system, the authors concluded that to be competitive with anaerobic digesters producing methane gas for electricity generation, MFC's need to obtain at least 160 W/m^3 or more.

A single chamber cylindrical MFC was constructed by *Liu and Logan (2004b)* to test the necessity of PEM. The cell used carbon paper as anode on one side of the chamber and carbon cloth coated with platinum was used as the cathode. One side of the cathode was exposed to air while the platinum coated side was touching the substrate. The system produced 146 mW/m^2 using wastewater as the substrate. The coulombic efficiency of the system was reduced from 40-55% to 9-12%, this was due to the diffusion of oxygen into the system since no PEM was present. The predominant variations in SCMFC include the type of anode used/treatment on the anode, cathode catalyst used and variations on the PEM/CEM.

Plate MFC

Plate MFC design is a variation of SCMFC; it is used to stack up more than one MFC and connected in series to increase the power density. Plate MFC's have two non-conductive plates with a channel cut on two sides to allow substrate to flow on one side and air on the other side. Cathode is made by hot pressing a PEM and carbon cloth with

platinum and placed between the two plates. Carbon paper is used as the anode. *Min and Logan (2004)* used this design and produced 56 mW/m^2 using domestic wastewater as the substrate and 309 mW/m^2 when acetate was used as the substrate. The overall efficiency of this system was very less when compared to single chamber fuel cells, the authors concluded that it must have been due to the proximity of the anode and cathode, causing oxygen diffusion into the anode chamber.

Liu et al. (2008) designed a plate MFC with certain design characteristics of a cylindrical MFC. The anode contained baffles allowing for a plug-flow treatment system. A carbon cloth anode was attached to all surfaces of the baffled compartment. The cathode was placed on top of the anode and sealed with a plastic cover with holes drilled to enhance oxygen diffusion. The novel design produced 520 mW/m^2 in batch mode and 695 mW/m^2 in continuous mode. Graphite granules and plates were used to enhance power density in this cell; although it had only little effect on power since cathode was limiting power production.

Stacked MFC'S

To increase the power production in MFC's, more than two or three fuel cells can be connected in series or parallel to increase the voltage. *Shimoyama et al. (2008)* produced 899 mW/m^2 by connecting 12 separate MFC's termed as cassettes. Each cassette had, from left to right, an anode, PEM, cathode and plastic frame to allow air to reach the cathode, and another set of cathode, PEM and anode. A study by *Aelterman et al. (2006)* showed that a parallel-connected stack had a higher short circuit current than the stack connected in series. This implied that a higher maximum bioelectrochemical reaction rate could be achieved by connecting MFC's in parallel than in series. Hence for

a high COD removal, parallel connections are preferred if the MFC units are not independently operated. A study by the same group showed that a parallel connection between multiple MFC's produced higher current and series connection resulted in high voltage.

Shin et al. (2006) used a bipolar-plate type of shacked MC containing five cells; glucose was used as the substrate to grow *Proteus vulgaris* and thionin was used as the mediator. The reactors produced 1300 mW/m^2 when ferricyanide was used as the catholyte and 230 mW/m^2 when pure oxygen has was used.

Several studies on stacked MFC's have indicated that voltage reversal is the main obstacle for the successful increase in voltage. *Oh and Logan (2007)* investigated voltage reversal in stack MFC's using a two-cell stack with acetate as the substrate and oxygen in the air as the electron acceptor. Initially both cells produced same power, but after several feeding cycles one cell produced a lower voltage at the beginning of a feeding cycle, and then before the cycle was complete the cell voltage reversed. This resulted in a decrease in voltage from 0.38 V to 0.08 V. Substrate depletion was found to drive one cell into voltage reversal. The study also showed that when the cell undergoing voltage reversal was fed with substrate in the next cycle without feeding the first cell, the first cell underwent voltage reversal. These results again concluded that connecting cells in series to form stacks could be an improper way in increasing voltage, as variations of output in individual cells could drive the stack power output to rapidly falter.

2.7 Electrode material

Anode

The properties of the anode material used play an important role in power density.

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Previous studies have reported that the anode should have high surface area, thereby making more space available for microbes to grow on and transfer electrons. *Chaudhuri et.al (2003)* reported that graphite felt ($A_{an}=20*10^{-3}$ m^2, A_{an}=Anode surface area) produced three times more current than graphite rods ($A_{an}=6.5*10^{-3}$ m^2). It was mentioned that the effect was due to the increase in surface area, and not due to the difference in material. The porosity of the graphite felt was greater than the graphite rod. *Rabey et.al (2005)* used graphite granules whose diameters varied from 1.5 to 5 mm. The granules were conductive, but in order to make the complete bed conductive the granules had to make good electrical contact with each other. It was learnt that due to shape of the granules and bed porosity, they can connect at only a small fraction of their total surface area. *You et.al (2007)* obtained a power density of 50.2 Wm^{-3} using glucose based substrates in a graphite granular anode based up flow MFC. *Rabey et.al (2005)* obtained a power density of 49±8 Wm^{-3} using graphite granules as anode on glucose based substrates. Although both *Rabey et.al (2005)* and *You et.al (2007)* used the same anode material (granular graphite) but different catalyst they obtained more or less the same power density.

The anode has reached the highest level of development with the advent of graphite fiber brush electrodes. *Logan et.al (2007)* first reported that the highest surface areas and porosities for anode can be achieved by using graphite fiber brush electrodes. Due to the small diameter of the graphite fiber (5-7 μm) extremely high specific surface areas can be obtained. An experiment by *Logan et.al (2007)* showed that a bottle MFC inoculated with wastewater had a power density of 1430 mW/m^2 when graphite fiber brush (length-7cm; diameter-5cm) anode was used versus a power density of 600 mW/m^2

when plan carbon paper anode was used. When the graphite fibers were arranged in a tow formation the MFC produced a power density of 1100 mW/m^2. *Logan et.al (2007)* studied that tests with different masses of fibers did not produce a clear trend of power per mass loading, suggesting that fiber clumping was a factor in the performance of the system. Use of a smaller graphite fiber brush (length-2.5cm; diameter-2.5cm) in a cube type MFC produced the highest power density yet achieved for an air cathode MFC. The power was 73 W/m^3 and the coulombic efficiency (CE) ranged from 40%-60% depending on the current density.

The use of conductive polymers as anode materials was first investigated by *Logan et.al (2006)*. The work by *Logan et.al (2006)* showed that polymers were not as effective as carbon paper or graphite granules. The maximum voltage produced by the best performing polymer anode was 99.4 ± 1.9 mV. This voltage was only 55% of that achieved using a carbon cloth anode (181±15 mV) with the same experimental set up. It was also reported that the performance of these polymer based systems were erratic and inconsistent, indicating that both maximum power densities and stability of these polymers will be a concern.

The use of metals and metal coatings on different carbon materials as anode is another approach to try and increase the power density of an MFC. In a study by *Kim et.al (2005)* it was shown that addition of vapor-deposited iron oxide to a carbon paper cathode decreased acclimation time of a reactor but did not affect the maximum power. After certain period the iron coating dissolved in the solution, leaving only the carbon paper electrode.

A study by *Park et.al (2002)* showed that a self made Mn^{4+} - graphite anode (graphite, manganese ion, nickel and a binder) produced a power density of 10.2 mW/m^2 compared to 0.02 mW/m^2 by a woven graphite electrode with a pure culture of *Shewanella putrefacians* and lactate as substrate. Further experiments were done with same electrodes but with different and more complex medium and sewage sludge inoculum were used. A power density of 788 mW/m^2 was obtained with Mn^{4+} - graphite anode versus a 0.65 mW/m^2 using a woven graphite electrode. The absence of a traditional cation exchange membrane (CEM) may have produced a lower internal resistance, resulting in greater power densities.

Cheng at.al (2006) came up with one of the most successful method in terms of maximizing power production by treating the anode material. They treated the carbon cloth using 5% NH_3 gas in a helium carrier gas at 700°C for 60 minutes. This treatment increased the surface charge of the carbon cloth from 0.38 to 3.99 meq/m^2 and thereby reduced the acclimation time by needed for a wastewater culture to produce power. The power was increased from 1330 mW/m^2 to 1970 mW/m^2 and the treatment also reduced the acclimation time by 50%. Adding cysteine to the system can also reduce the acclimation time, it works by scavenging the oxygen in the system.

Cathode

Carbon cloth cathode loaded with platinum catalyst is the most commonly used cathode currently for research purposes. Platinum based carbon cathodes have given the highest power densities. Catalyst binders are commonly used in the cathodes to hold the catalyst on the applied region and to allow the transfer of protons, electrons and oxygen.

Nafion is the most commonly used because of its high oxygen permeability and proton conductivity. It is used at the cathode to allow only the protons from the anode chamber. Polytetrafluroethylene (PTFE) suspension can also be used, but Nafion gives better results when compared to PTFE. A study by *Cheng et.al (2006)* was done were they prepared platinum based cathodes and used Nafion in one set and PTFE in another set of experiments. They found that Nafion produced slightly greater potentials in electrochemical tests than those prepared by using PTFE. In another experiment an air cathode MFC with Nafion produced 480 ± 20 mW/m^2 power versus an MFC with PTFE based cathode produced only 360 ± 10 mW/m^2.

Park et.al (2002) were the first use to non-precious metal as catalyst in a carbon based air cathode MFC. They made a cathode by combining ferric sulfate, fine graphite, kaolin and nickel chloride. These iron-based cathodes produced power up to 4 times as much as plain-woven graphite cathodes, but these results were not compared to platinum based cathodes of similar dimensions.

It has been reported that, cathode performance equal to that of platinum based carbon cathodes can be achieved using transition-metal carbon cathodes, thus eliminating the need for precious metals in MFC

2.8 Recirculation rate & feeding rate

Feeding the substrate to the MFC and the rate at which it is done is a crucial parameter when it comes to treatment efficiency and power sustainability. When the feeding rate is too low the bacteria doesn't have substrate to consume and hence the power density can go down. An optimized feeding rate and recirculation rate is therefore necessary for efficient performance of an MFC *(Schroder et.al (2003)*.

Zhang et.al (*2010)* conducted long-term tests on MFC with different feeding rates and recirculation rates. At a feeding rate of 1 ml/min and anolyte recirculation rate of 140 ml/min, a power density of 4.40 W/m^3 was obtained versus a power density of 4.28 W/m^3 when the recirculation rate was changed to 500 ml/min with the same feeding rate. The COD removal was 95% for a recirculation rate of 140 ml/min and 99% for 500 ml/min. There was no significant difference in the power density of the system. When the feeding was changed to 4 ml/min and the anolyte recirculation rate was 140 ml/min, the power density was 4.36 W/m^3, but when the recirculation rate was changed to 500 ml/min with the same feeding rate the power density increased to 7.11 W/m^3. There was a change in the COD removal rate, 67.7% for 140 ml/min and 83.7% for 500 ml/min.

An investigation by *You et.al (2007)* on a graphite granule membrane-less tubular air-cathode microbial fuel cell obtained a power density of 50.2 Wm^{-3} using glucose based substrate. The cell was inoculated with seed sludge from a local wastewater treatment facility. This is one of the highest power densities obtained in an up flow fuel cell.

An interesting study by *Zhang et.al (2008)* showed that, when the feeding rate was increased from 0.65 kg COD/m^3d to 5.2 kg COD/m^3d the power density increased from the 2 W/m^3 to 11.6 W/m^3 but the COD removal dropped from 89.4% to 58.5%. The performance in terms of power density of the system improved but the treatment efficiency decreased. The previously stated study by *Zhang et.al (2008)* reported that when the anolyte recirculation rate was increased the treatment efficiency (COD removal) was increased. Hence an optimum feeding rate and recirculation rate needs to be determined based on the substrate characteristics.

An intensive study done by *Lorenzo et.al (2009)* compared the effect of loading rate on power density and COD removal on a single chamber MFC with two different types of anode. A graphite disc anode and graphite granular anode was used. The results on the performance of the graphite disc anode & granular graphite anode are tabulated below

Table 2.3: Performance of MFC with graphite discs as anode by *Lorenzo et.al (2009)*

COD loading rate (Kg COD/m^3 d)	Power density (mW/m^2)	COD removal (%)
0.08	57.9	48
0.35	53.4	81
0.55	54.9	80

As seen in Table 1 there are no remarkable differences in the power density when the loading rate is changed, but there is huge difference in COD removal percentage when the feeding rate is increased. Even though the substrate used was wastewater, adding glucose to the system and not wastewater increased the organic loading.

From Table 2 we can see that the power density is going down as the COD loading rate is increased, but there is an increase in the COD removal. The treatment efficiency of the system is increased but the power isn't. In both the systems the loading rate was increased by adding glucose to the system and not by wastewater.

Table 2.4: Performance of MFC with graphite granules as anode by *Lorenzo et.al (2009)*

COD loading rate (Kg COD/m^3 d)	Power density (mW/m^2)	COD removal (%)
0.09	1.31	61
0.43	1.20	80
0.67	1.16	83

Aeltermann et.al (2006) conducted a study on 5 different MFCs to study the effect of different external resistance and loading rates on the power density of the MFC. They reported that, at an external resistance of 50Ω and when the loading rate was normalized from an irregular loading rate (< 1.6 g COD L^{-1} TAC d^{-1}) to a continuous loading rate of 1.6 g COD L^{-1} TAC d^{-1} the continuous power generation improved significantly. However when the loading rate was doubled from 1.6 to 3.3 g COD L^{-1} TAC d^{-1}, by doubling the flow rate, there was no significant increase in the power generation. At an external resistance of the 10Ω, doubling the loading rate as mentioned above increased the power generation significantly. They concluded from the results that, only at low resistance or near maximum currents, increasing loading rates can increase power generation.

CHAPTER 3

METHODS AND MATERIALS

3.1 Overview

Single chambered air-cathode microbial fuel cells were constructed to test various anode and cathode materials. Leachate was characterized for essential parameters before and after each MFC trial. The first set of experiments was operated in batch mode. Experiment 1 evaluated an alternative catalyst material, Experiment 2 evaluated an alternative anode material and Experiment 3 consisted of the design and operation of a semi-continuous MFC that was informed by first two experiments. Leachate used in the semi-continuous MFC was subjected to ammonia, nitrate, nitrite, sulfate, BOD assays and Inductively Coupled Plasma Mass Spectrometry (ICP - MS) analysis for specific metals before and after experiment 3.1 and 3.5. Influent and effluent levels were recorded for each cycle of the MFC to determine the percentage difference in specific parameters after MFC operation. Voltage data from the fuel cell was recorded on a computer every four minutes using LabVIEW data acquisition software.

3.2 Landfill leachate

Landfill leachate for all the MFCs was collected from the Oak Groove landfill in Barrow County, Georgia. The Oak Grove site is a RCRA approved Subtitle D landfill permitted to accept household, commercial and industrial wastes. The landfill is also licensed to collect contaminated soils, asbestos, sludge and other pre-approved non-hazardous wastes.

The Oak Grove landfill leachate is collected by an engineered leachate collection system and leachate from the entire landfill is held in a storage tank before it is transported off-site for treatment. Leachate for all the experiments was sampled from the storage tank. Sampling from the storage tank helped to obtain a more homogenized mixture of leachate from all cells of the landfill. The MFCs were started on the same day leachate was collected to utilize active bacteria and to prevent the loss of organic substrates. Leachate for subsequent runs of the same experiments were refrigerated until use and kept at room temperature overnight before use. Landfill leachate used in the batch MFCs was tested for conductivity, specific conductivity, dissolved oxygen, oxygen reduction potential, pH, resistance and COD before and after every cycle of operation.

3.3 Single chamber air-cathode MFC

A cylindrical single chambered air-cathode MFC was constructed to test different anodes and cathode catalysts in batch mode. The fuel cell was constructed with plastic and sealed using aquarium grade 100% silicone. All the batch fuel cells had the same dimensions, 13.2 cm high and a 10.16 cm diameter. Activated carbon granules from Sigma Aldrich and graphite rods and plates from graphitestore.com were used as the anode in the cell. Activated carbon granules (241 grams) filled only 75% of the height of

the cell, to create a void space and prevent leachate adsorption by activated carbon at the cathode. The granules were rinsed with distilled water and dried at room temperature prior use. Activated carbon granules have a high adsorbing capacity due to their large internal surface area. To make organic substrates readily available for bacteria and to correct for some COD reduction caused by activated carbon adsorption, activated carbon granules were soaked in leachate for seven days before starting the first cycle of Experiment 1. COD values of leachate before and after soaking the leachate in activated carbon was also recorded. The cathode and the substrate (leachate) must remain in constant contact in an MFC to complete the cathodic reaction and transfer protons to the cathode. In this case, the cells were constructed to hold 1000 ml leachate, and a working volume of 520 ml after adding activated carbon granules. The cell was fitted with a tap in the center to drain leachate out of the cell after each cycle without disturbing the biofilm.

A graphite rod was placed between the carbon granules to complete the anode by providing a transfer material for the electrons generated from leachate oxidation. The graphite rod was 11.4 cm long and had a diameter of 1.27 cm. Copper wires used for power transmission were glued to the rod using Silver epoxy (EE129-4, Epo-Tek).
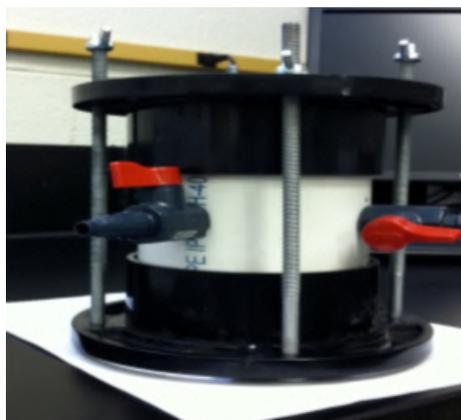
Figure 3.1: Single chamber air-cathode MFC

Carbon cloth was used as the cathode for the fuel cells in all the experiments. Platinum on carbon (Pt/C) and a method for nickel oxide deposited on carbon (NiO/C) were used as the two different cathode catalysts in Experiment 1. Platinum on carbon (Pt/C) was used as the cathodes in Experiment 2.
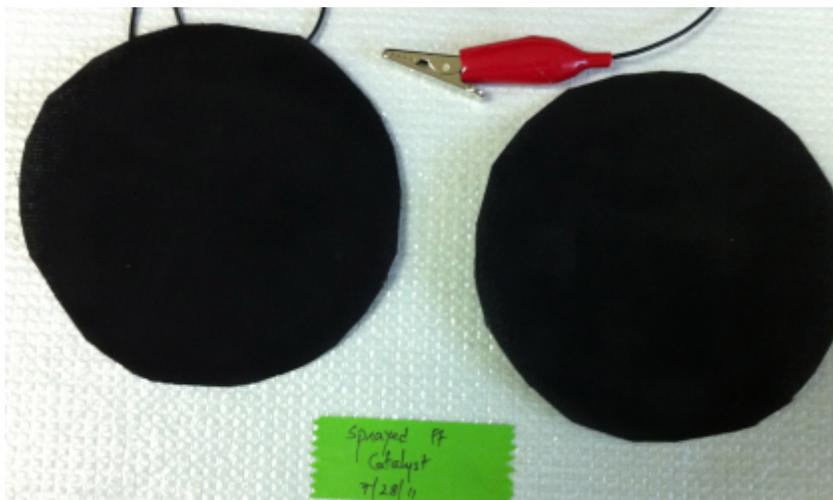
Figure 3.2: Sprayed Pt/C catalyst on carbon cloth

Carbon cloth from Fuel Cell Earth was used with 60% wet proofing, to limit the release of substrate through the cloth. Platinum was purchased as 10% HP platinum in Vulcan XC-72, a carbon black powder. The catalyst was prepared by mixing platinum on carbon powder (Pt/C) with a chemical binder, DuPont dispersion 5% (by weight) Nafion liquid solution to form a paste. 7ml of Nafion solution was mixed with 1gram of Pt/C powder and homogenized. The paste was then sprayed on the inner side of the cathode and allowed to dry for 48 hours before use. Individual copper wires from a multi-stranded wire were glued separately on the outer side of the catalyst using silver epoxy glue.

3.4 Nickel oxide on activated carbon

Nickel oxide was deposited on activated carbon based on the method described by *G. -h Yuan et.al (2005)*. Activated carbon granules was first oxidized with 98% H_2SO_4

and 10% HNO_3 solution for 1 hour and 4 hours respectively and dried overnight at room temperature. Dried activated carbon was then suspended in 30ml of $Ni(NO_3)_2$ solution and stirred for 4 hours using ultrasonic waves. After filtration and washing with distilled water, activated carbon was placed in 1mol/L NaOH solution and stirred for 30 minutes using ultrasonic waves to produce hydroxide from nitrate. The material was then filtered and allowed to dry for 48 hours before use. Nickel oxide on carbon (Ni/C) was also mixed with Nafion liquid solution to form a paste. Since activated carbon granules were used, Ni/C powder was not completely homogenous.

The catalyst paste prepared using the Ni/C granules and Nafion binder was painted on the inner side of the carbon cloth instead of spraying it due to a lack of homogeneity in the paste. Copper wires were glued on the outer side of the cloth using silver epoxy glue.

Figure 3.3: Painted Ni/C catalyst on carbon cloth

Alligator clips were attached at the end of the anode and cathode to connect them to an electrical breadboard. The breadboard contained a capacitor to compensate the electrical noise within the system, along with a 1 Ω resistor to overcome the resistance

from the data acquisition unit. A 470 Ω was also used to provide a load for the system. Resistance used as load in this research was based upon previous research (*Damiano 2009)*.
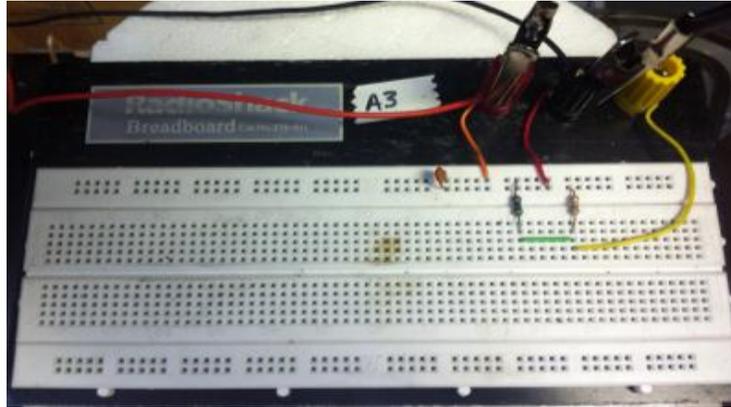
Figure 3.4: Electrical breadboard used to provide resistance and capacitance to the system

3.5 Semi-continuous air-cathode MFC

A semi-continuous microbial fuel cell was constructed using Plexiglas (37 cm × 32 cm × 6 cm) and sealed using 100% aquarium grade silicon. Dense fine grain graphite plates (25.4 cm × 5.08 cm × 0.635 cm) and graphite rods with a diameter of 1.27 cm and a length of 30.48 cm were used as the anode in this cell. The plates were arranged parallel to each other (2 inches apart) inside the cell and glued to the bottom using silicon glue. The plates were arranged in a specific pattern (as shown in **Figure 3.5**) allowing the flowing leachate to cover maximum surface area in the cell. Two graphite rods were placed between the plates passing through the plates at both sides of the cell; the rods were wired and connected with alligator clips. Carbon cloth sprayed with platinum catalyst (as used in the batch MFCs) was used as the cathode. The cathode was glued with multi-stranded wire using silver epoxy as done for the cathodes used in the batch MFCs.
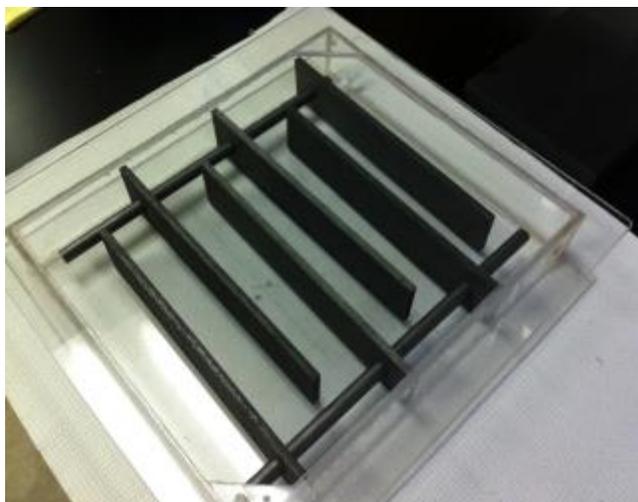
Figure 3.5: Top view of the semi-continuous MFC with graphite plates and rods

Leachate was pumped into the cell using a peristaltic pump (Variable – speed low flow pump); the effluent from the cell was recycled back into the fuel cell. The influent flow rate was maintained at 7ml/min throughout all the semi-continuous experiments. The effluent from the cell was arranged to flow such that the flow rate of the influent and effluent were similar. The semi-continuous cell had a working volume of 5.5 liters. The same electrical breadboards and resistance used in the batch MFC was also used for the semi-continuous MFC. Since leachate was continuously pumped into the cell, there was constant contact between the leachate and cathode thereby making the MFC reaction complete.

3.6 Methods

MFC Operation

Landfill leachate was used as the substrate and inoculum in this research. No additional bacteria, nutrients or mediators were used to enhance the efficiency of the system. The batch and semi-continuous cells were not cleaned between consecutive cycles of MFC operation to allow the continual growth of the biofilm. The subsequent

cycles of the MFC were started immediately to maintain optimum conditions for bacterial growth. Three experiments were conducted to accomplish the objectives of this research: Experiment 1 explored the performance of an MFC when different catalysts were used at the cathode, Experiment 2 evaluated the performance of different anodes in an MFC, and Experiment 3 was conducted to provide further information for the scale-up for leachate treatment with MFCs.

Single chamber air-cathode MFC

The batch MFCs were used to evaluate alternative cathode catalysts and anode materials relative to voltage generation and treatment efficiency (COD reduction). Experiment 1 was conducted three times (Cycle 1.1, 1.2 and 1.3), and Experiment 2 was conducted three times (Cycle 2.1, 2.2 and 2.3) Before and after every cycle of the batch MFC, pH, conductivity, specific conductivity, resistance, dissolved oxygen and oxygen reduction potential of the leachate was measured using an YSI 556 multi probe system. COD assays were conducted using HACH Methods TNT 822.

Semi-continuous air-cathode MFC

The effluent flowed to a reservoir and leachate from the reservoir was recycled back into the cell with a low flow, variable, peristaltic pump from Fisher Scientific (Model 3385). The reservoir was also filled with leachate before the cell was started.

The semi-continuous MFC was operated for five different runs (Experiment 3). Fresh leachate from the landfill was used as the substrate in cycle 3.1, 3.3, 3.4 and 3.5. Effluent from cycle 3.1 was mixed with unused leachate and used as substrate for cycle 3.2 due to the unavailability of fresh leachate. This was done to maintain an active biofilm for future cycles. Influent and effluent of the semi-continuous MFC was

measured for ammonia, nitrate, nitrite, sulfate, COD, BOD and heavy metals. BOD and heavy metals analysis were conducted at the University of Georgia Lab for Environmental analysis.

The oxidation of organic substrates produces carbon dioxide and water as byproducts, causing the leachate to evaporate during operation. Evaporation lowers the liquid surface and can result in an incomplete circuit connection, as the leachate and cathode need to be in contact for the circuit to be complete. Moreover when there is no contact between the cathode and leachate, protons from the anode cannot reach the cathode resulting in an incomplete MFC reaction. The batch reactors were checked every day of operation for proper contact between the cathode and leachate. Leachate was added to the cells periodically to prevent the loss of contact (volumes provided in appendix B). The operation of batch MFCs was terminated when the voltage decreased to less than 150 mV. This voltage was chosen so that the microbial community within the cell could be sustained; yet batch conditions for effluent testing of the leachate could be created. Hindrance in starting a new cycle immediately after a run occurred at times due to the unavailability of fresh leachate.

3.7 Data Acquisition

Data from the fuel cells were measured and recorded using a data acquisition unit connected to a desktop computer. A National Instruments (USB 6210) multifunction I/O unit with 16 inputs, 16-bit, 250 kS/s was used to measure voltage data from the cells every 4 minutes. LabVIEW 8.5 was used to store data in a "text" file; the data was imported to MS Excel for further analysis.

3.8 Leachate chatacterization

Leachate characterization was conducted before and after every cycle of the batch and semi-continuous MFC. All analyses except BOD and ICP for heavy metals were conducted at the Driftmier Engineering Center.

Probe readings

Dissolved oxygen (% and mg/L), conductivity (μS/cm), specific conductivity (μS/cm), resistance (Ω – cm), pH and oxidation-reduction potential (mV) were measured using an YSI professional plus multi probe system. YSI 5580 confidence solution was used to check if ORP and specific conductivity required calibration prior analysis. YSI 3822 and YSI 3823 buffer solutions were used to calibrate the pH to 7.0 and 10.0 respectively. The electrode of the probe was rinsed with distilled water prior analysis.

Chemical oxygen demand

COD is a common assay to indirectly measure the amount of organic compounds present in a substance. COD is measured in mg/L, denoting the mass of oxygen consumed per liter of solution. COD analysis were performed on all samples using USEPA approved HACH COD methods. In the HACH 8000 method, the sample is digested for two hours at 120° C with potassium dichromate, a strong oxidizing agent. The oxidizable organic compounds reduce the dichromate ion ($Cr_2O_7^{2-}$) to chromic ion (Cr^{3+}). The amount of Cr^{3+} present is the determined using a spectrophotometer at 620 nm. The HACH COD method used had a COD measuring range of 0-1500 mg/L. All samples were diluted before analysis, as leachate often had high COD values.

HACH method TNT 822 was used to measure the COD of leachate from all experiments. 0.3 ml of leachate was diluted with 2.7 ml of distilled water. 2 ml from this

mixture were added to the COD vials containing the digestion reagent. The vials were inverted gently several times to homogenize the sample and the reagents. The samples were then digested at 120° C for two hours using a HACH COD reactor (DR 200). After digestion, the vials were allowed to cool to less than 120°C in the digester. The vials were allowed to cool further to room temperature and then analyzed using a HACH DR 3800 spectrophotometer. When the vials are placed in the spectrophotometer, the instrument reports the COD of the sample. The displayed value was multiplied by the appropriate dilution factor (e.g., 10) accordingly.

Ammonia

High concentrations of ammonia in leachate are a constituent of concern because of free ammonia and free nitrous acid toxicity. Discharging leachate with high levels of ammonia into water bodies can affect water quality and the aquatic system. Ammonia was measured using HACH Methods TNT 832. Ammonia is measured using the Salicylate method. In this method, ammonium ions from the sample react with hypochlorite ions and salicylate ions to form indophenol. The reaction occurs at 12.6 pH using sodium nitroprusside as the catalyst. A green colored solution is formed as a result of the catalyst and excess reagents; the amount of color formed is directly proportional to the ammonia nitrogen present in the sample. Test results are measured at 620nm.

0.2 ml of undiluted sample was added to TNT 832 barcoded vial and inverted gently till the reagents in the vials are dissolved. After 15 minutes the outside of the vial was cleaned and placed on the cell holder of the DR 3800 spectrophotometer. The instrument reads the barcode and selects the ammonia high range program to measure the

concentration of ammonia in the sample in mg/L. The ammonia method detection limits ranged from $2 - 47$ mg/L $NH_3 - N$.

Nitrate and nitrite

Nitrate (NO_3^-) and Nitrite (NO_2^-) are found in landfill leachate as metabolic products of microbial digestion of nitrogen containing wastes. Nitrate and nitrite concentrations were determined using HACH Method TNT plus 836 and TNT plus 840 to measure nitrate and nitrite, respectively. Leachate nitrate concentration was measured using the Dimethylphenol method. Nitrate ions in the sample react with $2,6 -$ Dimethylphenol to form $4 - nitro - 2,6 -$ Dimethylphenol in solutions containing sulfuric and phosphoric acids. The sample is measured at a wavelength of 345 nm. 0.2 ml of undiluted sample was added to the barcoded vial. Solution A, a part of the nitrate method was required to be added (1ml) immediately after adding the sample. The vial was inverted gently until no streaks were visible. The vial was placed in the DR 3800 spectrophotometer after 15 minutes to record the concentration of nitrate in mg/L.

Nitrite concentration in leachate was analyzed using the Diazotization method. Nitrite in the sample is made to react with a primary aromatic amine in acidic solution to form a diazonium salt. The salt couples with an aromatic compound to form a colored complex that is directly proportional to the amount of nitrite present in the sample. The sample is then measured at 515 nm. 0.2 ml of undiluted sample was added to the TNT plus 840 vials to analyze the amount of nitrite in the sample. The TNT plus 840 vials came with a special cap that needed to be flipped and screwed back after adding the sample. The cap was flipped and inverted until the reagents in the cap were dissolved.

After 10 minutes, the vial was cleaned thoroughly and placed in the DR 3800 spectrophotometer to display nitrite concentrate in mg/L.

Biochemical oxygen demand

Biochemical Oxygen Demand (BOD) is a measure of the amount of molecular oxygen that aerobic biological organisms will utilize when degrading organic matter. It is a commonly used test to predict oxygen demand associated with a release of substrate into a water body. High BOD concentrations in a water body can cause dissolved oxygen depletion and affect the ecosystem negatively.

The Feed and Environmental Water Lab at the University of Georgia performed BOD analysis on the influent and effluent of cycles 3.1 and 3.5 of the semi-continuous MFC. The analysis lab followed the procedure as described in the Standard Methods for the Examination of Water and Wastewater 20th Edition. In this method, the sample is seeded with mixed liquor from a biological treatment system for microbial oxidation of the sample. The seed and the sample are incubated in airtight bottle for five days. The sample is measured for dissolved oxygen initially and after incubated for dissolved oxygen (DO). The BOD of the sample is then computed from the difference between the initial and final DO concentration. The samples were incubated at 20°C for 5 days and excluded from sunlight to prevent the photosynthetic production of dissolved oxygen. BOD is measured in units of mg/L.

Trace metal analysis

A trace heavy metal analysis was conducted on the influent and effluent of cycles 3.1 and 3.5 of the semi-continuous MFC at the University of Georgia Lab for Environmental Analysis. Leachate was analyzed for the concentration of magnesium,

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potassium, phosphorous, calcium, boron, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, cadmium, barium and lead.

Inductively Coupled Plasma – Mass Spectrometry (ICP – MS) was used to detect trace inorganic metals in the total metal analysis test. The ICP – MS method is a highly sensitive method capable of detecting metals and several non-metals at concentrations even below parts per trillion (10^{12}). A Perkin Elmer 9000 instrument was used following the USEPA 3051 method - Microwave assisted acid digestion of sediments, sludges, soils and oils. In this method the sample is dissolved in concentrated nitric acid and heated in a sealed fluorocarbon polymer or quartz microwave vessel to oxidize the organic compounds present. After cooling, the contents in the vessel are filtered, centrifuged and then diluted to analyze using ICP – MS machine. Heavy metal concentration was measured in parts per million in this experiment.

3.9 Cyclic voltammetry (CV)

Cyclic voltammetry is a potentiodynamic electrochemical measurement in which information about the analyte is obtained by measuring the current as the potential is varied. In this research CV is used to measure the oxygen reduction capability of the Pt/C and Ni/C catalysts. A CH instruments electrochemical analyzer was used along with silver –silver chloride reference electrode and platinum counter electrode to measure the CV of the catalysts. A potassium-phosphate buffer with pH 7 was used as the electrolyte for this experiment. The NiO/C catalyst was tested in an electrolyte saturated with oxygen to test its oxygen reduction ability. Later a nitrogen-saturated electrolyte was used to reaffirm that only oxygen reduction is occurring.

The working electrode was polished with 0.05 micron MicroPolish powder from CH instruments before loading the sample on it. The polished working electrode was then rinsed with distilled water and sonicated for 5 minutes to remove any left over polish powder using a Ultrasonic cleaner from Sharper Tek.

0.015 g of nickel catalyst was mixed with 100 μl of Nafion solution, 16 μl of this mixture was loaded on to the working electrode and dried at 70°C using a air oven. The Pt/C catalyst and carbon powder were also prepared and loaded on to the working electrode using the same procedure.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview

Microbial fuel cells were operated in batch and semi-continuous conditions using landfill leachate as the substrate in all the cells. Types of data collected include data obtained from voltage production, influent and effluent leachate characterization and cyclic voltammetry performed on the cathode catalysts. Experiment (1) and (2) were performed in batch MFCs to evaluate cathode catalysts and an alternative anode. The semi-continuous MFC was constructed to analyze the performance of MFC in scalable conditions. Influent and effluent leachate in all experiments was subjected to various assays to measure the concentration of organic matter, inorganic parameters and trace metals in the leachate.

4.2 Experiment1

Table 4.2.1 outlines the reactors names, electrodes and operating conditions for ease of interpretation.

Table 4.2.1: Reactor names, electrodes and operating conditions

	Experiment 1		Experiment 2		Experiment 3
	Reactor A *(Cycles 1.1, 1.2 and 1.3)*	**Reactor B** *(Cycles 1.1, 1.2 and 1.3*	**Reactor C** *(Cycles 2.1, 2.2 and 2.3)*	**Reactor D** *(Cycles 2.1, 2.2 and 2.3)*	**Reactor E** *(Cycles 3.1, 3.2,3.3,3.4 & 3.5)*
Cathode catalyst	Pt/C	NiO/C	Pt/C	Pt/C	Pt/C
Anode	Activated carbon and rod	Activated carbon and rod	Activated carbon and rod	Biochar and rod	Graphite plate and rod
Operation	Batch	Batch	Batch	Batch	Semi - continuous

The impact on treatment efficiency and power density was assessed in this experiment when Pt/C and an alternative cathode (NiO/C intended as catalyst) was used. As will be discussed later in subsequent sections, there is some doubt that the NiO/C was present on the cathode. It later appeared that activated carbon itself could provide some catalytic properties. Therefore, it is not known if it was Ni/C or C providing catalyst properties being evaluated in this experiment. Reactor B is still referred to as the "nickel" or NiO/C MFC or cell in some cases, but this is not a true evaluation of NiO/C, only an alternative cathode. Three continuous cycles of operation were completed in this experiment to ensure data consistency. Experiment 1 voltage data is plotted against time

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in Figures 4.2.1, 4.2.2 and 4.2.3. Influent and effluent leachate values of various parameters of Experiment 1 along with the percent difference are presented in the Appendix in Tables 4.2 – 4.7.
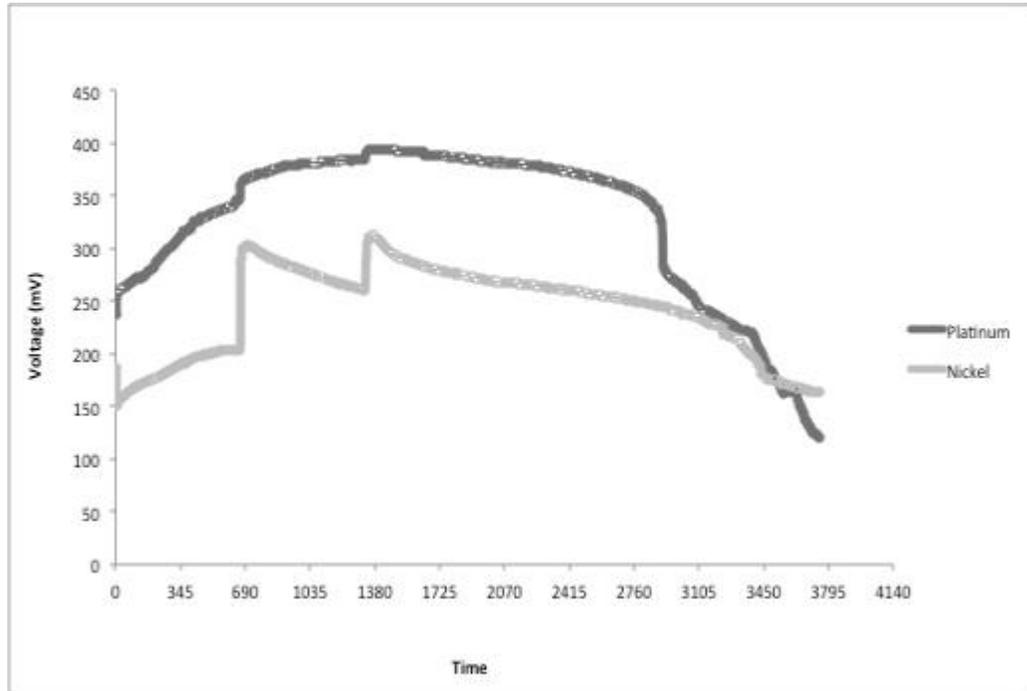

Figure 4.2.1: Experiment 1, cycle 1.1, MFC voltage

The voltage versus time plot illustrated in Figures 4.2.1, 4.2.2 and 4.2.3 illustrates the phases that are typical in bacterial growth curve. The bacterial growth curve begins with a lag phase during which bacteria get accustomed to the environment; very little growth and multiplication occurs in this phase. Electricity generation was minimal at the beginning of this phase and steadily increased at the end of the lag phase. An exponential growth phase follows the lag phase, microbial population increases exponentially in this phase. The exponential and stationary phase account for the majority of electrons produced in a microbial fuel cell. In the stationary phase, there is little or no growth observed but living cells are maintained active. Electricity production remained

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stable in this phase. The last phase is a death phase, in which bacteria die, if the carbon and nutrient sources are not replenished. Electricity production started to decline in this stage.
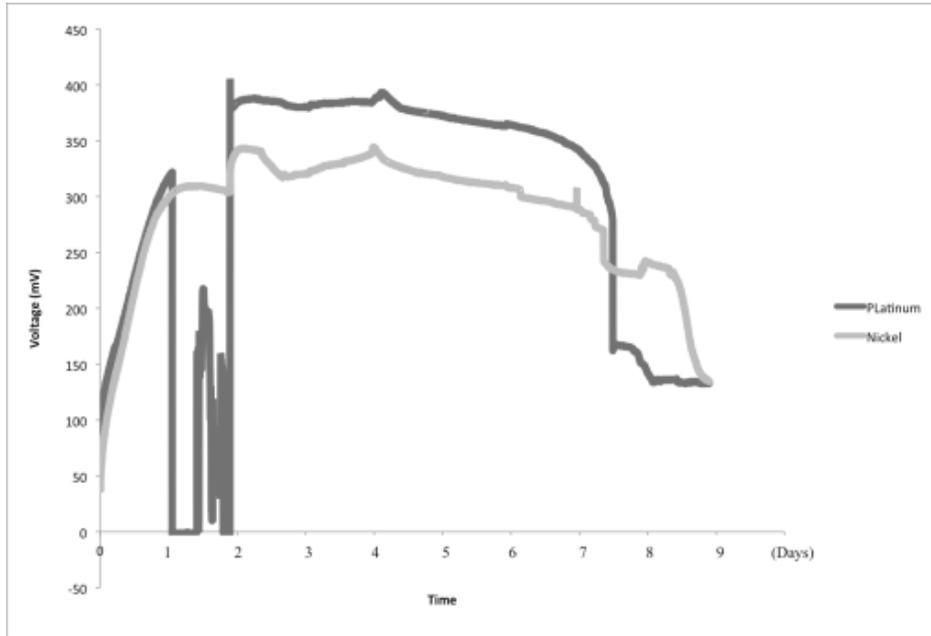

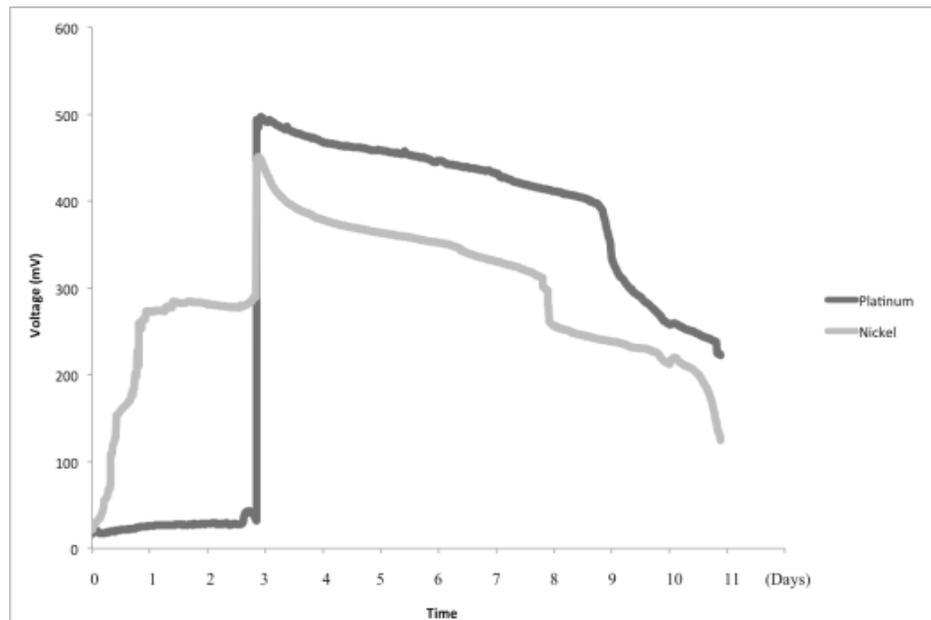Figure 4.2.2: Experiment 1, cycle 1.2, MFC voltage

Figure 4.2.3: Experiment 1, cycle 1.3, MFC voltage

MFC operation periods varied for each cycle and experiment. Cycle time of the MFCs in Experiment 1 varied from 8 to 10 days. The batch MFCs were stopped once the voltage reduced to less than 150 mV ensuring an active biofilm is present for the next cycle. The maximum voltage reached in each cell improved over time in each run. Reactor A with the platinum catalyst peaked at 394 mV in cycle 1.1 and improved to 490 mV at the end of cycle 1.3. Reactor B with alternative cathode also showed similar results in which the maximum voltage increased from 312 mV in cycle 1.1 to 451 mV in cycle 3.3. Table 4.2.2 summarizes the dates and days of operation of each MFC along with the voltage at the start, peak and end of each cycle.

Table 4.2.2: Experiment 1, Overview of the batch MFC

	Cycle 1.1		Cycle 1.2		Cycle 1.3	
Start Date	7/2/2011		7/13/2011		7/29/2011	
End Date	7/13/2011		7/22/2011		8/9/2011	
Cycle Time (hours)	260		213		260	
	Pt cell	**Ni cell**	**Pt cell**	**Ni cell**	**Pt cell**	**Ni cell**
Start Voltage (mV)	238.3	151.7	91.5	37.8	16.9	21.8
Peak Voltage (mV)	394.5	312.1	402.7	344.3	496.6	451.1
Time to peak (mV) voltage (hours)	94	95	45	95	69	69
End Voltage (mV)	120.0	163.1	132.8	134.5	222.1	125.1

With the successive improvement of voltage in each run, the data in Table 4.2.2, indicates the establishment of an active exoelectrogen community within the MFC, since an increase in maximum voltage was observed as each cycle progresses. Negative voltage

values were obtained occasionally in some cycles of the batch MFC owing to the loss of contact between the cathode and leachate or loose connection in the breadboard. Leachate was added in the batch MFCs to compensate for evaporation thereby bringing contact between the cathode and leachate. Volumes of leachate added in the batch MFCs during operation can found in Appendix A. The platinum (Reactor A) and nickel (Reactor B) based MFCs reached their maximum voltage in almost similar time frames with an exception of cycle 1.2 in Experiment 1. Tables in appendix A show the various parameters measured before and after cycle 1.1, as well as percent change in each.

Negative values in the percent difference column indicate that there was an increase of that particular parameter during MFC operation. Effluent from Reactor A and B showed different chemical characteristics in spite of being subjected to reactors with same dimensions, leachate and electrodes.

Landfill leachate is an anaerobic fluid with low dissolved oxygen concentration. DO concentration in leachate can increase during sampling or when transferring leachate into a microbial fuel cell. Since all cycles of Experiment 1 operated in this research had an open-air cathode, the DO concentration could increase during operation. Dissolved oxygen concentration of the effluent increased in Cycles 1.1(Reactor A) and Cycle 1.2. An Aerobic zone could have been formed around the cathode in these MFCs causing an increase in DO concentration. Dissolved oxygen decreased in Cycle 1.1 (Reactor B) and Cycle 1.3. Anaerobic zones within theses MFCs would have been more efficient in maintaining the substrate anaerobic, thereby retaining low DO concentration.
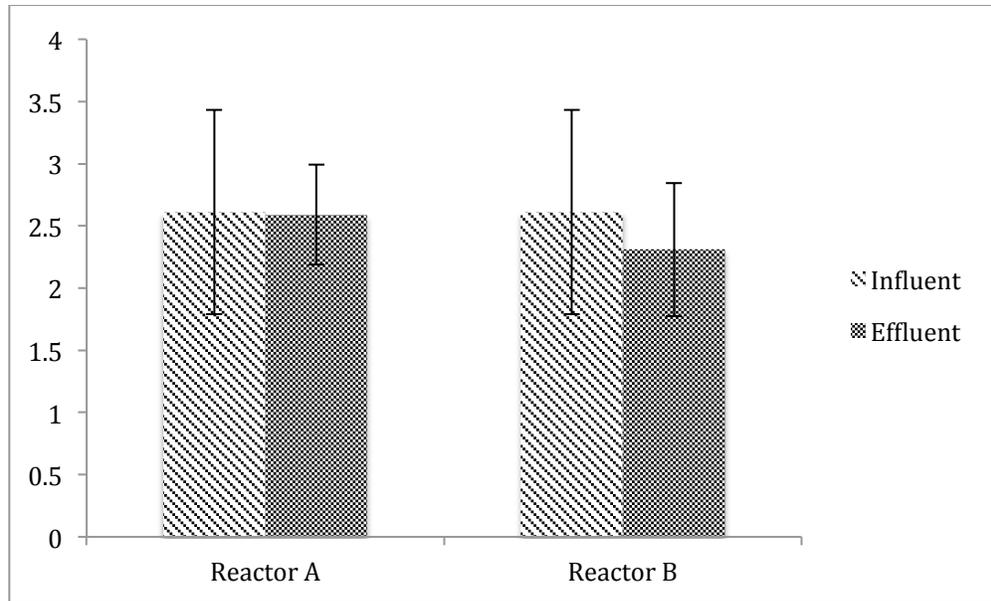
Figure 4.2.4: Average influent and effluent DO value of Experiment 1 with one

standard deviation error bars

The error bars overlap between the influent and effluent of both Reactors A and B, indicating that there is no statistically significant difference in influent and effluent DO concentrations. There was no significant difference between the effluent of Reactor A and B as well.

pH:

The pH of the effluent decreased in all cycles of Experiment 1. pH of the substrate in an MFC us subjected to change based upon the efficiency of the anode and cathode. An incomplete cathode reaction can cause proton build up within the MFC making the pH more acidic. Figure 4.2.5 presents the average influent and effluent pH value of Reactor A and B from all cycles of Experiment 1.
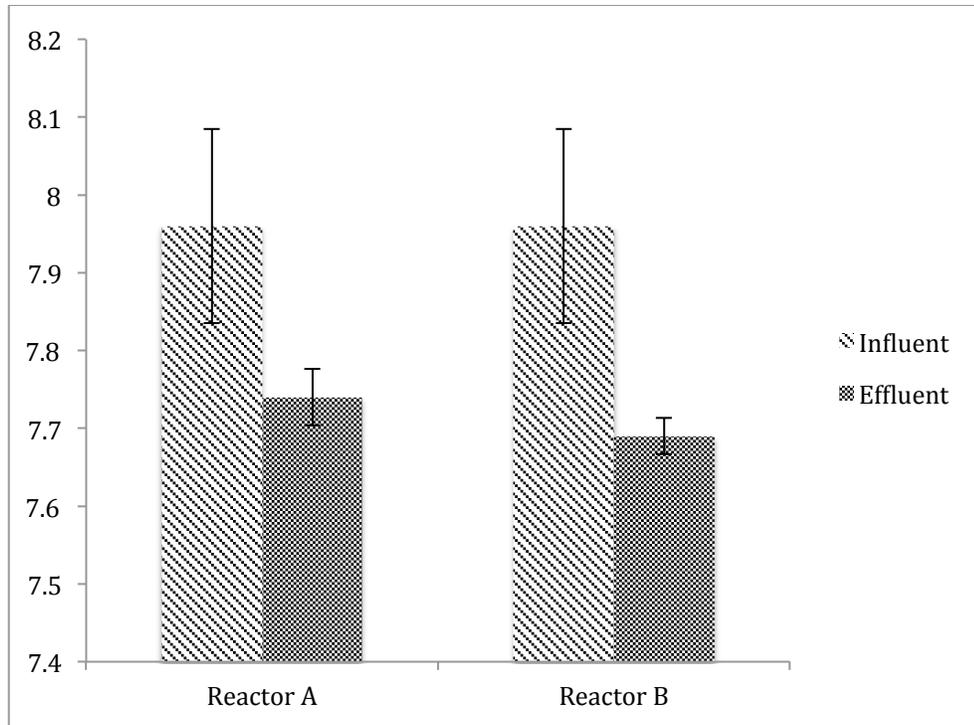
Figure 4.2.5: Average influent and effluent pH value of Experiment 1 with one

standard deviation error bars

Leachate being a highly buffered fluid with high sulfide concentrations is capable of accepting protons and limit pH decrease. pH increase in certain cycles can be because of an efficient cathode reaction, in which the protons are efficiently transferred to the cathode. Other studies that used landfill leachate as substrate suggested that the removal of acid components present in the leachate like volatile fatty acids could increase the pH of the system. (*Greenman et al. 2009)*. In all cycles of Experiment 1, there was significant difference within the influent and effluent pH value in Reactors A and B, but no significant difference was observed between the effluents of both the reactors.

Oxidation reduction potential

Oxidation-reduction potential (ORP) measures the tendency of a solution to lose or gain electrons. An aerobic system shows a positive ORP reading, while an anaerobic

system displays negative reading. Landfills are generally anaerobic systems; as a result the leachate flowing through them is usually anaerobic with negative ORP values. ORP values of all influent leachate used in this experiment were anaerobic with negative ORP values. . Figure 4.2.6 shows the average influent and effluent leachate OPR values from all cycles of Experiment 1 with one standard deviation error bars.

In Cycle 1.1 (Reactor B) the ORP value decreased at the end of MFC operation, indicating the presence of electrons in leachate. It must be noted that low ORP values cannot be indicative that no dissolved oxygen was present in the leachate. The oxidation-reduction potential of effluent from all the other cycles had higher ORP readings (towards the positive scale) than their influents implying the loss of electrons during MFC operation.
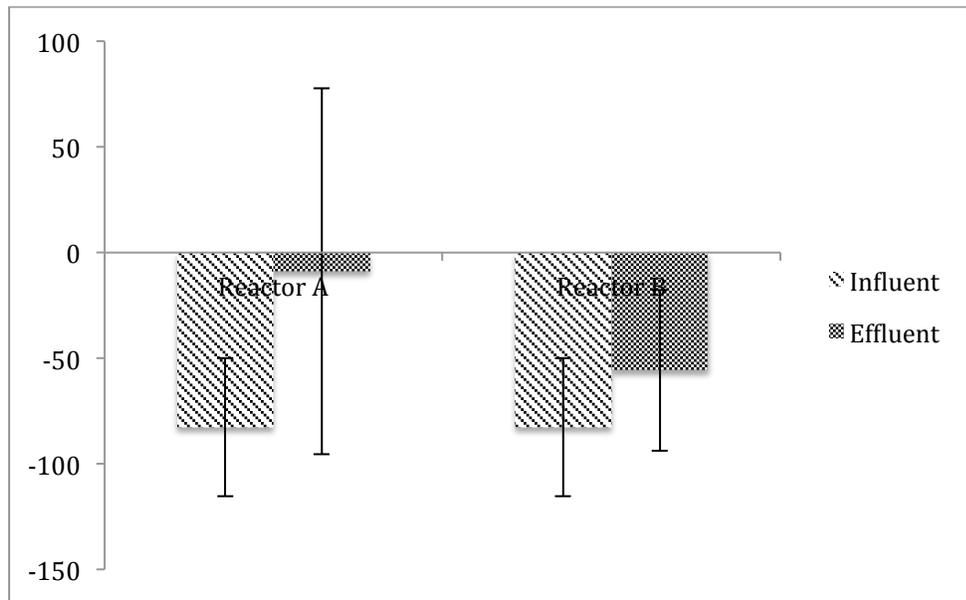

Figure 4.2.6: Average influent and effluent ORP value of Experiment 1 with one standard deviation error bars

Conductivity

Conductivity is the ability of a solution to conduct electricity. The conductivity of a solution depends on the ion concentration of the solution as the electric charge is

carried by ions in a solution. Influent and effluent leachate conductivity of all cycles in Experiment1 is presented in the Appendix. Conductivity of the effluent decreased in all cycles of Experiment 1, except in Reactor C of Cycle 1.2. Decrease in ion concentration due to MFC reaction, could explain low conductivity in the effluent. Figure 4.2.7 shows the average influent and effluent conductivity value of leachate of all Cycles in Experiment 1 with one standard deviation error bars. No statistically significant difference can be observed between the influent and effluent of both reactors. The effluents of both reactors are also not significantly different suggesting that the difference in catalyst might not affect the conductivity of the solution.
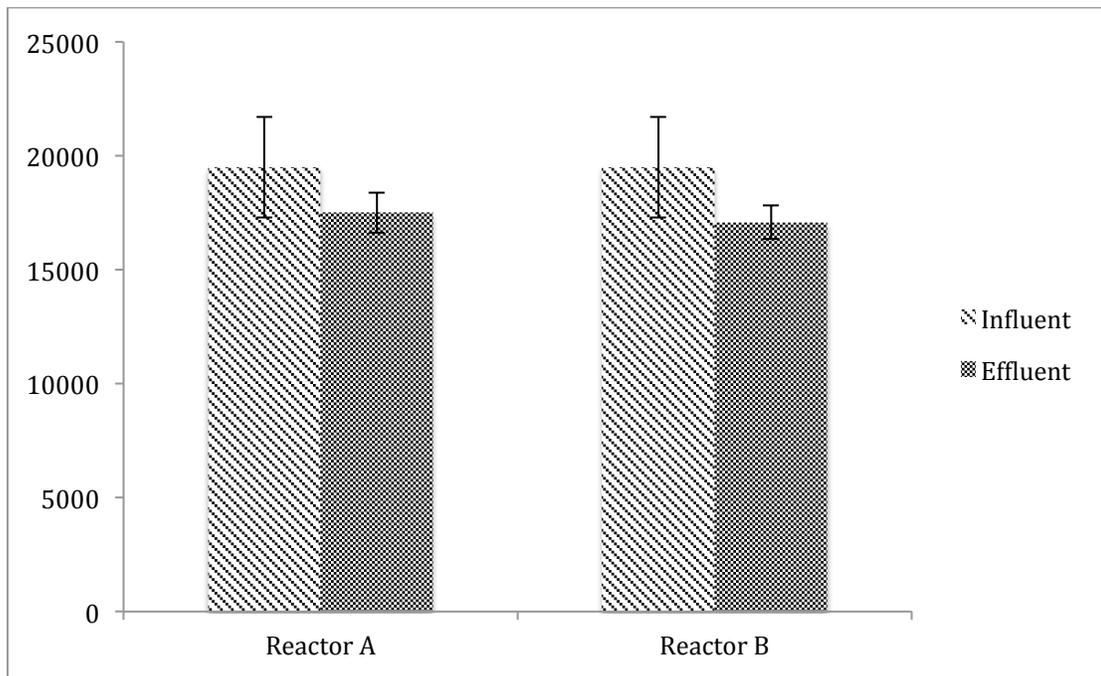

Figure 4.2.7: Average influent and effluent conductivity values of Experiment 1 with one standard deviation error bars

Chemical oxygen demand

Chemical oxygen demand (COD) assay was used in this research to measure the amount of organic substrate present in leachate. COD was used to test the concentration

of organic compounds present in the influent and effluent of all cycles in all experiments of this research. Tables in Appendix A present the influent and effluent values of leachate COD along with the percent difference form all cycles of Experiment 1.

Figure 4.2.8 shows the influent and effluent COD in mg/L of Reactor A in Cycles 1.1, 1.2 and 1.3. The MFC with platinum on carbon catalyst (Reactor A) reduced COD marginally better than the alternative cathode MFC (Reactor B).

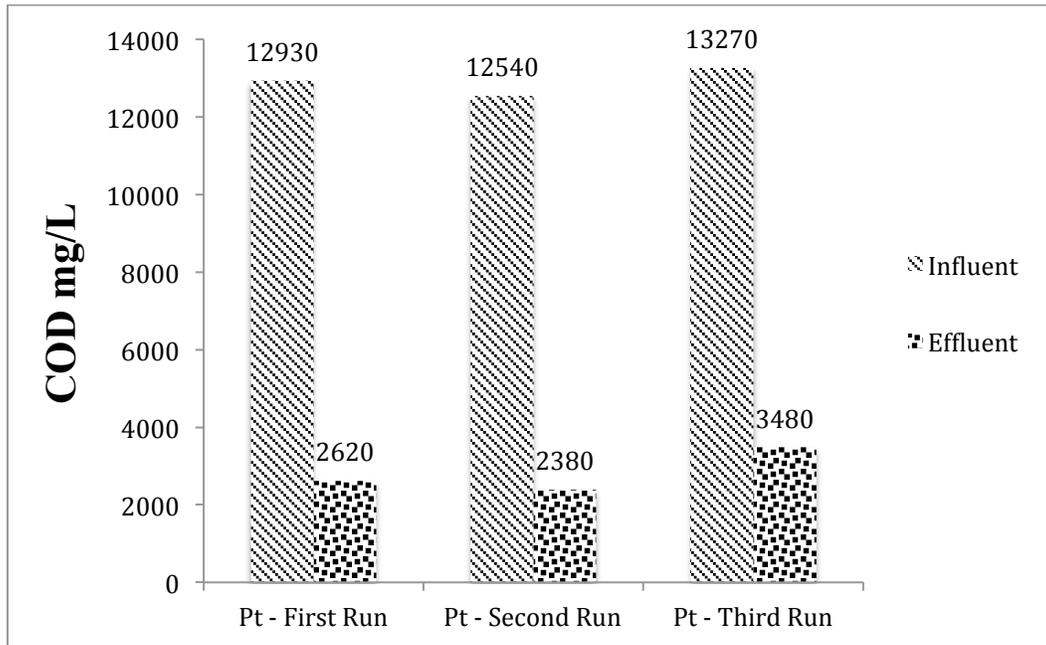

Figure 4.2.8: COD comparison of Cycles 1.1, 1.2 and 1.3 of the Platinum MFC

The efficiency of Reactor B in reducing COD of the influent wasn't as good as Reactor A, although significant amount of COD reduction was observed. Figure 4.2.9 displays the influent and effluent COD in mg/L of reactor B. The influent COD was reduced by 79.8 % in cycle 1.1, 62.2% in cycle 1.2 and 75.6 % in cycle 1.3.
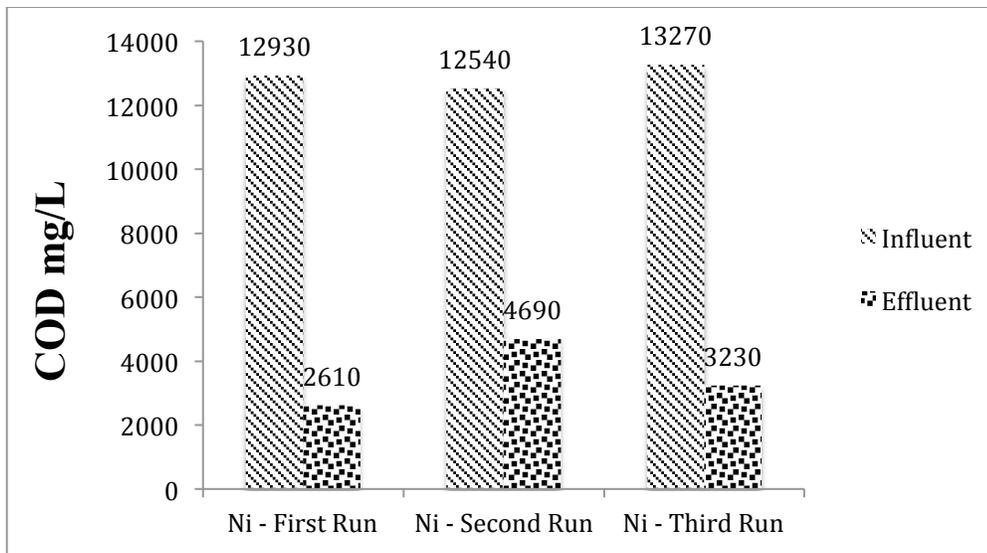
Figure 4.2.9: COD comparison of Cycles 1.1, 1.2 and 1.3 of the Nickel MFC

The platinum MFC displayed better performance than the alternative cathode MFC with better efficiency in terms of treatment as well as voltage production. The percentage difference was greater than 20% when the average effluent values were used to calculate the differences. Interestingly, when the percent difference values of the effluents were used to check the selection criterion (less than 20% difference), less than 20 % difference in effluent COD was observed. The sheer magnitude of the numbers (raw COD value in 3500 – 4500 mg/L and percent difference in 72-78%) explains the variation in the results.

Figure 4.2.10 shows the average COD values of influent and effluent obtained from the platinum and alternative cathode MFC. The error bars represent one standard deviation of the mean. Based upon this standard deviation there is a significant difference between the mean influent and effluent COD values for each MFC, since the error bars do not overlap each other.
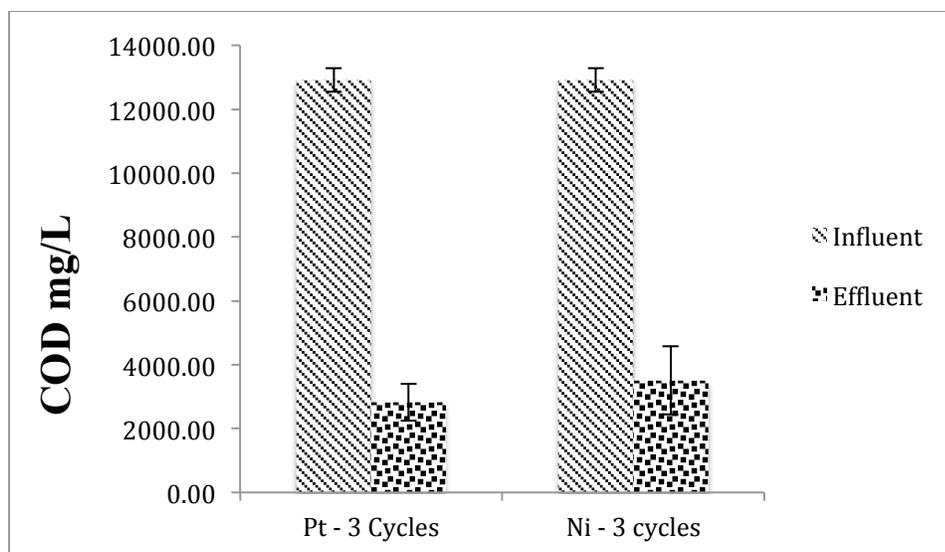
Figure 4.2.10: COD of Influent and Effluent of all cycles in experiment 1, with standard deviation error bars

The average COD values of influent and effluent leachate from all cycles of Experiment 1 are presented in Figure 4.2.9, using one standard deviation as error bars. In this case, the error bars overlap indicating there is no significant difference in the mean percent reduction of COD, i.e., both Pt/C and NiO/C (alternative cathode) performed similarly in terms of percent reduction of COD. Although there was no significant difference in percent reduction (Figure 4.2.11), the experiment was confounded by the fact that activated carbon was providing adsorption to also reduce COD values. Activated carbon has been a widely used to remove recalcitrant organic compounds from landfill leachate for its adsorbing capabilities. *(Kurniawan T. A et. al 2005)*. Activated carbon can efficiently adsorb readily available organic and inorganic pollutants from wastewater due to its large surface area, micro porous structure, high adsorption capacity and reactivity. Considerable amount of COD reduction in Experiment 1 might be due to activated carbons capacity to readily adsorb less biodegradable organics. Due to this confounding phenomenon, Pt/C was again chosen as the cathode material for both Experiment 2 and 3.

Since a new material was being tested in Experiment 2 (biochar as anode) a proven efficient catalyst was used as the cathode catalyst. Then to further explore NiO/C as a cathode catalyst, other methods (e.g., CV, XRD, SEM) were used to evaluate it.
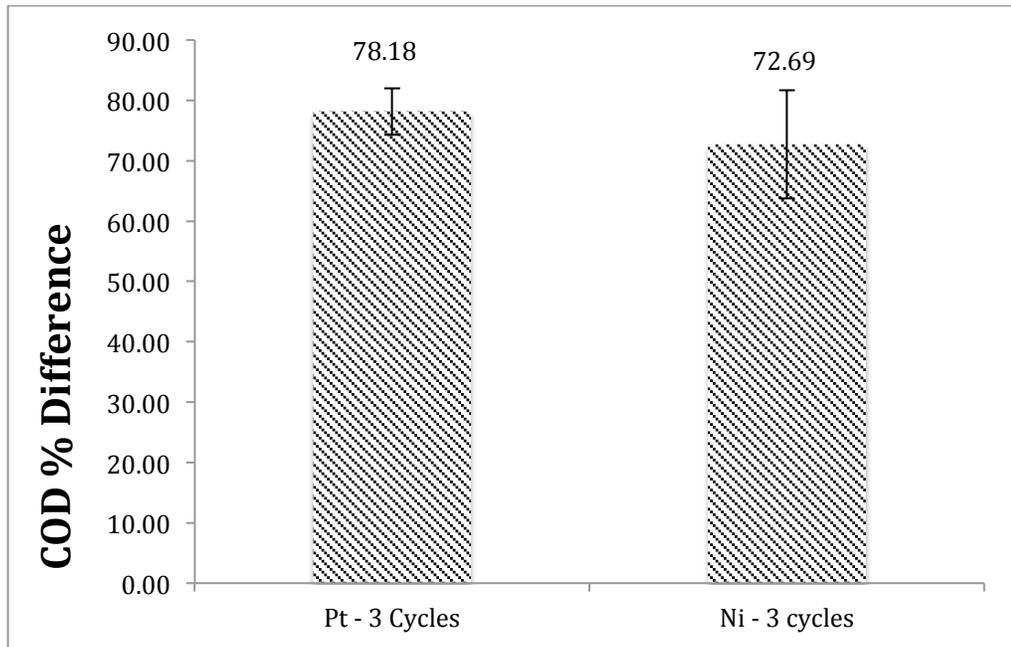
Figure 4.2.11: Average percent reduction of influent and effluent COD values of Experiment1

4.3 Experiment 2

Experiment 2 was conducted to test the performance of activated carbon and biochar when used as an anode in a microbial fuel cell. For consistency, Experiment 2 was also conducted in three continuous cycles of operation. Reactors with same dimensions used in experiment 1 were utilized in this experiment as well. Platinum on carbon was chosen as the catalyst for all cycles of this experiment. Activated carbon granules were used as the anode in Reactor C and biochar was used as the anode in Reactor D. Voltage

versus time plot of all cycles of Experiment 2 are presented in Figures 4.3.1, 4.3.2 and 4.3.3.
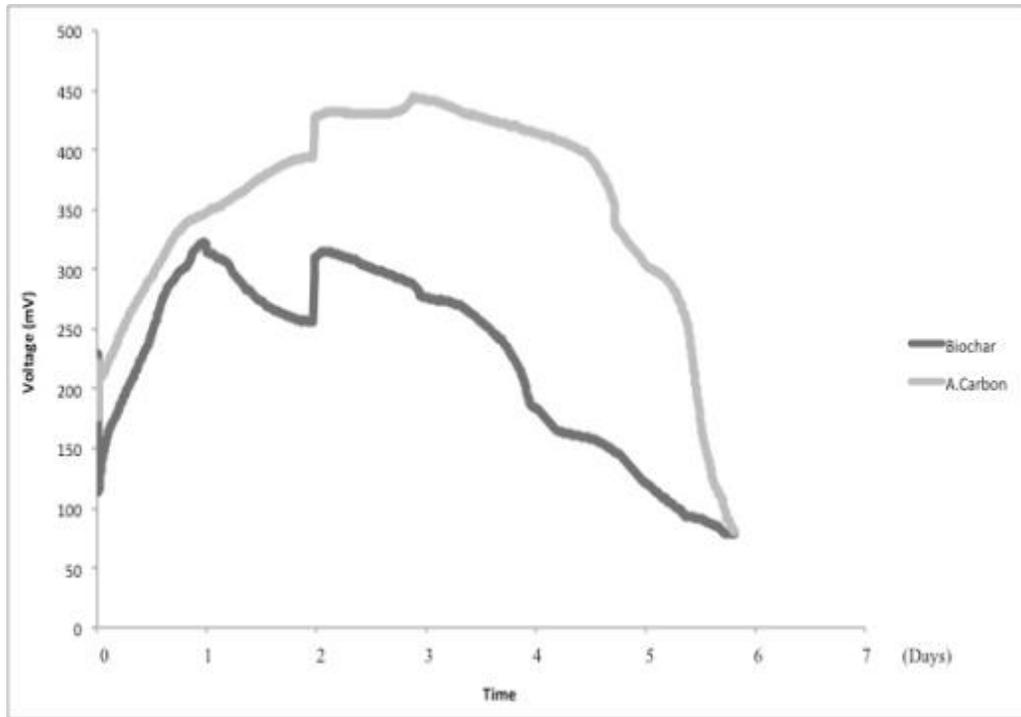
Figure 4.3.1: Experiment 2, cycle 2.1, MFC voltage

The maximum voltage obtained by Reactor C reduced from 444 mV in cycle 2.1 to 424 mV in cycle 2.3. Saturation of activated carbon over time may be attributed towards the reduction in voltage production over time. As the surface area of activated carbon gets saturated with recalcitrant COD or other trace metals, it cannot provide an ideal environment for an efficient MFC reaction. Even a thick inactive biofilm can be a limiting factor for efficient electron transfer to the anode.

Figure 4.3.2: Experiment 2, cycle 2.2, MFC voltage
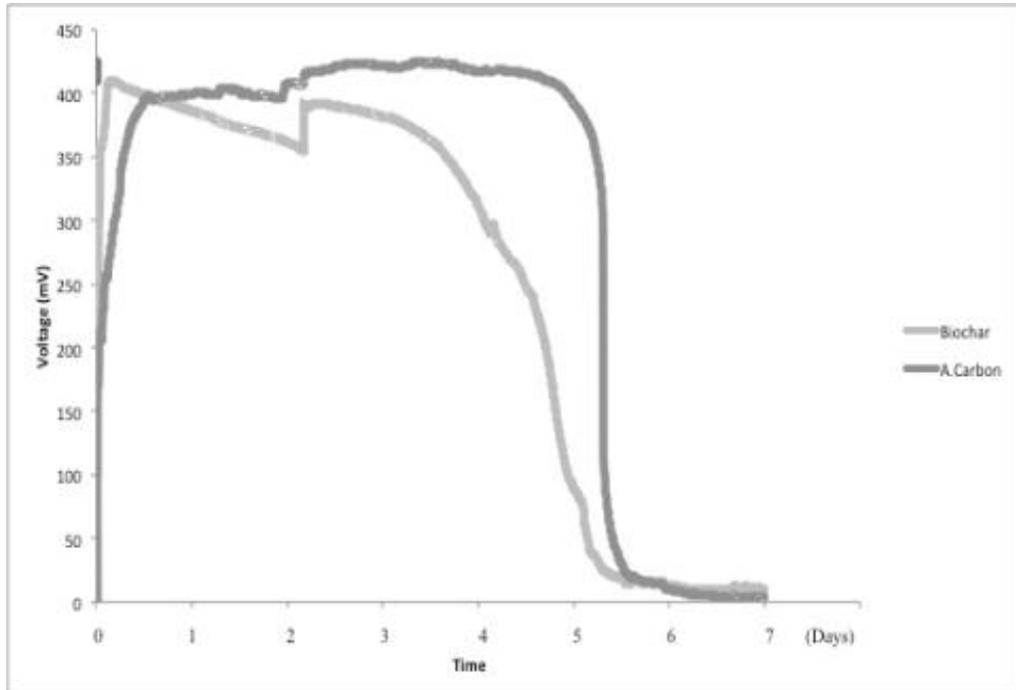
Figure 4.3.3: Experiment 2, cycle 2.3, MFC voltage

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Table 4.3.1 presents an overview of the voltage results obtained from all cycles. The operation period of each cycle in this experiment was shorter than observed in Experiment 1.

Table 4.3.1: Experiment 2, Overview of batch MFC results

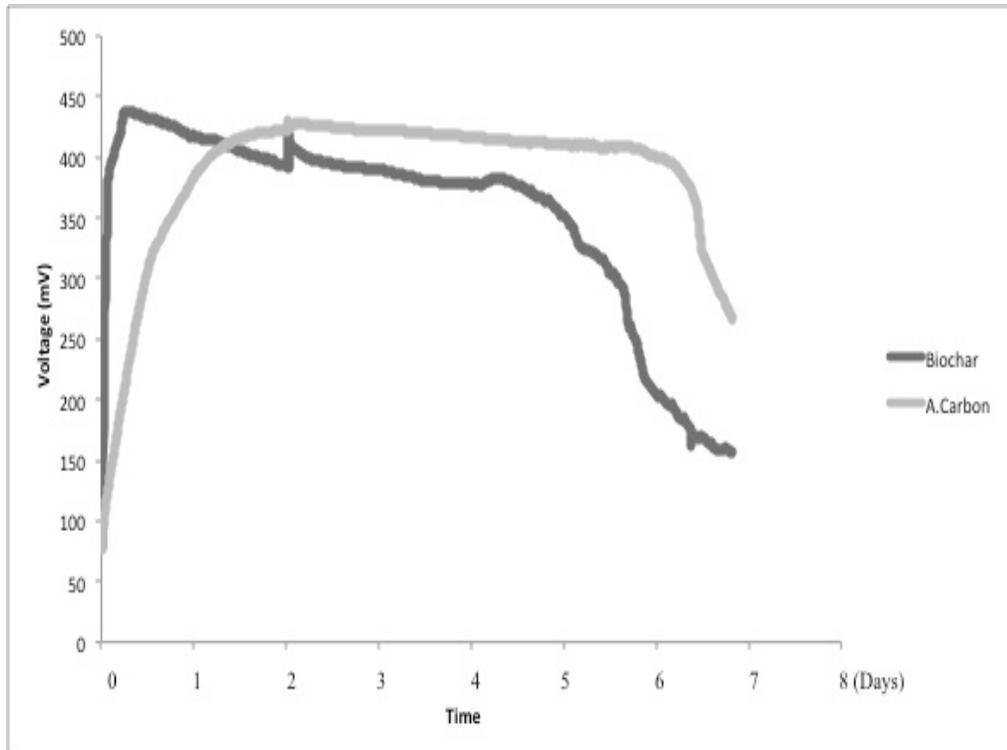|	Cycle 2.1		Cycle 2.2		Cycle 2.3	
Start Date	9/13/2011		9/19/2011		9/26/2011	
End Date	9/19/2011		9/26/2011		10/3/2011	
Cycle Time (hours)	139		163		167	
	A.C cell	BC cell	A.C cell	BC cell	A.C cell	BC cell
Start Voltage (mV)	176	113	76	79	171	101
Peak Voltage (mV)	444	322	430	437	424	409
Time to peak voltage (hours)	68	23	48	6	81	9
End Voltage (mV)	80	77	264	155	10	3

The peak voltage obtained in reactor D increased from 322 mV in cycle 2.1 to 437 mV in cycle 2.2. Activated carbon, known for having a high internal surface area in comparison to biochar is a more efficient anode. In cycle 2.2, reactor D with biochar as the anode had a higher maximum voltage than reactor C that used activated carbon as the anode. Reactor C also took a longer time to reach the maximum voltage than reactor D.

Dissolved oxygen

Dissolved oxygen concentration is required to be low in the substrate for efficient power production in an MFC. Effluent dissolved oxygen concentration increased in all

cycles of Experiment 2 except Cycle 2.1 in Reactor D and in Cycle 2.2. This might be because of an aerobic zone formed around the cathode as a result of the MFC design. Reactor C in Cycle 2.1 and both reactors in Cycle 2.3 showed decreased DO levels in the effluent. Figure 4.3.4 presents the influent and effluent dissolved oxygen concentrations of all cycles from Experiment 2 with one standard deviation error bars.
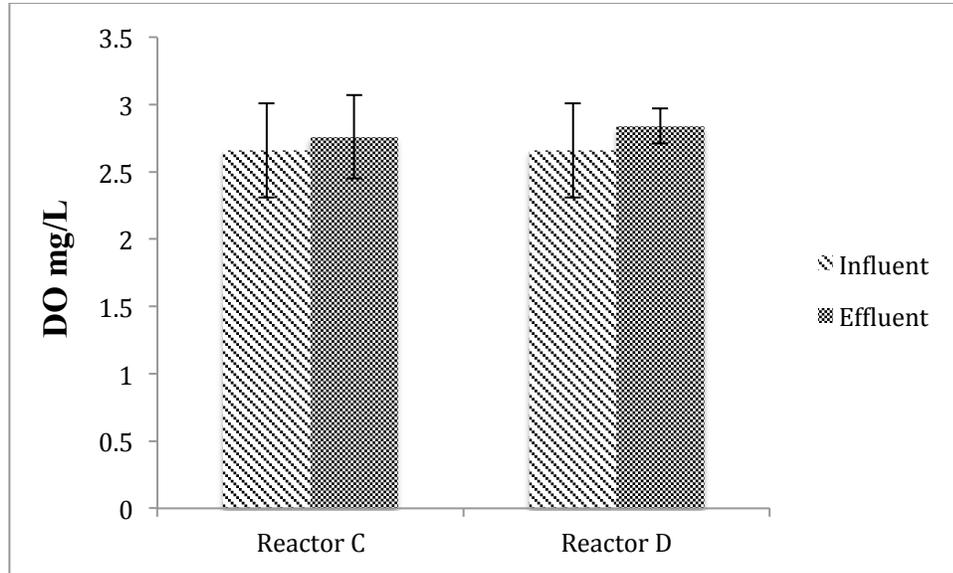
Figure 4.3.4: Average influent and effluent DO values of Experiment 2 with one standard deviation error bars

pH:

The pH of the effluent decreased on cycles of Experiment 2, except Cycle 2.1, Reactor D in Cycle 2.2 and Cycle 2.3. Increase in pH value of the effluent could be associated with an incomplete cathode reaction that can cause proton build up within the MFC. Removal of acidic components within the leachate could also increase the pH of the system.
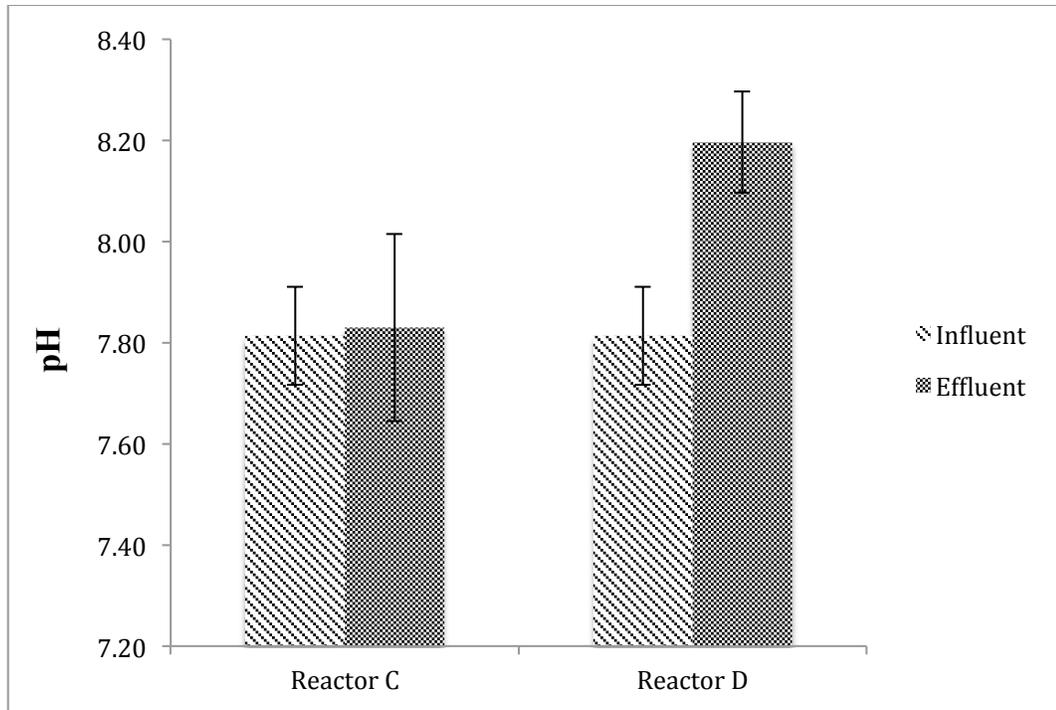
Figure 4.3.5: Average influent and effluent pH values of Experiment 2 with one standard deviation error bars

Figure 4.3.5, presents the average influent and effluent pH values from all cycles of Experiment 2 with one standard deviation error bars. The error bars overlap in Reactor C suggesting that there was no significant difference in influent and effluent pH in Reactor C. Reactor D showed significant increase in effluent pH concentration. Individual pH values along with the percent difference of all cycles in Experiment 2 are presented in the appendix.

Oxidation reduction potential

ORP indicates the ability of a solution to donate electrons. Influent with leachate with low ORP (negative) values are preferred for efficient voltage production in an MFC. Influent leachate used in all cycles of Experiment 2 had negative ORP values.
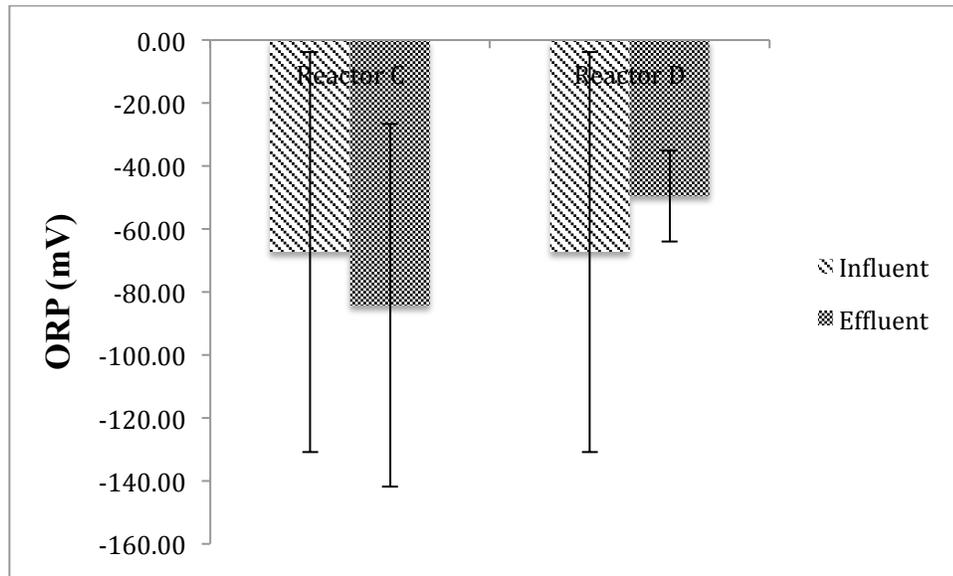
Figure 4.3.6: Average influent and effluent ORP values of Experiment 2 with one standard deviation error bars

Figure 4.3.6 illustrates that there was no statistically significant difference in influent and effluent leachate of Reactor C and D. Raw ORP values of all cycles from Experiment 2 are presented in the Appendix.

Conductivity

The conductivity of the effluent was increased in both reactors of Cycle 2.2 and Reactor C of Cycle 2.3. Figure 4.3.7 presents the average conductivity of influent and effluent leachate from all cycles of Experiment 2.
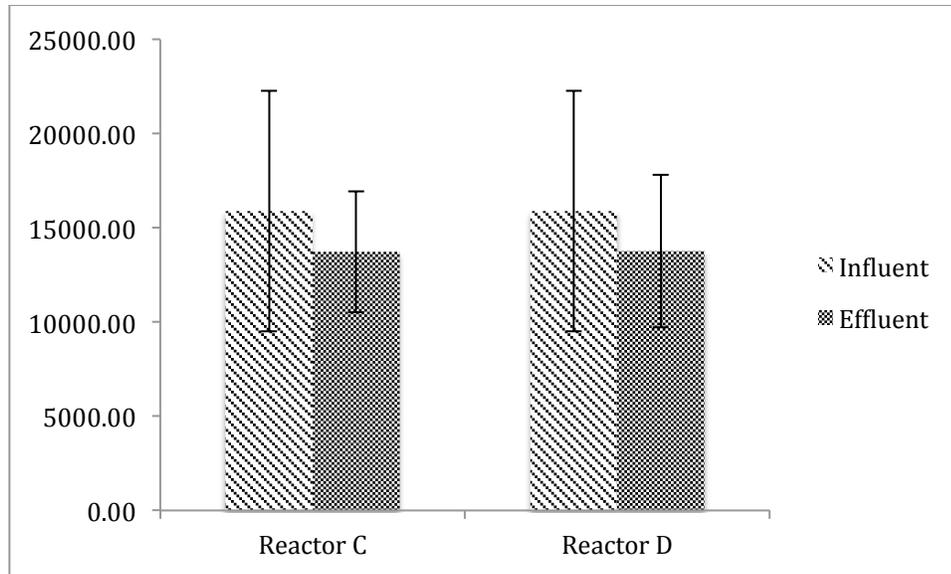
Figure 4.3.7: Average influent and effluent conductivity values of Experiment 2
with one standard deviation error bars

Conductivity of the effluents in this experiment was similar to those observed in Experiment 1. Despite reduced individual effluent values, these were not statistically significant.

Chemical oxygen demand

COD assay was used to measure the concentration of organic substrates of all influent and effluent leachate in this experiment. Reactor C with activated carbon granules as anode reduced influent COD better than reactor D with biochar anode. Reactor C had COD removals of 69 – 79 % while reactor D achieved a COD reduction within the range of 22 – 38 %. COD reduction of influent leachate may also be caused by the adsorption of leachate by activated carbon granules and biochar. Activated carbon granules have higher surface area than biochar. Surface area of both these materials was measured using a Quantachrome Autosorb 1-C, using the 11-point BET method.

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Activated carbon granules used as anode in this research had a surface area of 579.2 m^2/g while the biochar had 15.8 m^2/g surface area.

Effluent COD value appeared to be increasing over time after every cycle in both the reactors. In reactor C, effluent COD increased from 3320 mg/L in cycle 2.1 to 3830 mg/L in cycle 2.2 and finally to 4790 mg/L in cycle 3.3. A similar effluent COD increase was observed in reactor D. Such an increase in effluent COD could be associated with the saturation of activated carbon and biochars' surface area with organic materials and other substances.
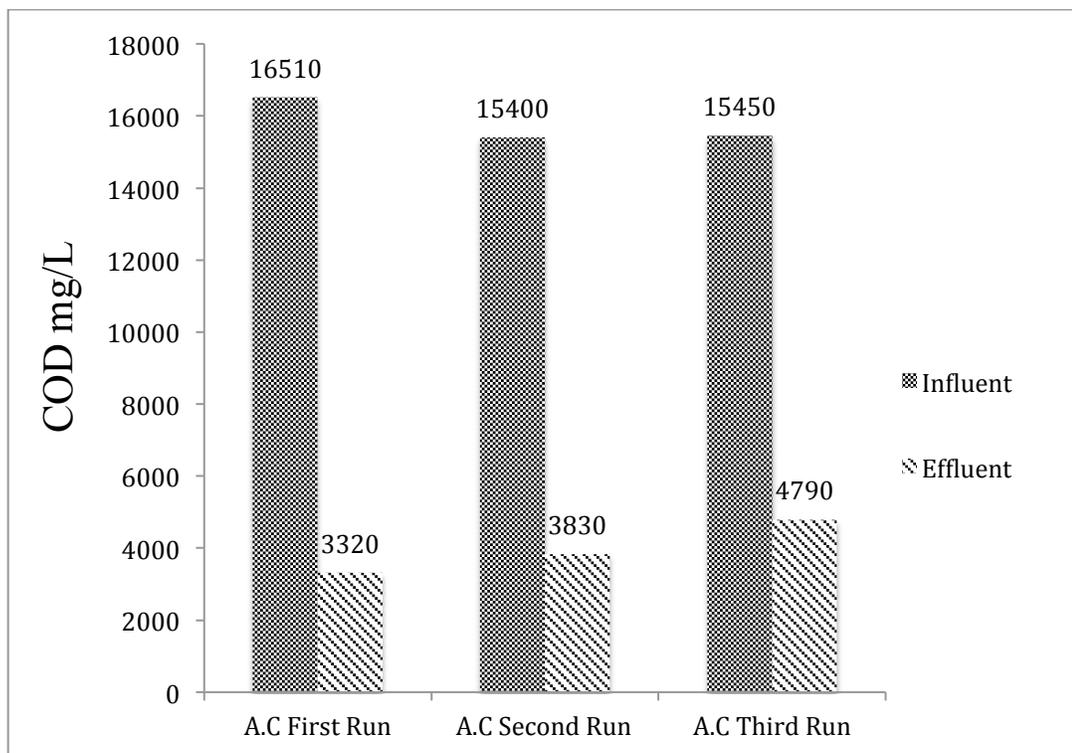
Figure 4.3.8: COD comparison of Cycles 2.1, 2.2 and 2.3 of the Activated carbon MFC
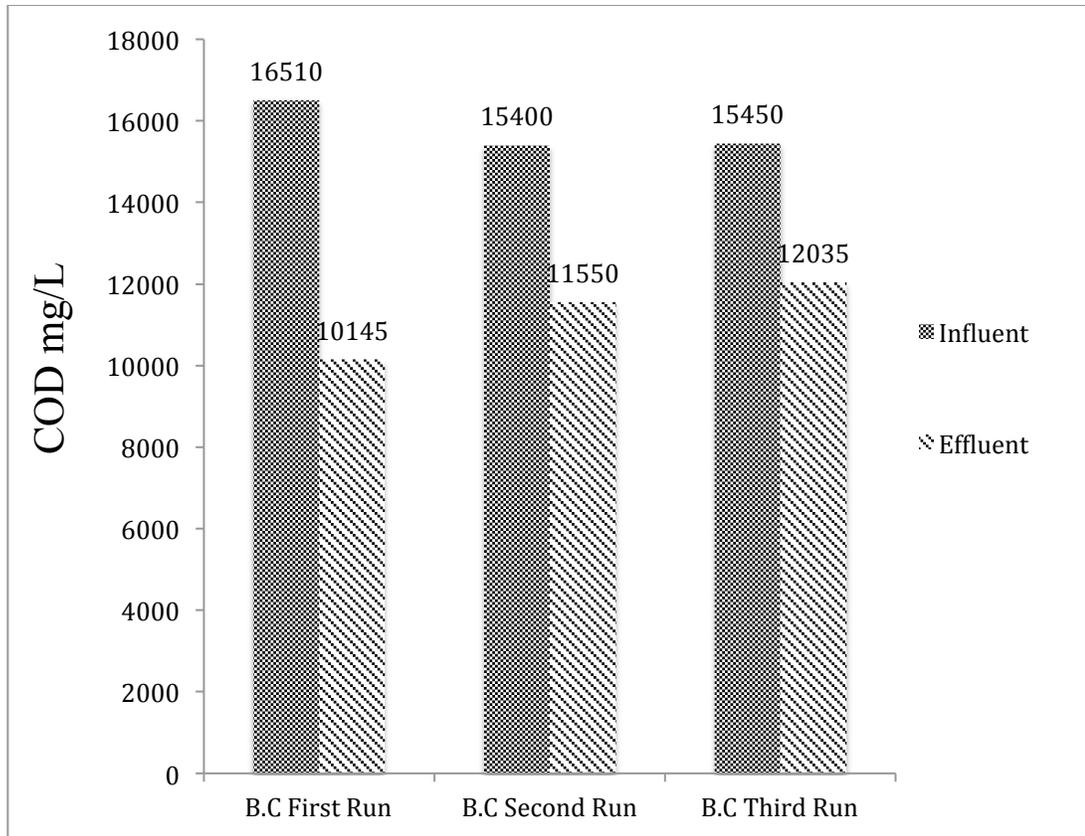
Figure 4.3.9: COD comparison of Cycles 2.1, 2.2 and 2.3 of the Biochar MFC

Dissimilarity in COD reduction in Reactors C and D could be because of activated carbons' ability to readily adsorb recalcitrant COD. Since activated carbon has a higher surface area than biochar, it can reduce the concentration of organics more efficiently. The use of activated carbon as the anode in an MFC can still be advantageous as it can efficiently adsorb recalcitrant COD and other trace metals. Although, when COD reduction is due to microbial oxidation, more electrons are produced and this could increase voltage production.

The average percent difference of influent and effluent COD from all cycles of Experiment 2, showed that there was a statistically significant difference in effluent COD in Reactors C and D.
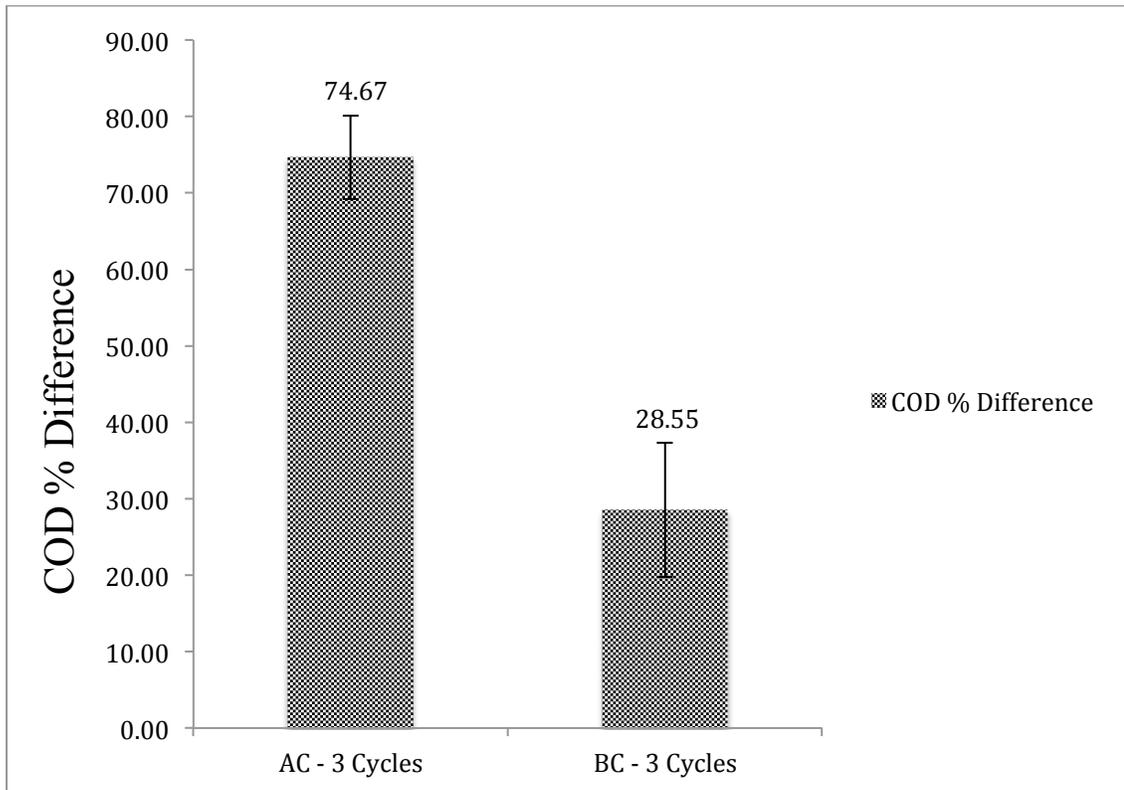
Figure 4.3.10: Average percent difference of COD of all cycles from Experiment 2 with standard deviation error bars

4.4 Experiment 3

The semi-continuous MFC was operated from January through March 2012. All the cycles, except Cycle 3.2 were operated using fresh leachate in the semi-continuous MFC. In Cycle 3.2, effluent from Cycle 3.1 was mixed with stored leachate (collected for Cycle 3.1) due to the unavailability of fresh leachate. Table 4.4.1 presents an overview of voltage data obtained from all cycles of Experiment 3.

Table 4.4.1: Experiment 3, Overview of Semi-continuous MFC results

	Cycle 3.1	Cycle 3.2	Cycle 3.3	Cycle 3.4	Cycle 3.5
Start Date	1/27/12	2/1/12	2/9/12	2/23/12	2/28/12
End Date	2/1/12	2/8/12	2/23/12	2/28/12	3/7/12
Cycle Time (hours)	118	163	330	116	187
Start Voltage (mV)	96	87	105	203	233
Peak Voltage (mV)	454	579	566	575	573
Time to peak voltage (hours)	63	45	276	20	42
End Voltage (mV)	204	288	515	526	547

Stable voltage production was observed in all cycles of Experiment 3. Due to time constraints all cycles in Experiment 3 were terminated once voltage production became stable and to also ensure an active biofilm is present for future cycles. Cycle 3.2 with the mixed leachate obtained the highest peak voltage, followed by Cycle 3.4 and 3.5.

Cycle 3.3 took 330 hours to reach its peak voltage, the longest when compared with other cycles, whereas Cycle 3.4 and 3.5 obtained their maximum voltage in a very

short period, 20 and 42 hours respectively. In Cycle 3.3 a highly active biofilm could have been formed over time and since the next cycle were started immediately, Cycle 3.4 and 3.5 reached their peak voltage in a very short period. Cycle 3.5 took double the time than cycle 3.4 to reach its peak voltage, suggesting a decline in biofilm activity. Figures 4.4.1- 4.4.5 shows voltage data plotted against time for all cycles in Experiment 3.
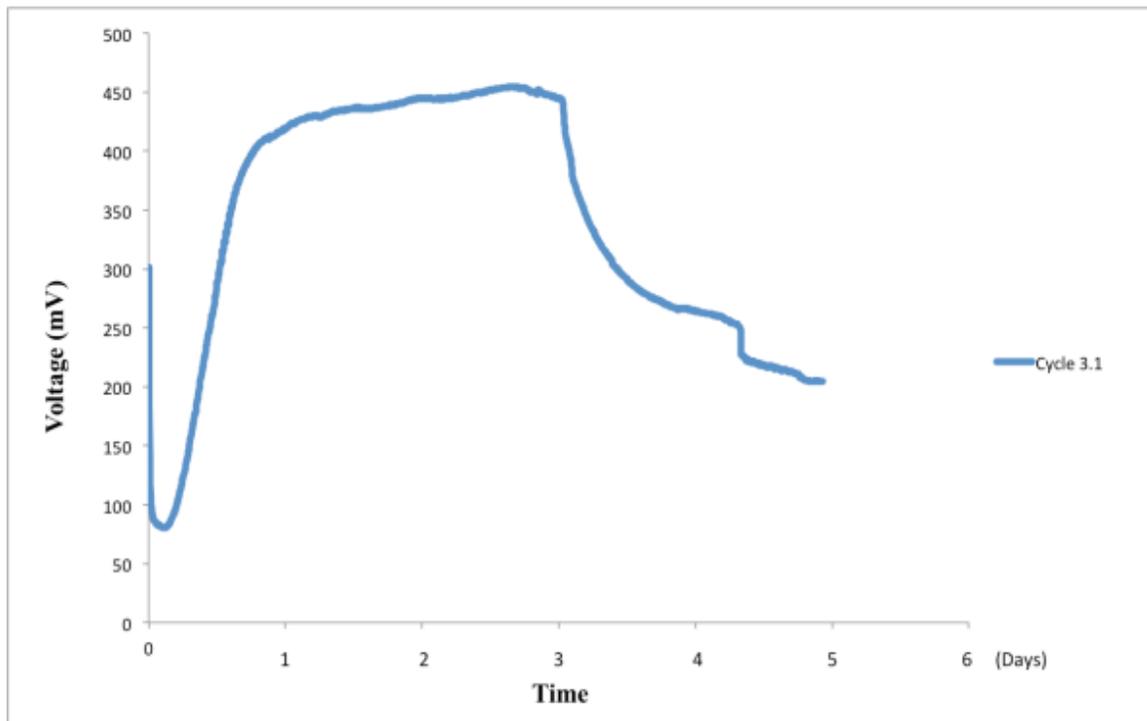
Figure 4.4.1: Experiment 3, cycle 3.1, MFC voltage
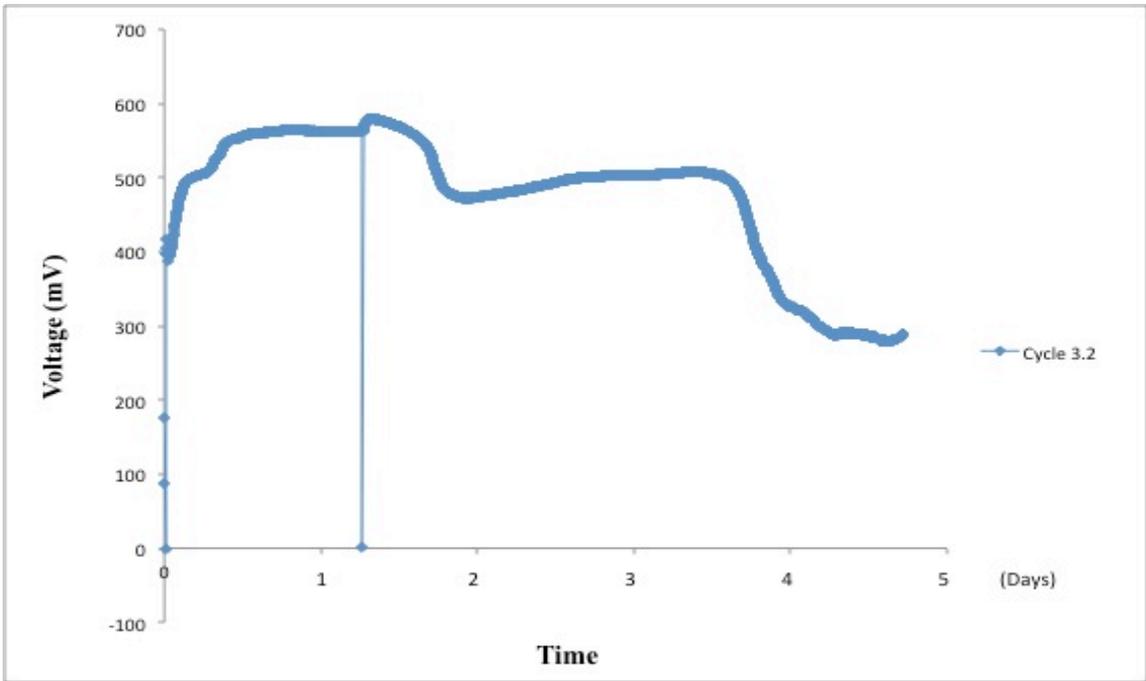
Figure 4.4.2: Experiment 3, cycle 3.2, MFC voltage
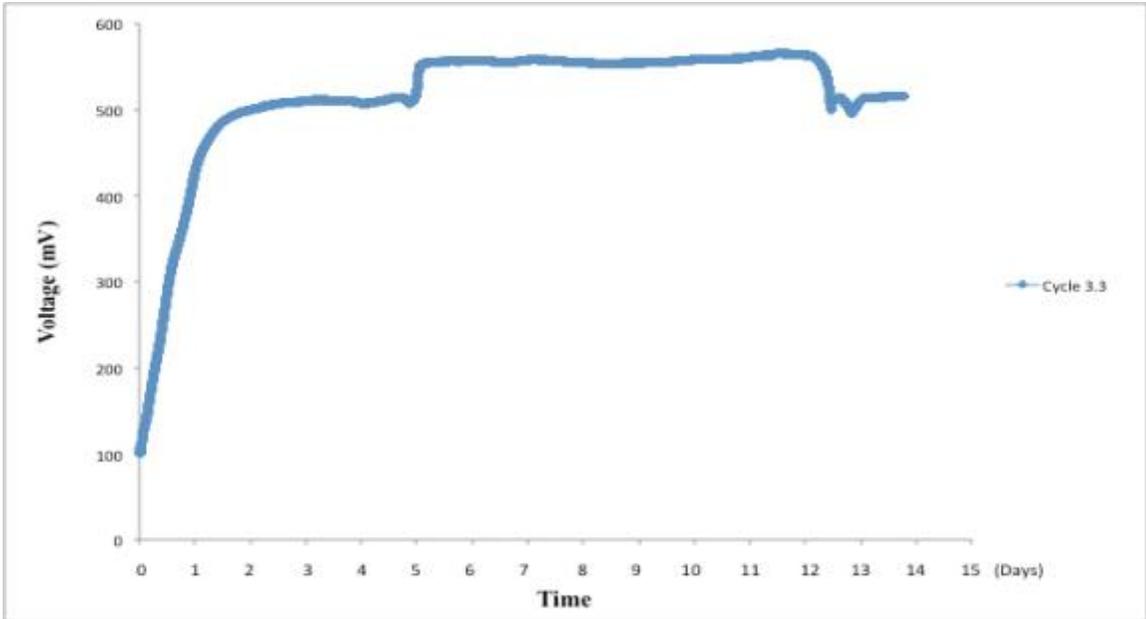
Figure 4.4.3: Experiment 3, cycle 3.3, MFC voltage
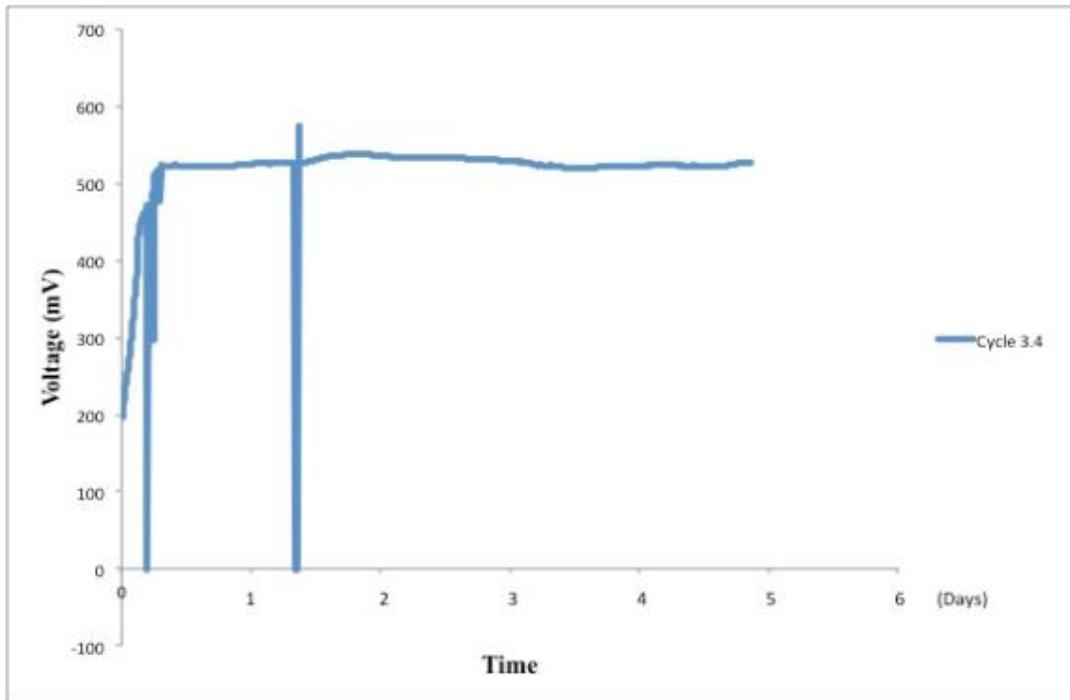
Figure 4.4.4: Experiment 3, cycle 3.4, MFC voltage
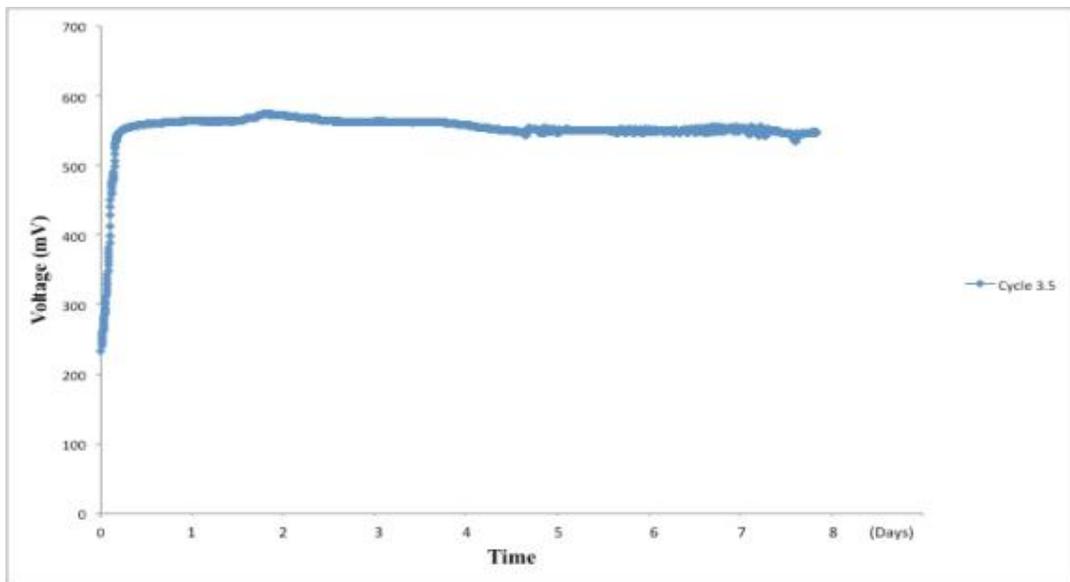
Figure 4.4.5: Experiment 3, cycle 3.5, MFC voltage

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Dissolved oxygen

Influent leachate for all cycles in this experiment had very low dissolved oxygen concentration. As the semi-continuous MFC had an open-air cathode as well, dissolved oxygen concentration could increase in the system during operation. Dissolved oxygen concentration decreased in the effluent of Cycles 3.2 and 3.4. Anaerobic zones within these MFCs would have been more efficient in maintaining the substrate anaerobic, thereby retaining low DO concentration. Effluent from Cycles 3.1 and 3.4 had high DO levels. Figure 4.4.6 presents the average influent and effluent DO concentrations of all cycles from Experiment 3 with one standard deviation error bars.
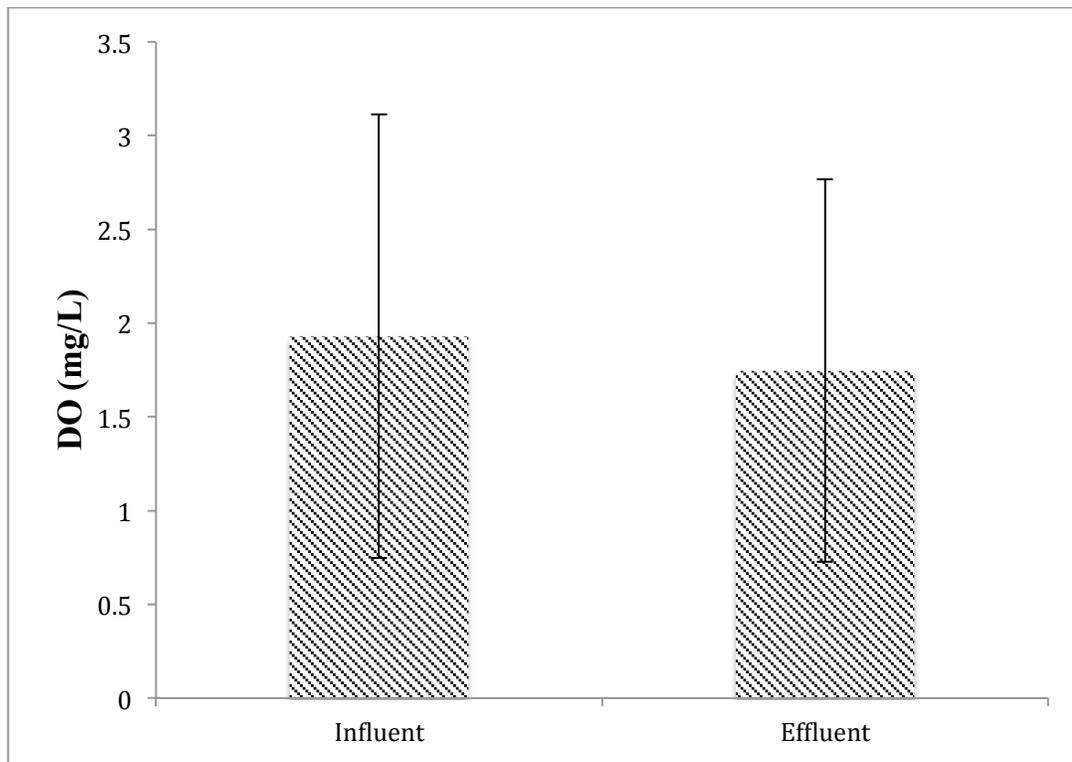
Figure 4.4.6: Average influent and effluent DO values of Experiment 3 with one standard deviation error bars

The error bars overlap each other indicating no significant difference in influent and effluent DO concentrations in all cycles of Experiment 3.

pH:

The pH of the effluent increased in cycles of Experiment 3, except cycle 3.2. An efficient cathode reaction and removal of acid components in the leachate during MFC operation could increase the pH of the system. Figure 4.4.7 presents the average influent and effluent pH values of leachate from all cycles along with one standard deviation error bars.
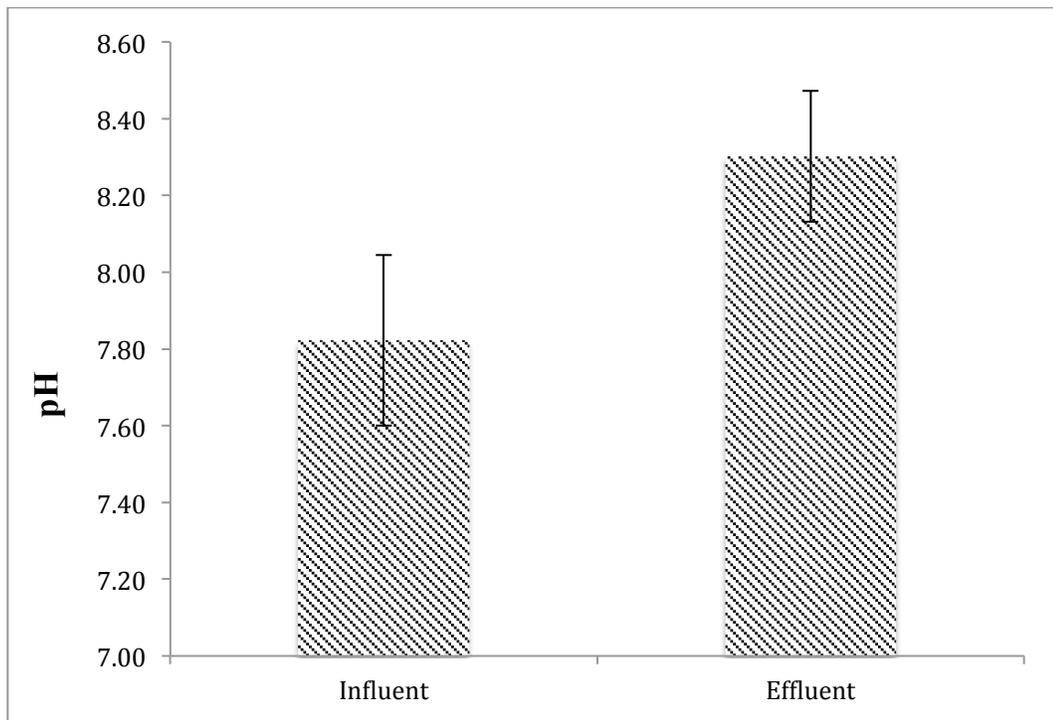
Figure 4.4.7: Average influent and effluent pH values of Experiment 3 with one standard deviation error bars

Influent and effluent leachate from all cycles showed statistically significant differences in pH. Effluent leachate pH was higher than influent pH.

Oxidation reduction potential

ORP of influent leachate used in all cycles had negative ORP values with an exception of the influent used in Cycle 3.1. It had an influent value of 46 mV indicating that the influent leachate was aerobically active. The OPR of the effluent in that cycle was reduced to12 mV. Influent and effluent ORP did not show any statistical difference
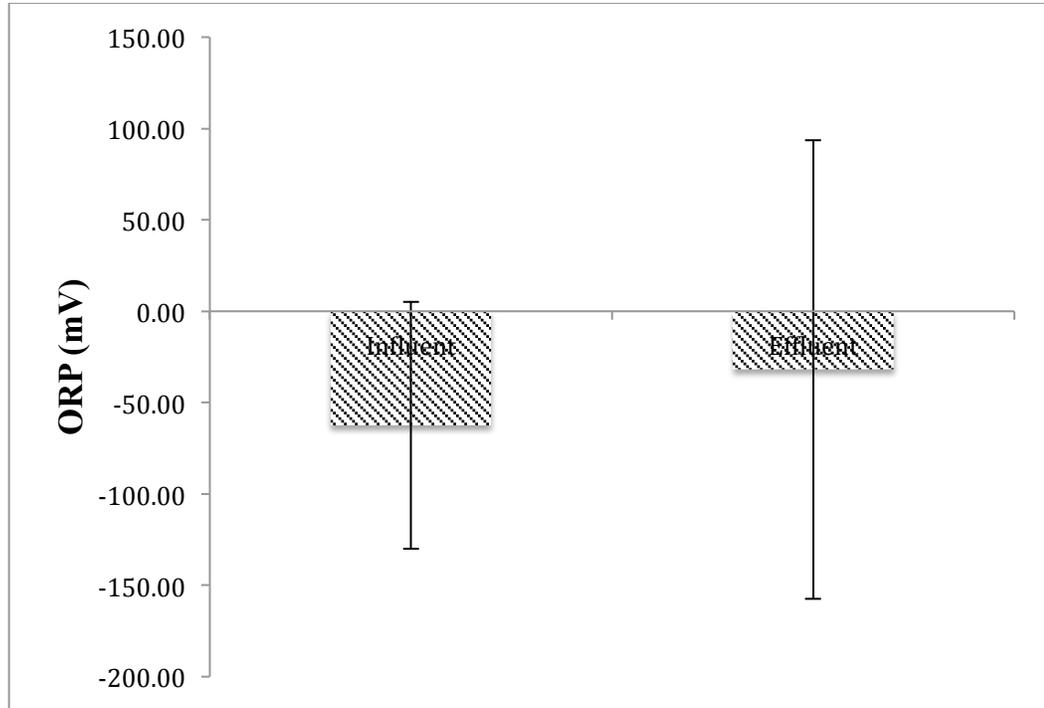
Figure 4.4.8: Average influent and effluent ORP values of Experiment 3 with one standard deviation error bars

Conductivity

The conductivity of the effluent increased in Cycles 3.1, 3.3 and 3.4. The ion concentration in the leachate could have increased during operation causing an increase in conductivity. Figure 4.4.9 shows the average influent and effluent leachate conductivity of all cycles in Experiment 3.
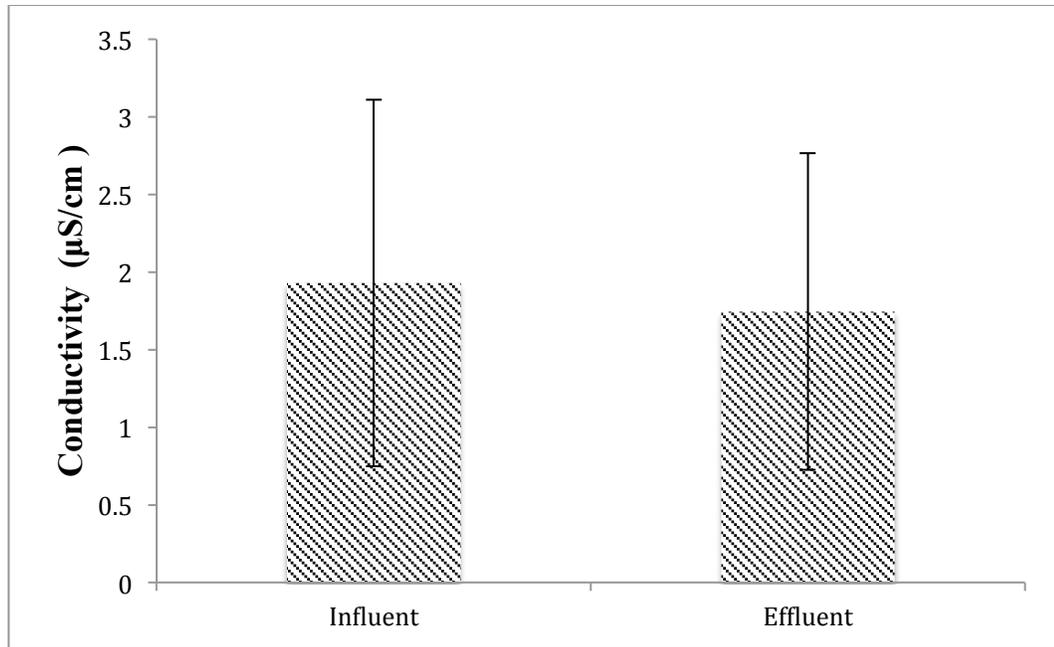
Figure 4.4.9: Average influent and effluent conductivity values of Experiment 3

with one standard deviation error bars

Ammonia, Nitrate and nitrite

High concentrations of ammonia in leachate can cause loading issues at receiving wastewater treatment plants and also can limit leachate recirculation activity at a landfill. Nitrate and nitrite concentrations in leachate are related to ammonia, since the amount of nitrate, nitrite and ammonia can vary depending on the efficiency of processes such as nitrification and denitrification. Nitrate and nitrite concentrations can also vary based upon the types of waste present in the landfill. Ammonia and nitrate concentrations of influent and effluent from all cycles in Experiment 3 are presented in figures 4.4.10, and 4.4.11. Nitrite concentrations were found to be below detection limits (< 0.6 mg/L NO_2 - N) in influent and effluent leachate in all cycles of Experiment 3.
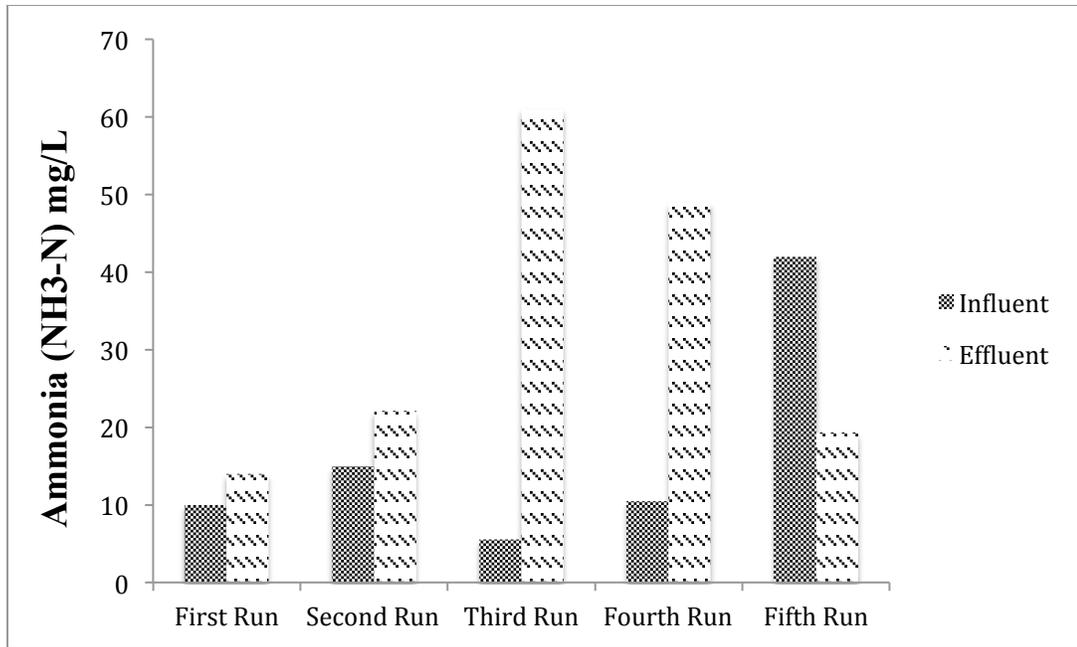
Figure 4.4.10: Influent and effluent concentrations of Ammonia
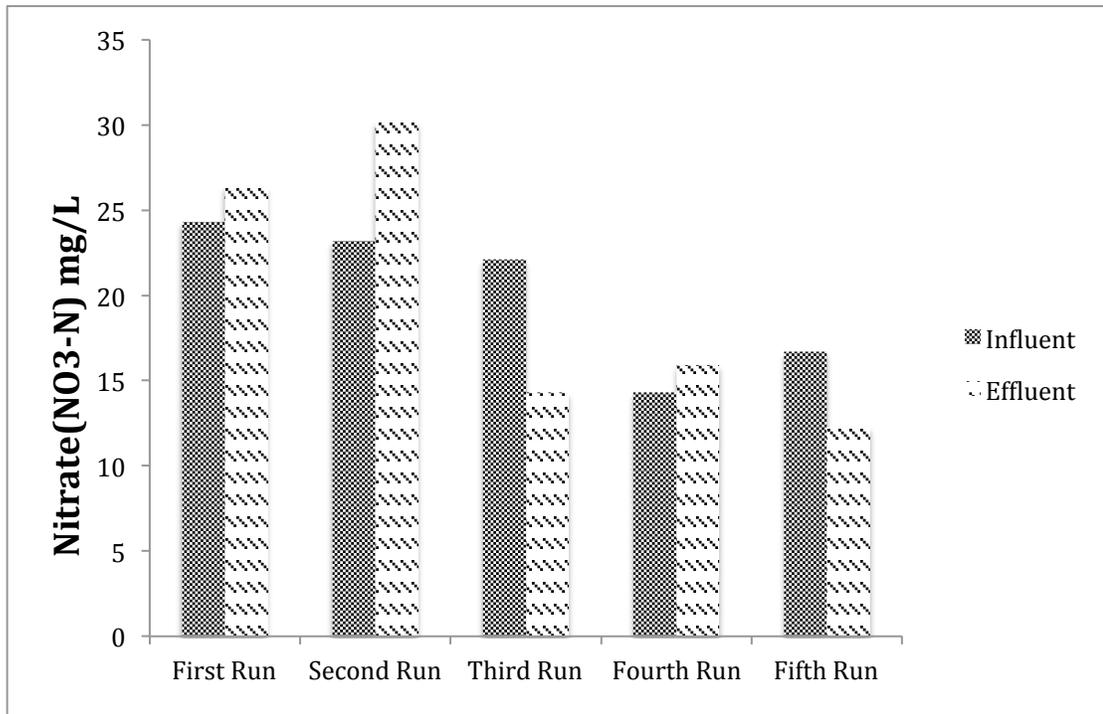
Figure 4.4.11: Influent and effluent concentrations of Nitrate

Concentration of ammonia increased in the effluent at the end of all cycles, except cycle 3.5. Cycle 3.5 showed 54 % reduction in ammonia. The decrease of ammonia in Cycle 3.5 could be due to mixture of certain mechanisms that can cause ammonia removal. Nitrifying bacteria could have oxidized ammonia in the aerobic region near the cathode coupled with denitrification. Ammonia oxidizing bacteria could also be oxidizing ammonia in conjunction with ammonia oxidation and nitrite reduction by anaerobic ammonia oxidation bacteria.

Biodegradation of organic matter produces carbon dioxide water, ammonia and new microbial biomass *(Wiszniowski. J et. al 2005)*. This is a common phenomenon in an activated sludge process. The reaction is shown in equation 1.

$$CHONS + O_2 + Nutrients è CO_2 + NH_3 + C_5H_7NO_2 \qquad \text{Eqn. - 1}$$

(Organic matter)   (New bacterial cells)
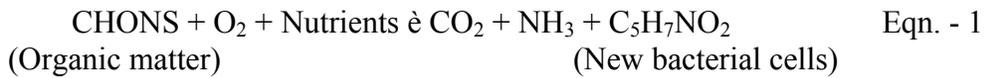
This process could be occurring at the aerobic cathode, and the absence of nitrifying bacteria in the system could be associated with an increase in ammonia concentration.
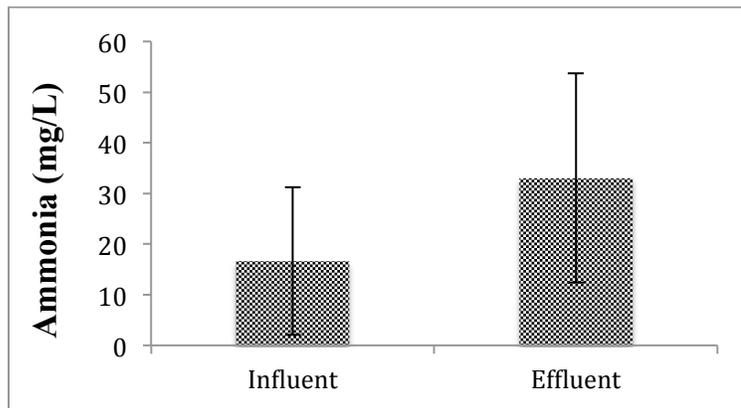
Figure 4.4.12: Average influent and effluent ammonia values of Experiment 3 with one standard deviation error bars

Even though Figure 4.4.12 shows that there is no statistically significant difference in influent and effluent ammonia concentrations, values from individual cycles showed difference in concentrations. Nitrate concentrations in the effluent increased after cycle 3.1, 3.2 and 3.4. Absence of denitrifying bacteria might be the reason for high levels of nitrate in the effluent.

Sulfate

The concentration of sulfate increased at the end of every cycle. Sulfate concentration increase in the effluent was in the range of 5 – 65%. This was most likely due to the sulfur oxidation with the MFC. Figure 4.4.13 shows influent and effluent sulfate concentrations from all cycles on this experiment. The error bars indicate a statistically significant difference in influent and effluent sulfate concentrations.
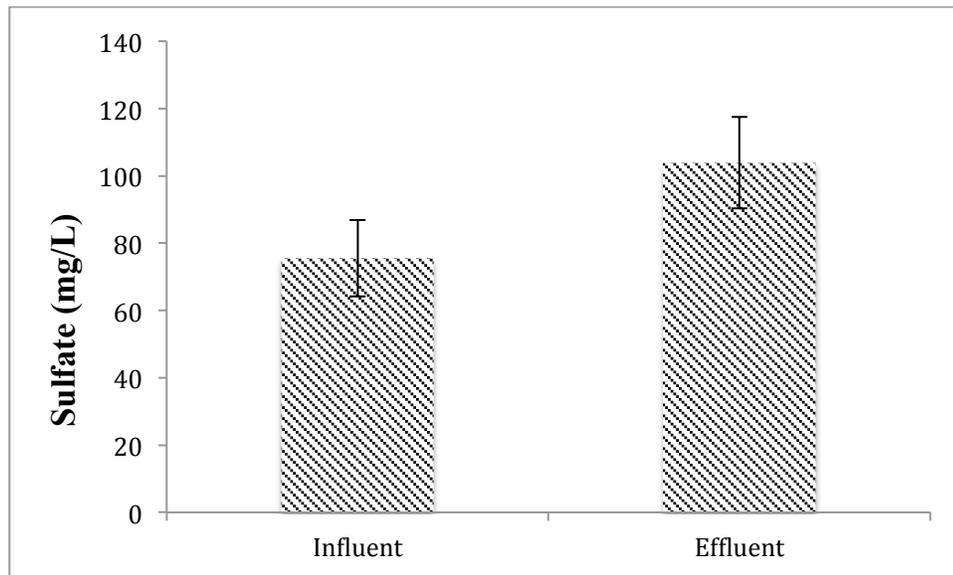
Figure 4.4.13: Average influent and effluent Sulfate concentrations of Experiment 3 with one standard deviation error bars

COD and BOD:

In the semi-continuous MFC, graphite plates and rods as the anode (without activated carbon) better represent COD reduction due to microbial oxidation. Percent reduction of COD ranged from 9% to 37%. COD removal was highest in cycle 3.4 with 37.7 % reduction and lowest in cycle 3.2 with 9.8 % reduction. It should be noted that the influent for cycle 3.2 was made by mixing effluent from cycle 3.1 with stored and unused leachate, because of this, COD removal might not have been as efficient since readily degradable COD was already reduced. Figures 4.4.14 and 4.4.15 represent the influent and effluent COD leachate values and COD percent difference of all cycles of Experiment 3.
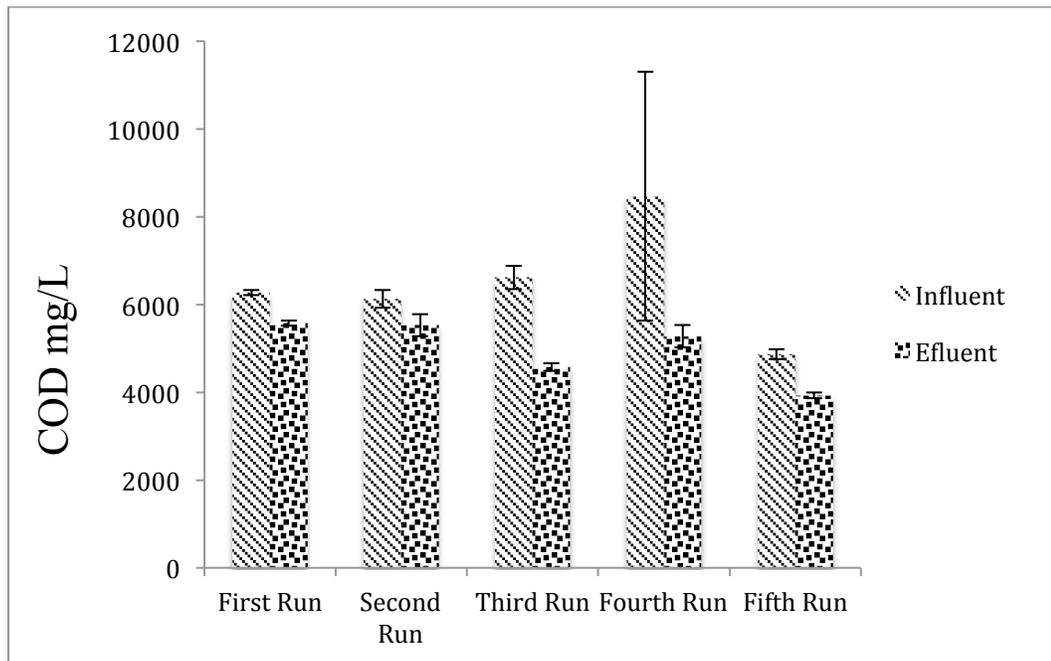
Figure 4.4.14: Influent and Effluent COD values of all 5 cycles of Experiment 3
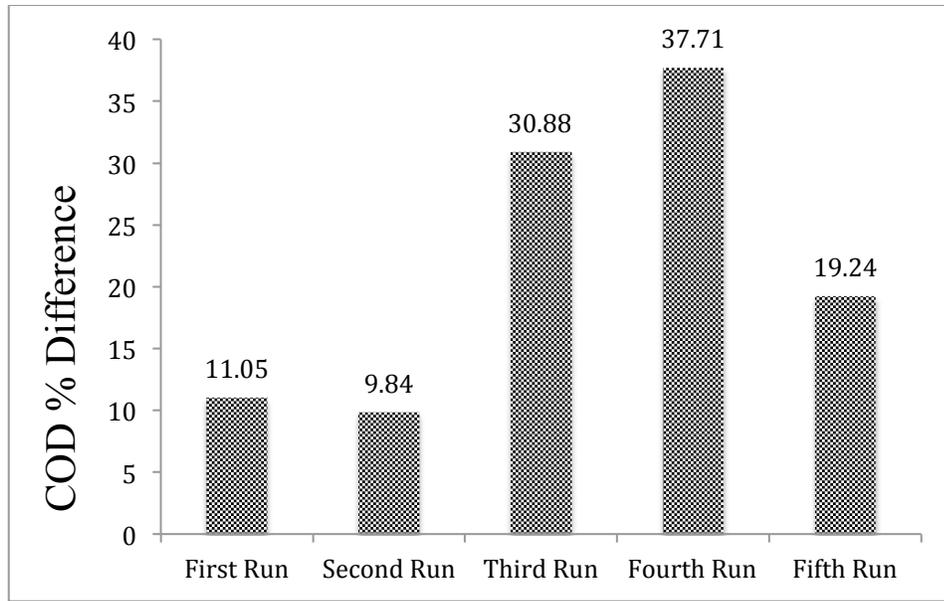
Figure 4.4.15: Influent and effluent COD percent difference of all five cycles

Cycle 3.4 had the highest COD removal percentage and took the least time to reach peak voltage (575 mV), in 20 hours. A very active biofilm could have been formed as this cycle also had the second highest maximum voltage. Reactor E had an average percent COD reduction of 21.74 ±12.2 %.
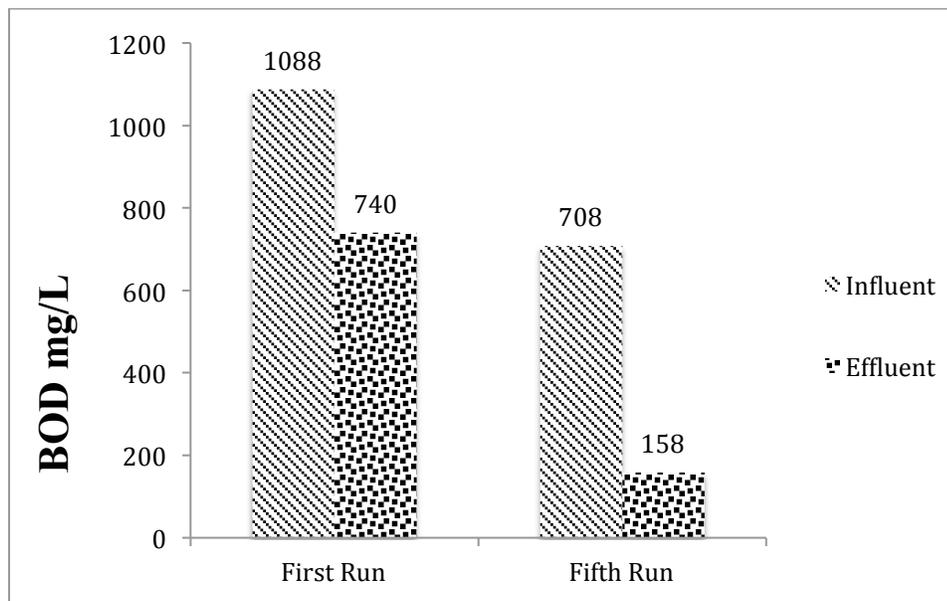
Figure 4.4.16: Influent and effluent BOD values of cycle 3.1 and 3.5

BOD was conducted on the influent and effluent of Cycles 3.1 and 3.5. Both cycles provided a significant decrease in BOD concentrations. Cycle 3.1 decreased BOD by 31.9% and Cycle 3.5 decreased BOD 77%. Figure 4.3.16 shows influent and effluent BOD of Cycles 3.1 and 3.5

Trace metal analysis

An analysis of trace metals of the influent and effluent leachate from cycle 3.1 and 3.5 was completed using ICP, as discussed in chapter 3.

Table 4.4.8 Cation concentration of influent and effluent leachate of cycle 3.5

Metal	Influent	Effluent	Increase/decrease
B (ppm)	31.73	43.72	Increase
Mg (ppm)	36.34	45.45	Increase
P (ppm)	3.47	3.04	Decrease
K (ppm)	255.49	350.69	Increase
Ca (ppm)	111.58	35.49	Decrease
Cr (ppm)	0.05	0.07	Increase
Ni (ppm)	0.10	0.14	Increase
Cu (ppm)	0.01	0.17	Increase
Zn (ppm)	0.02	< 0.0040	Decrease
As (ppm)	0.07	0.10	Increase
Se (ppm)	< 0.0500	< 0.0500	Negligible
Mo (ppm)	0.01	0.01	Increase
Cd (ppm)	< 0.002	0.00	Decrease
Ba (ppm)	0.24	0.07	Decrease
Pb (ppm)	0.00	0.00	0.00

Table 4.4.9: Metal concentration for influent and effluent leachate of cycle 3.5

Metal	Influent	Effluent	Concentration difference
Mg (ppm)	40.1106	43.1685	Increase
P (ppm)	4.2065	3.2861	Decrease
K (ppm)	315.3885	348.9433	Increase
Ca (ppm)	167.5093	64.7870	Decrease
B (ppm)	37.4360	40.9773	Increase
Cr (ppm)	0.0676	0.0624	Decrease
Mn (ppm)	4.4576	0.8277	Decrease
Co (ppm)	0.0387	0.0420	Increase
Ni (ppm)	0.1179	0.1508	Increase
Cu (ppm)	0.0234	0.1604	Increase
Zn (ppm)	0.0631	0.1923	Increase
As (ppm)	0.0707	0.0731	Increase
Se (ppm)	0.0424	0.0431	Increase
Mo (ppm)	0.0063	0.0071	Increase
Cd (ppm)	0.0002	< 0.0006	Increase
Ba (ppm)	0.2969	0.1043	Decrease
Pb (ppm)	0.0031	0.0025	Decrease

There was variation in the influent metal concentration in both the cycles. Certain metals' concentrations were found to be high in the effluent of one cycle but not in the other. Influent and effluent metal concentration from cycle 3.1 and 3.5 are presented in Tables 4.3.8 and 4.3.9 respectively. This analysis was done to determine if any major changes in cation concentration occurred during MFC operation.

4.5 Cyclic voltammetry CV)

CV is a commonly used assay for investigating the electrochemical behavior of a substance or system. In this research CV was used to further compare the oxygen reduction efficiency of the catalysts used in Experiment 1: Pt/C and NiO/C (especially

since the activated carbon, while providing treatment of COD, potentially confounded the difference between the catalysts). Figure 4.5.1 illustrates a "classic" oxygen- reduction curve of a platinum reference electrode. In fuel cells, an efficient cathode catalysts oxygen reduction curve is usually attained at a more positive potential in a CV reflecting on the oxygen reducing ability of the catalyst.
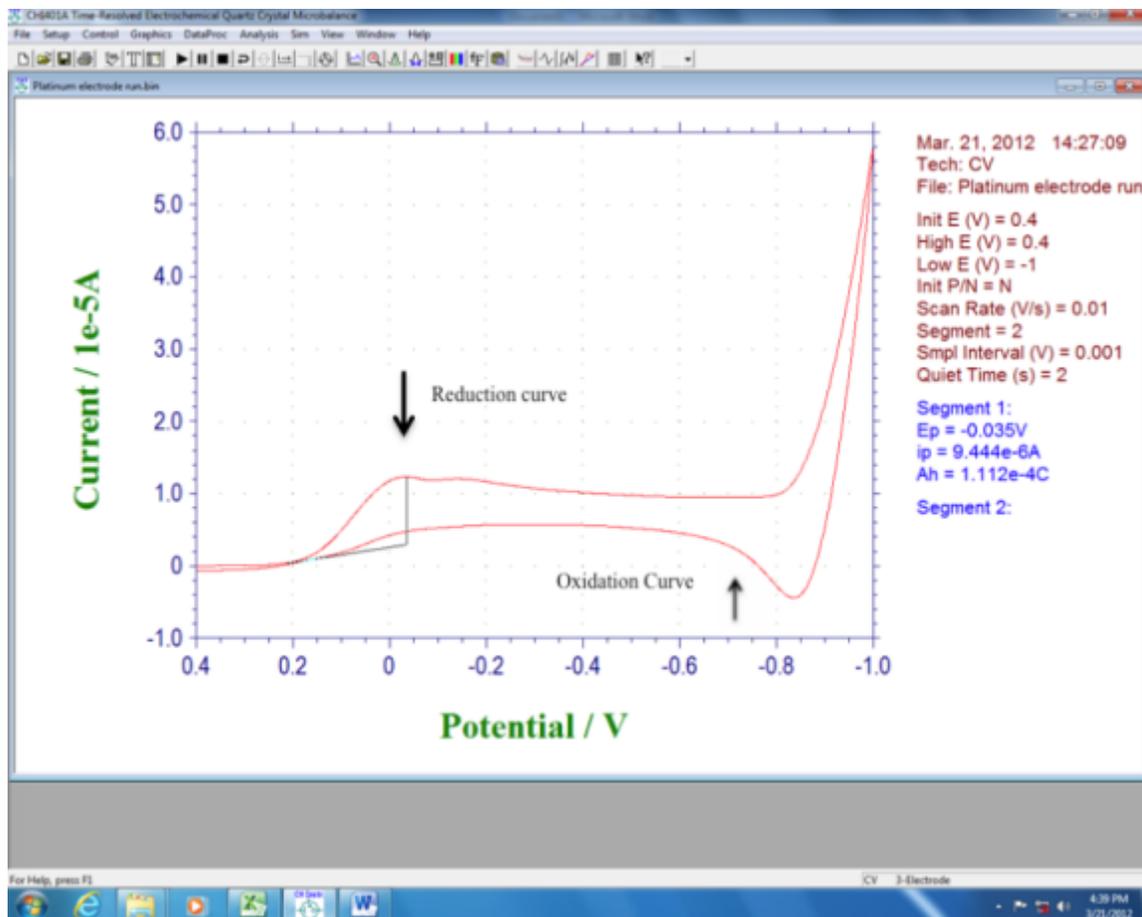
Figure 4.5.1: Cyclic voltammogram of platinum reference electrode

The alternative cathode catalyst was tested with an electrolyte saturated with oxygen and nitrogen in separate experiments to ensure that the observed reduction peak is due to oxygen reduction. From Figure 4.5.2 it can be observed that the nickel on carbon catalyst showed a reduction peak when saturated with oxygen but did not show any peak

when the electrolyte was saturated with nitrogen. NiO/C-oxygen experiments' reduction peak potential was attained around – 0.5 V. When the Pt/C catalyst was subjected to same conditions, the reduction peak was attained close to – 0.7 V in oxygen saturated electrolyte.
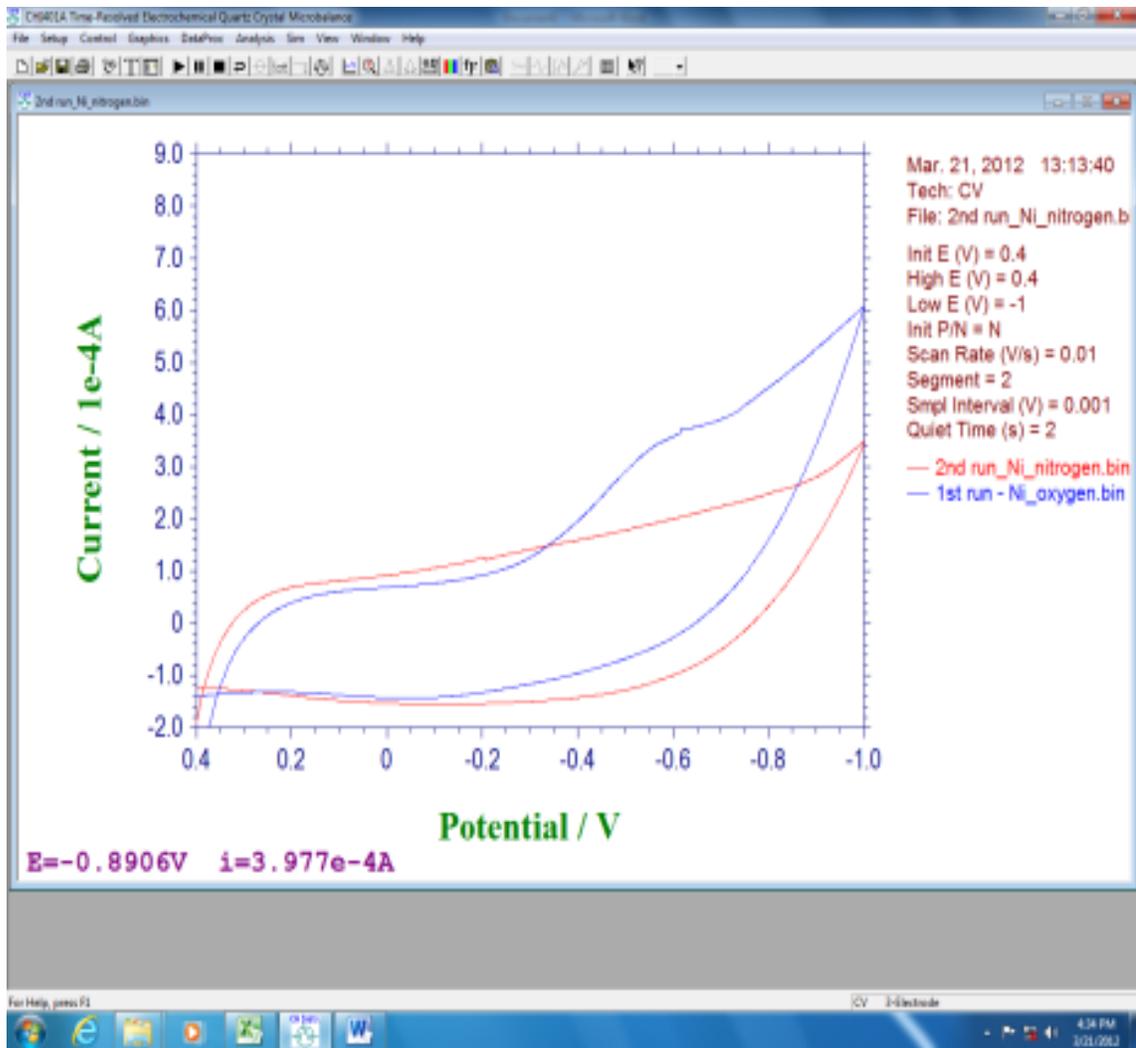
Figure 4.5.2: Cyclic voltammogram overlay of NiO/C-oxygen and NiO/C-nitrogen experiment
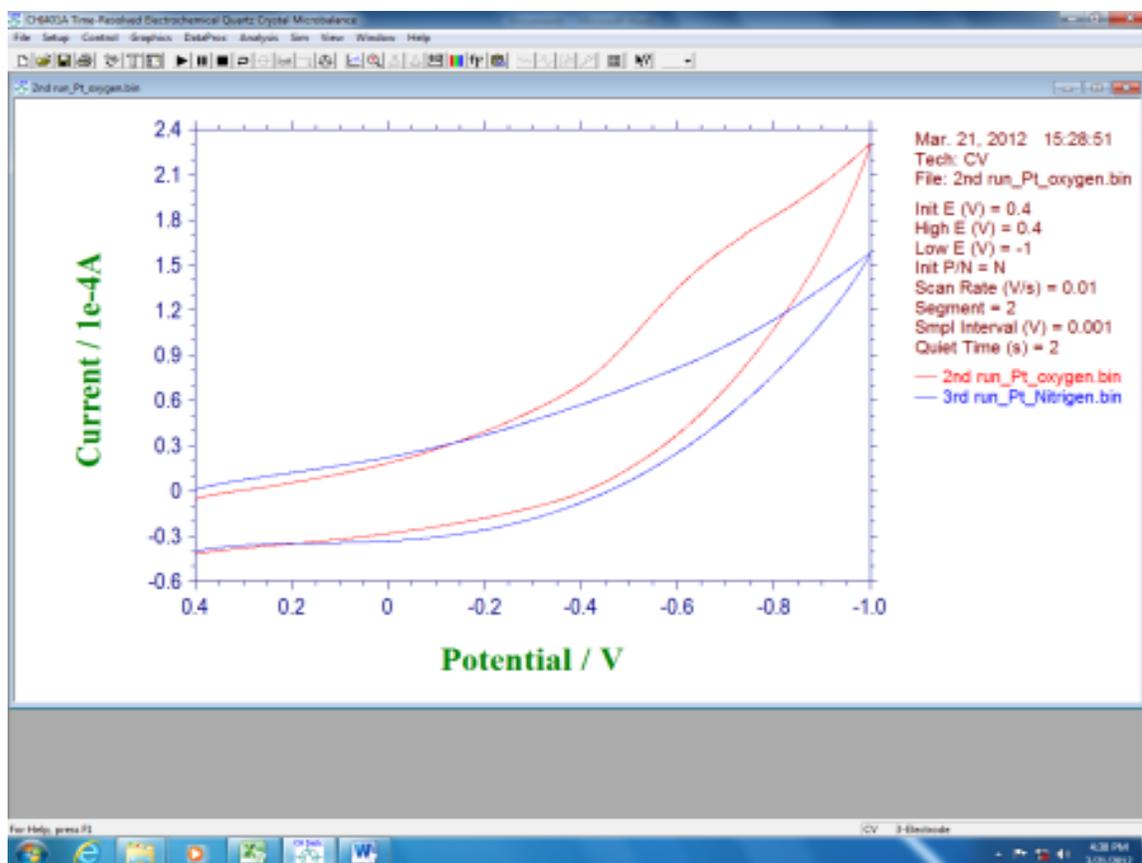
Figure 4.5.3: Cyclic voltammogram overlay of Pt/C-oxygen and Pt/C-nitrogen

When both the results were compared, alternative cathode catalyst (NiO/C) had a higher potential than Pt/C catalyst. Pt/C known for its oxygen reduction ability is expected to have higher potential than NiO/C. To confirm if the peak shown by nickel on carbon catalyst was solely due to reduction capability of nickel catalyst, the carbon granules used to deposit nickel oxide were also tested. An overlay of the alternative cathode catalyst and carbon powder cyclic voltammogram is shown in Figure 4.5.4. The potential exhibited by plain activated carbon powder was higher than that obtained by nickel on carbon catalyst, suggesting that the reduction peak shown by nickel on carbon catalyst was partly due to carbons oxygen reducing power.
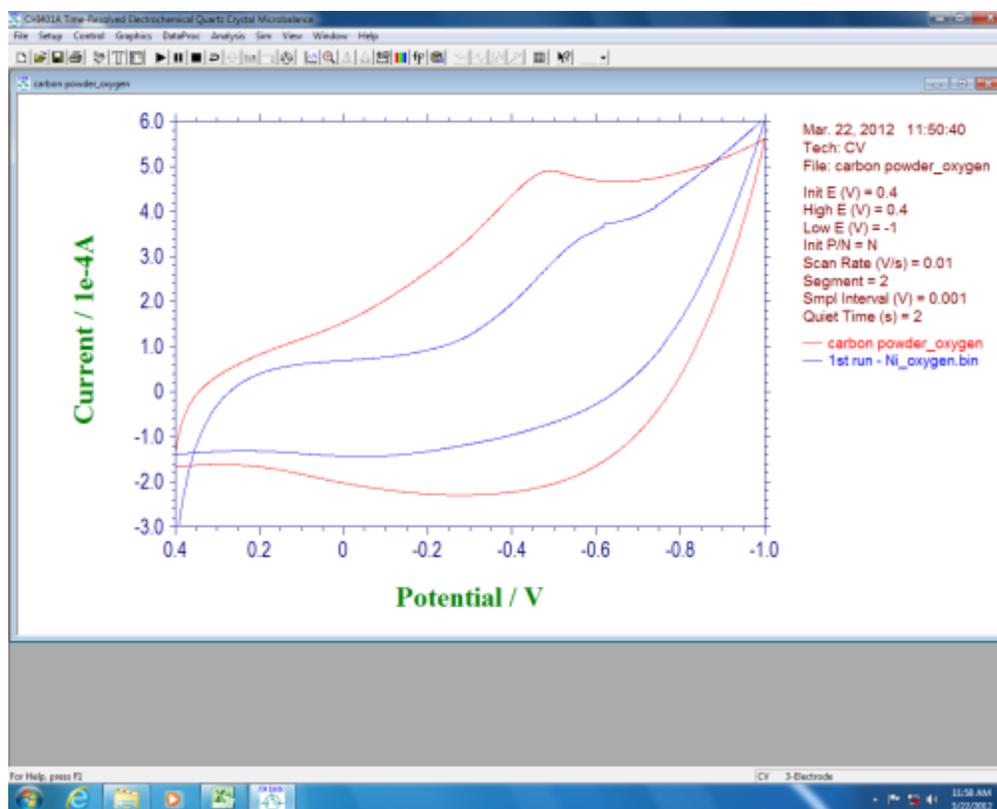
Figure 4.5.4: Overlay of the cyclic voltammogram of carbon powder and NiO/C-oxygen run

X-ray diffraction (XRD) AND Scanning electron microscopy (SEM)

Since CV results demonstrated the ability of plain activated carbon to reduce oxygen, XRD and SEM analysis were done on alternative cathode catalyst to test for the presence of Ni on carbon.

In a SEM, the electrons interact with the atoms that make up the sample. The interaction produces signals that contain information about the sample's surface topography, composition and other properties. SEM images presented in Figure 4.5.5 and 4.5.6 show structural differences in morphology, which might indicate the presence of nickel in the catalyst.
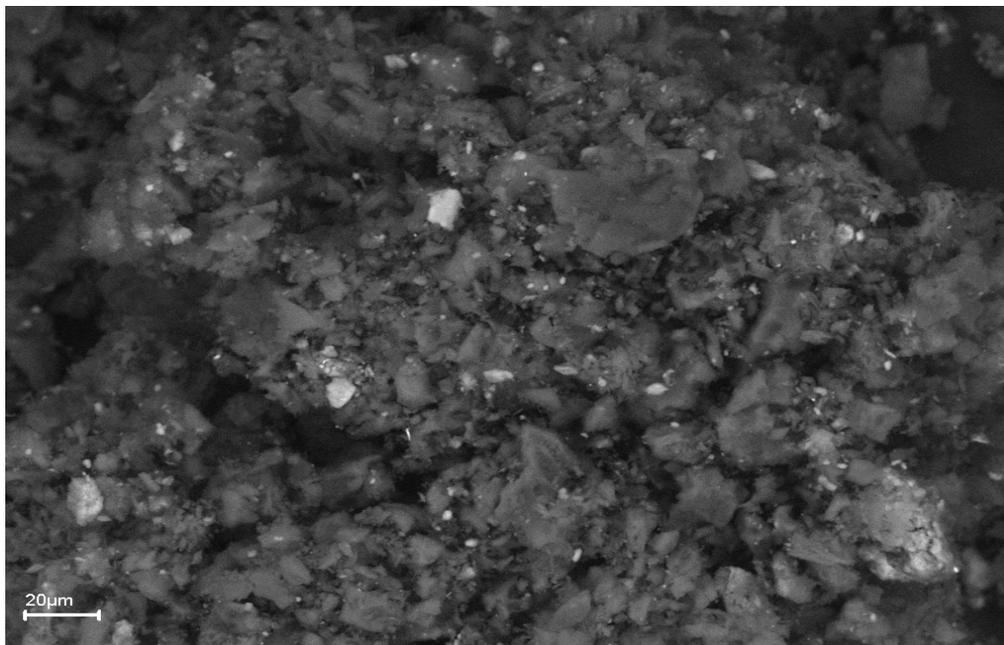
Figure 4.5.5: SEM image of activated carbon
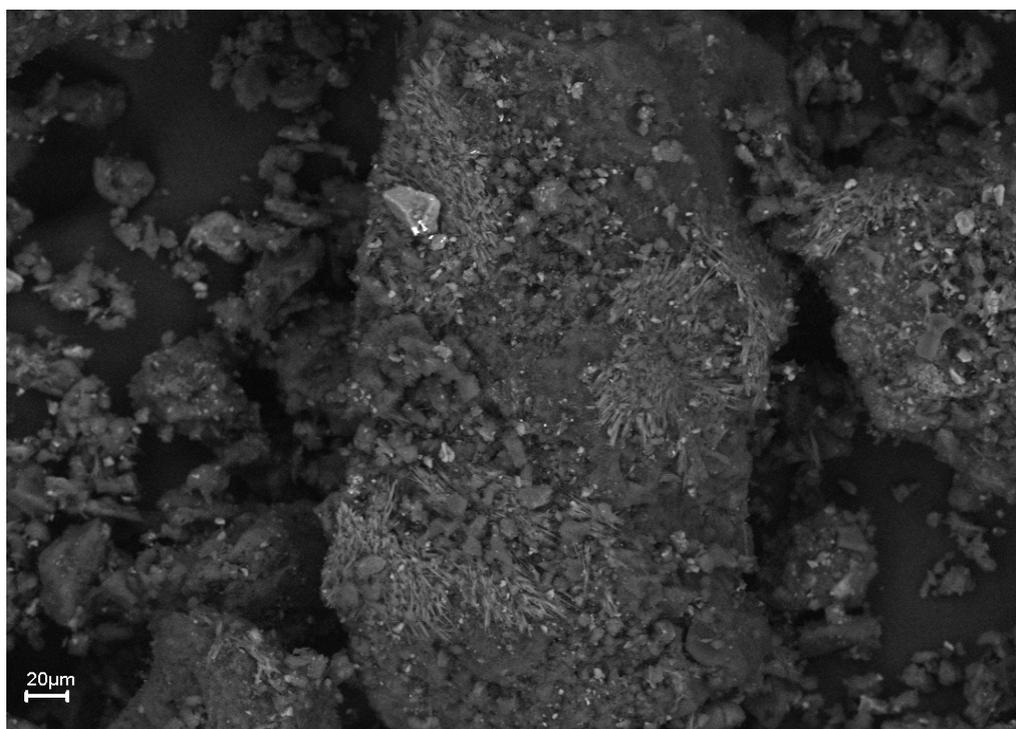
Figure 4.5.6: SEM image of NiO on activated carbon (alternative catalyst)

XRD can provide information about the order, spacing and angles in the structure

of crystals. The atomic planes of a crystal can cause an incident beam of X-rays to

interfere with another as they leave the crystal, causing X-ray diffraction. *Juan et. al (2005)* reported that peaks obtained at 59.14° of 2θ in an XRD (Figure 4.5.7) correspond to 110 diffraction of β − $Ni(OH)_2$. A similar peak can be observed in Figure 4.5.7. Moreover only 0.002 grams of left over catalyst (NiO/C) was used as sample for both these experiments, the sample might no be a true representation of the original catalyst used for the MFCs.

Morphological changes in the physical structure of the alternative cathode catalyst and a peak at 59.14° of 2θ in the XRD manifests the possibility for NiO presence in activated carbon.
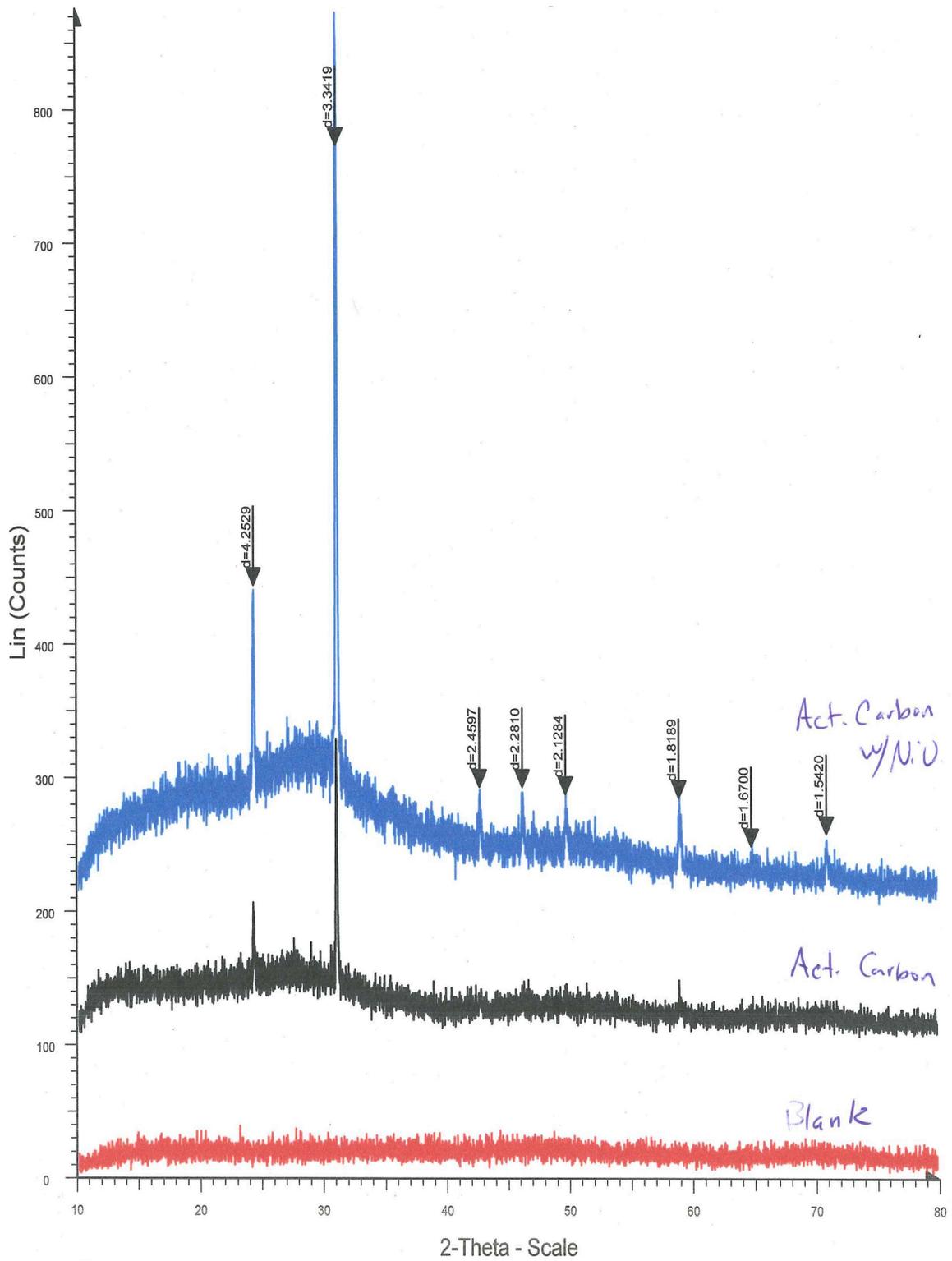
Figure 4.5.7: XRD pattern of NiO/C (in blue), activated carbon (black) and zero

background plate (red)

CHAPTER 5

SUMMARY DISCUSSION AND CONCLUSIONS

Microbial fuel cells are a promising alternative technology that can be used for treating landfill leachate and producing electricity. In this research, two different designs were tested using landfill leachate as a substrate without any external source of inoculation. Batch and semi-continuous designs were operated with a working volume of 520 ml and 5.5 L respectively. Two experiments were performed on the batch MFC to test an alternative cathode catalyst and biochar as the anode. The final experiment was performed on a semi-continuous MFC using graphite plate anode and platinum on carbon cathode catalyst. Cycles with platinum on carbon catalyst (reactor A) showed COD removals in the range of 73 – 81 % while alternative cathode catalyst MFC (reactor B) had COD removals within 62-79 %. Much of the COD reduction in Experiment 2 can be attributed towards the huge internal surface area of activated carbon when compared to biochar. Reactor C showed COD removals in the range of 69-79 % while reactor D removed COD within the range of 22-38%.

The platinum catalyst MFC (reactor A) achieved the highest peak voltage and power density than the alternative cathode catalyst MFC (reactor B). Reactor A in experiment 1 attained a peak voltage of 496 mV with a power density of 0.524 W/m^3. Reactor B reached a maximum voltage of 451 mV with a power density of 0.43 W/m^3. Reactor C in experiment 2 had a higher maximum voltage than reactor D with 444 mV and 437 mV respectively.

Table 5.1: Overview of Experiments 1 and 2

| | Experiment 1 | | | | | | Experiment 2 | | | | | |
	Reactor A			Reactor B			Reactor C			Reactor D		
Cycle	1.1	1.2	1.3	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3
Anode	A.C	A.C	A.C	A.C	A.C	A.C	A.C	A.C	A.C	B.C	B.C	B.C
Catalyst	Pt/C	Pt/C	Pt/C	NiO/C	NiO/C	NiO/C	Pt/C	Pt/C	Pt/C	Pt/C	Pt/C	Pt/C
Mode	Batch	Batch	Batch	Batch	Batch	Batch	Batch	Batch	Batch	Batch	Batch	Batch
Peak Voltage (mV)	395	402	497	312	344	451	444	430	424	322	437	409
Power Density (W/m^3)	0.33	0.344	0.524	0.206	0.251	0.432	0.419	0.394	0.383	0.211	0.407	0.356

Table 5.2: Overview of Experiment 3

	Experiment 3				
Anode	Graphite plate and rod				
Cathode catalyst	Pt/C				
Mode	Semi-continuous				
Cycle	3.1	3.2	3.3	3.4	3.5
Peak Voltage (mV)	454	579	566	575	573
Power density (W/m^3)	0.079	0.129	0.123	0.127	0.127

The semi-continuous MFC had stable power production in all five cycles with a peak voltage of 454 mV, 579 mV, 566 mV, 575 mV and 573 mV. BOD was reduced by 31 % in the first cycle and by 77% in the fifth cycle of the semi-continuous run. Ammonia concentration was increased in the effluent of all cycles except cycle 3.5 in which 52 % ammonia reduction was observed. Nitrite concentration was very negligible in the influent and effluent of all cycles in this research. Nitrate amount was increased in the effluent of Cycles 3.1, 3.2 and 3.4. Throughout all cycles of the semi-continuous MFC, power production was maintained stable for a longer time and a significant reduction in effluent volume was observed. Effluent volume decrease was in the range of 27 – 41% and occurred mainly due to evaporation and leakage in one instance (Cycle 3.5). Such a reduction in leachate volume during MFC operation is an added advantage in using MFCs for leachate treatment. Although power production did not increase linearly with the increase in volume, power production was higher than the batch MFCs and voltage was maintained constant for a longer time.

Table 5.3: Comparison of results with values from other research

Type	Substrate	Volume (ml)	Power density (W/m^3)	COD removal	Source
Single chamber cylinder	Landfill leachate (diluted)	40	6.817	70-98	You et al. 2006
Single chamber column	Landfill leachate	900	0.00138	57-66 (BOD)	Greenman et al. 2009
Single chamber square	Landfill leachate	995	0.004	43	Damiano 2009
Single chamber circular	Landfill leachate	934	0.031	48	Damiano 2009
Two-chamber MFC with CEM	Landfill leachate	850	4.2	78	Yan et al. 2010
Single chamber column	Landfill leachate (diluted)	167	0.0061	32	Puig et al. 2011
Single chamber (Pt/C & A.C)	Landfill leachate	520	0.523	73-81%	This research
Single chamber (Pt/C & Biochar)	Landfill leachate	520	0.407	22-38%	This research
Single chamber (NiO/C & A.C)	Landfill leachate	520	0.432	62-79%	This research
Semi-continuous	Landfill leachate	5500	0.129	9-37%	This research

Cyclic voltammetry experiments on the catalysts indicated that considerable amount of oxygen reduction by the alternative cathode catalyst was due to the carbon

powder. Although these results cannot conclude that oxygen reduction wasn't due to nickel in the catalyst, SEM and XRD results didn't show the presence of nickel in the catalyst. These results can still not comprehensively explain the absence of nickel in the catalyst, as the sample used for SEM and XRD experiments might not be representing the true catalyst mixture used in the MFCs. Only 0.002 grams of catalyst was left after using as catalyst in the MFC, such a small amount cannot be a real representation of the true catalyst mixture. Further research needs to be conducted on the proper deposition of nickel on activated carbon to increase the efficiency of the catalyst. It would be interesting to analyze the performance of an MFC using activated carbon as the cathode catalyst.

MFCs used in this study removed organic substrates efficiently demonstrating its capability in being a viable option for leachate treatment. The larger scale semi-continuous MFC showed consistent power production and significant BOD reduction. Even though the presence of nickel in the NiO/C catalyst can be debated, its performance in terms of COD reduction and voltage production was on par with Pt/C catalyst. Future studies should be directed towards the use of efficient and less expensive cathode catalysts, as they have been a huge obstruction in the real world application of microbial fuel cells. To achieve practical implementation, MFCs still need to be scaled-up by several orders of magnitude from the laboratory scale (10^{-6} to 10^{-3} m^3) to a scale suitable for wastewater treatment (1 to 10^3 m^3). Several complexities like microbiological, technological and economical aspects have hindered the use of MFC for wastewater treatment. One of the most important challenges in deploying MFCs for real-world application is to find a balance between treatment efficiency, electricity production and

capital costs. It is also important to compare the costs incurred by current wastewater treatment facilities and other energy generating treatments along with MFCs to compare capital costs, revenue and profits. *Rozendal et. al (2008)* compared the capital costs and product revenues of microbial fuel cells, microbial electrolysis cells (MEC), activated sludge treatment and anaerobic digestion. In this study the costs (for MFC & MEC) were developed based on the currently used laboratory materials as well as on predicted future capital costs based on less expensive substitute materials. Table 5.4 presents a comparison of these technologies along with estimated capitals costs and product revenues.

Table 5.4: Comparison of estimated capital costs and product revenues of different wastewater treatment systems

System	Product	Capital costs (€/kg COD)	Product revenue (€/kg COD)	Offset (product revenue minus capital costs) (€/kg COD)
Activated sludge	N/A	0.1	-0.3	-0.4
Anaerobic digestion	Methane	0.01	0.1	0.1
Microbial fuel cell	Electricity	$8^1/0.4^2$	$0.2^{3,4}$	-0.2
Microbial electrolysis cell	Hydrogen	$8^1/0.4^2$	$0.6^{3,5}$	0.2

[1]Capital cost based on materials currently used in laboratory experiments
[2]Predicted future capital costs based on less expensive substitute materials
[3]Assuming an electricity price of 0.1 €/kWh
[4]Assuming an MFC voltage of 0.5 V
[5]Assuming an electricity requirement of 1lWh/m^3 H$_2$ and a hydrogen price of 0.5 €/Nm^3 H$_2$

Via improved designs and innovative materials, these capital costs can be reduced significantly in the future, but because of their inherent complexity in design, the capital costs of MFCs are expected to be higher than conventional wastewater treatment systems.

MFCs have the potential to be cost effective and energy-efficient alternative to activated sludge system in the future (Table 5.4). They can also be advantageous over anaerobic digesters as they MFCs can operate at low COD concentrations with integrated nitrogen removal.

Effluent from MFCs are still high in organics and other constituents, they still require further treatment before discharge. While research on improving overall treatment efficiency using less expensive materials is required, this technology can still be used as a pre-treatment for recirculation or to reduce energy use for further treatment. It also has the potential to be used as a standalone treatment process. Different leachate from various landfills could be utilized as substrate and treatment efficiencies should be evaluated to find methods to overcome problems due to leachate variation. Advances should also be made in using more efficient anodes and less expensive cathode catalysts. Finally, a more in-depth microbial community analysis should also be completed to examine characteristics of the community within the MFC in detail. A better understanding on the specific bacterial species providing exocellular electron transfer along with developing efficient inexpensive cathode catalysts can accelerate the real-world use of microbial fuel cells for leachate and other waste water treatment.

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APPENDICES

Appendix A

Influent and effluent leachate values of various parameters of all MFC cycles operated in this study.

Experiment 1:

Leachate characterizations, Nickel MFC, Cycle 1.1

Parameter	Influent	Effluent	% Difference
DO (% L)	33	21.6	34.5
DO (mg/L)	2.45	1.8	26.5
SPC (μS/cm)	20613	18475	10.3
C (μS/cm)	22064	16949	23.1
Resistance(Ω – cm)	45.15	58.99	-30.6
pH	7.84	7.7	1.7
ORP (mV)	-45.3	-84.6	-86.7
COD (mg/L)	12930	2610	79.8

Leachate characterization, Platinum MFC, Cycle 1.1

Parameter	Influent	Effluent	% Difference
DO (% L)	33	28.3	14.2
DO (mg/L)	2.45	2.75	-12.2
SPC (µS/cm)	20613	19173	6.9
C (µS/cm)	22064	17746	19.5
Resistance(Ω – cm)	45.15	56.36	-24.8
pH	7.84	7.78	0.7
ORP (mV)	-45.3	85.5	288.7
COD (mg/L)	12930	2620	79.7

Leachate characterization, Nickel MFC, Cycle 1.2

Parameter	Influent	Effluent	% Difference
DO (% L)	21.7	27.8	-28.1
DO (mg/L)	1.88	2.26	-20.2
SPC (µS/cm)	21448	19262	10.1
C (µS/cm)	18162	17876	1.5
Resistance(Ω – cm)	55.04	55.91	-1.5
pH	7.96	7.7	3.2
ORP (mV)	-96.7	-70	27.6
COD (mg/L)	12540	4690	62.2

Leachate characterization, Platinum MFC, Cycle 1.2

Parameter	Influent	Effluent	% Difference
DO (% L)	21.7	25.5	-17.5
DO (mg/L)	1.88	2.13	-13.3
SPC (μS/cm)	21448	19657	8.3
C (μS/cm)	18162	18248	-0.4
Resistance(Ω – cm)	55.04	54.78	0.4
pH	7.96	7.73	2.8
ORP (mV)	-96.7	-84.5	12.6
COD (mg/L)	12540	2380	81.0

Leachate characterization, Nickel MFC, Cycle 1.3

Parameter	Influent	Effluent	% Difference
DO (% L)	41	34.1	16.8
DO (mg/L)	3.5	2.87	18.0
SPC (μS/cm)	21290	17844	16.1
C (μS/cm)	18293	16433	10.1
Resistance(Ω – cm)	54.6	60.43	-10.6
pH	8.09	7.66	5.3
ORP (mV)	-106	-12.6	88.1
COD (mg/L)	13270	3230	75.6

Leachate characterization, Platinum MFC, Cycle 1.3

Parameter	Influent	Effluent	% Difference
DO (% L)	41	34.3	16.3
DO (mg/L)	3.5	2.88	17.7
SPC (μS/cm)	21290	17950	15.6
C (μS/cm)	18293	16533	9.6
Resistance(Ω – cm)	54.6	60.37	-10.5
pH	8.09	7.71	4.7
ORP (mV)	-106	-27.8	73.7
COD (mg/L)	13270	3480	73.7

Experiment 2

Leachate characterization, Activated Carbon MFC, Cycle 2.1

Parameter	Influent	Effluent	% Difference
DO (% L)	29.87	28.3	5.2
DO (mg/L)	2.59	2.42	6.5
SPC (μS/cm)	20779	16463	20.7
C (μS/cm)	22568	14972	33.6
Resistance(Ω – cm)	44.13	66.69	-51.1
pH	7.73	7.82	-1.1
ORP (mV)	-36.2	-21	41.9
COD (mg/L)	16510	3320	79.8

Leachate characterization, Biochar MFC, Cycle 2.1

Parameter	Influent	Effluent	% Difference
DO (% L)	29.87	32.6	-9.1
DO (mg/L)	2.59	2.8	-8.1
SPC (μS/cm)	20779	17271	16.8
C (μS/cm)	22568	15743	30.2
Resistance(Ω – cm)	44.13	63.8	-44.5
pH	7.73	8.19	-5.9
ORP (mV)	-36.2	-34.5	4.7
COD (mg/L)	16510	10145	38.5

Leachate characterization, Activated Carbon MFC, Cycle 2.2

Parameter	Influent	Effluent	% Difference
DO (% L)	28.45	35.7	-25.4
DO (mg/L)	2.35	3.03	-28.9
SPC (μS/cm)	16784	17454	-3.9
C (μS/cm)	15264	16120	-5.6
Resistance(Ω – cm)	60.2	61.84	-2.7
pH	7.79	7.65	1.8
ORP (mV)	-25.4	-98.3	-297.0
COD (mg/L)	15400	3830	75.1

Leachate characterization, Biochar MFC, Cycle 2.2

Parameter	Influent	Effluent	% Difference
DO (% L)	28.45	32	-12.4
DO (mg/L)	2.35	2.74	-16.6
SPC (µS/cm)	16784	17755	-5.7
C (µS/cm)	15264	16439	-7.7
Resistance(Ω – cm)	60.2	60.7	-0.8
pH	7.79	8.1	-3.9
ORP (mV)	-25.4	-50.8	-100
COD (mg/L)	15400	11550	25

Leachate characterization, Activated Carbon MFC, Cycle 2.3

Parameter	Influent	Effluent	% Difference
DO (% L)	39.5	31.7	19,75
DO (mg/L)	3.04	2.82	7.24
SPC (µS/cm)	10648	11221	-5.38
C (µS/cm)	9846	10077	-2.35
Resistance(Ω – cm)	101.5	99.32	2.15
pH	7.92	8.02	-1.26
ORP (mV)	-140.4	-133.5	4.91
COD (mg/L)	15450	4790	69.00

Leachate characterization, Biochar MFC, Cycle 2.3

Parameter	Influent	Effluent	% Difference
DO (% L)	39.5	33.4	15.4
DO (mg/L)	3.04	2.99	1.6
SPC (μS/cm)	10648	10151	4.6
C (μS/cm)	9846	9111	7.4
Resistance(Ω – cm)	101.5	109.74	-8.1
pH	7.92	8.3	-4.8
ORP (mV)	-140.4	-63.3	54.9
COD (mg/L)	15450	12035	22.1

Experiment 3:

Leachate characteristics of Cycle 3.1

Parameters	Influent	Effluent	% Difference
DO (% L)	30.4	23.9	21.3
DO (mg/L)	3.44	2.18	36.6
SPC (μS/cm)	10984	9937	9.5
C (μS/cm)	7456	8724	-17.0
Resistance (Ω - cm)	143.22	114.49	20.0
pH	7.89	8.28	-4.9
ORP (mV)	46	12	73.9
COD (mg/L)	6273	5580	11.0
Ammonia (NH3 - N)	9.98	14	-40.2
Nitrate (NO3 - N)	24.3	26.4	-8.6
Nitrite (NO2 - N)	0.208	0.482	-131.7
Sulfate (SO4)	67	111	-65.6
Leachate Volume (L)	8.8	5.15	41.4
BOD mg/L	1088	740	31.9

Leachate characteristics of Cycle 3.2

Parameters	Influent	Effluent	% Difference
DO (% L)	25.3	34.4	-35.9
DO (mg/L)	2.4	3.08	-28.3
SPC (μS/cm)	5334	5213	2.2
C (μS/cm)	4741	4572	3.5
Resistance (Ω - cm)	210.77	218.27	-3.5
pH	8.16	8.04	1.4
ORP (mV)	-95	102.5	207.8
COD (mg/L)	6133	5530	9.8
Ammonia (NH3 - N)	15	22.1	-47.3
Nitrate (NO3 - N)	23.2	30.2	-30.1
Nitrite (NO2 - N)	0.386	0.362	6.2
Sulfate (SO4)	90.1	95.5	-5.9
Leachate Volume (L)	8.3	5.25	36.7

Leachate characteristics of cycle 3.3

Parameters	Influent	Effluent	% Difference
DO (% L)	8.4	13.2	-57.1
DO (mg/L)	0.76	1.6	-110.5
SPC (μS/cm)	7619	9625	-26.3
C (μS/cm)	6812	8654	-27.0
Resistance (Ω - cm)	146.93	115.5	21.3
pH	7.56	8.51	-12.5
ORP (mV)	-132.3	-20.2	84.7
COD (mg/L)	6616	4573	30.8
Ammonia (NH3 - N)	5.55	61	-999.1
Nitrate (NO3 - N)	22.1	14.3	35.2
Nitrite (NO2 - N)	0.207	0.296	-43.0
Sulfate (SO4)	61.4	87	-41.6
Leachate Volume (L)	8.4	5.11	39.1

Leachate characteristics of cycle 3.4

Parameters	Influent	Effluent	% Difference
DO (% L)	23.9	3	87.4
DO (mg/L)	2.36	0.28	88.1
SPC (μS/cm)	10210	9947	2.5
C (μS/cm)	7893	8862	-12.2
Resistance (Ω - cm)	126.66	112.87	10.8
pH	7.75	8.32	-7.3
ORP (mV)	-49.3	-238.2	-383.1
COD (mg/L)	8470	5276	37.7
Ammonia (NH3 - N)	10.5	48.9	-365.7
Nitrate (NO3 - N)	14.3	15.9	-11.1
Nitrite (NO2 - N)	0.337	0.268	20.4
Sulfate (SO4)	79.4	122	-53.6
Leachate Volume (L)	7.25	5.24	27.7

Leachate characterization of Cycle 3.5

Parameters	Influent	Effluent	% Difference
DO (% L)	8.5	17.8	-109.4
DO (mg/L)	0.69	1.59	-130.4
SPC (μS/cm)	10174	9649	5.16
C (μS/cm)	9494	8610	9.3
Resistance (Ω - cm)	105.32	116.15	-10.2
pH	7.75	8.36	-7.8
ORP (mV)	-81.7	-15.8	80.6
COD (mg/L)	4866	3930	19.2
Ammonia (NH3 - N)	42	19.3	54.0
Nitrate (NO3 - N)	16.7	12.3	26.3
Nitrite (NO2 - N)	0.491	0.415	15.4
Sulfate (SO4)	79.8	104	-30.3