SIMULTANEOUS WASTEWATER TREATMENT AND ENERGY HARVESTING IN MICROBIAL FUEL CELLS

PRESENTED BY

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CERTIFICATION

This project work on **Simultaneous Wastewater Treatment And Energy Harvesting In Microbial Fuel Cells** carried out by **Ihenacho, Chizobam Chikeziri** under the strict supervision of PROF. C.O.AKUJOBI and DR. K.O.MEJEHA has been read and certified as having met the necessary conditions for the award of Master of Science (M.Sc) degree in Environmental Microbiology, Federal University of Technology Owerri.

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DEDICATION

This thesis is dedicated to the Most high God for his grace, wisdom, understanding, providence and the enablement to complete this work.

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ABSTRACT

The prohibitively high cost of treating certain wastewaters has often resulted in their indiscriminate disposal without treatment, especially in most developing countries. This has contributed to the presently heightened environmental problems. Microbial fuel cells (MFCs) have increasingly attracted attention as a viable tool to address these challenges. The dual chambers MFCs were used to treat piggery wastewater samples for 25days, and selected physicochemical parameters were monitored. The effects of surface area of anode ($0.005m^2$ to $0.015m^2$), surface area of cathodes ($0.005m^2$ to $0.015m^2$) and volume of anode (750ml to 1125ml) on voltage generation, were optimized. Optimization was designed with Box Behnken Design, which gave 30 runs of dual chambers MFCs. All components of MFCs were set up according to the design. Following 25 days running of the MFCs and daily recording of voltage production (morning and evening), the average voltages, taken across 10,000Ω resistance, as produced by the 30 MFCs were optimized with Minitab® 17. Results showed that $0.011m^2$, $0.015m^2$ and 1500ml were the optimum surface area of anode, cathode and volume of anode respectively, with estimated highest average voltage production of 41.83mV. When these optimums were used to set MFCs, the highest average voltage obtained 52.5mV, which is 25% higher than estimated highest average voltage, while the lowest was 20.13mV. These were higher than highest average voltage of 34.32mV and lowest of 7.76mV obtained without optimization. The BOD, COD of the wastewaters reduced from 1705.33mg/l and 5311.67mg/l in original wastewater to 1383.33mg/l and 3643.33mg/l respectively, after treatment with MFC. These represent 18.89% and 31.41% reduction in BOD and COD respectively. In the control (untreated) sample, they only reduced to 1583.33mg/l and 4699.67mg/l respectively. Similarly, concentrations of NO_3^+, PO_4^{3+} and NH_4^+ in the wastewater also decreased after treatment, from 28mg/l, 2.34mg/l and 2.77mg/l to 8.33mg/l, 1.83mg/l and 1.52mg/l respectively. The pH of treated wastewater increased from 7.1 to 8.33 after treatment. These were different from 7.37 recorded in pH, 23.33mg/l, 2.02mg/l and 2.23mg/l recorded in control samples, for nitrate, phosphate and ammonium respectively. Initial piggery wastewater samples used and swab of biofilm on anode surface recorded a total viable bacterial counts ranging from 1.0×10^6Cfu/ml to 9.75×10^7Cfu/ml. Species of *Bacillus, Pseudomonas, Enterococcus, Klebsiella, Serratia, Staphylococcus, Enterobacter, Corynebacterium, Salmonella, Shigella, Micrococcus* and *Escherichia coli* include isolates found on the samples. Consequently, MFCs hold great promises as a cheaper tool for treatment of wastewater, and factors affecting its potentials should be further investigated.The results of this study are recommended for further studies on scale up of MFCs for commercial applications.

Keywords: Microbial fuel cell, Wastewater, Voltage, Bioelectricity, Optimization.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Wastewater treatment also called **sewage treatment**, is the removal of impurities from wastewater or sewage, before it reaches aquifers or natural bodies of water such as rivers, lakes, estuaries, and oceans. Since pure water is not found in nature (i.e., outside chemical laboratories), any distinction between clean water and polluted water depends on the type and concentration of impurities found in the water as well as on its intended use. In broad terms, water is said to be polluted when it contains enough impurities to make it unfit for a particular use, such as drinking, swimming, or fishing. Although water quality is affected by natural conditions, the word *pollution* usually implies human activity as the source of contamination. Water pollution, therefore, is caused primarily by the drainage of contaminated wastewater into surface water or groundwater, and wastewater treatment is a major element of water pollution control.

The growing population of the world and an ever-increasing quest for industrialization have made global water access and energy demand the preferential topics of discussion (Stern, Sovacool, & Dietz., 2016;Kober, Schiffer, Densing, &Panos., 2020). The population is increasing at an enormously high rate and is anticipated to increase by 21% by 2040 (Koberet al., 2020). In order to meet this growing energy demand, fossil fuels are a major contributor that dominates about 80%–85% of the global energy production market (Liu, 2015). According to recent research, our planet has entered a new era called the Anthropocene era (Stern et al., 2016;Crutzen, 2006;Lewis &Maslin, 2015;Ruddiman, 2013;Keys et al., 2019;Wagner, 2020). Anthropocene refers to changes in the earth's environment, ecosystem and lifecycle due to the anthropogenic activities of humans. Several anthropogenic activities are responsible for Anthropocene, but according to many studies fossil fuel burning and accompanied release of CO_2 are the main contributors (Stern et al., 2016;

Ruddiman, 2013; Council, 2011; Change, 2013). Therefore, to ensure a sustainable future, clean and green energy sources are required.

Another eminent issue faced globally is water scarcity. With increasing population, global water demand is increasing but resources are depleting at a higher pace. In the next decade, freshwater availability may decrease by 40% (Gupta et al., 2020). In this regard, waste treatment has obtained much attention. But existing waste treatment technologies require a lot of energy and also lack the work–energy ratio for effluents discharge (Gupta et al., 2020; Siddiqi et al., 2018). Since organic waste serves as a viable source of energy (Zaidi et al., 2018;Siddiqi et al., 2019; Mushtaqet al., 2022; Siddiqi et al., 2020), wastewater (that contains organic wastes) can be seen as a renewable source of energy that has minimal detrimental impacts on the environment (Gupta et al., 2020;Caiet al., 2019). In this regard, microbial fuel cell (MFC) has emerged as a promising technology. In nutshell, MFC can be touted as a single solution catering to two eminent global issues, renewable energy production and fulfilment of water demand.

Microbial fuel cell is a bio-electrochemical cell that generates electricity by imitating natural bacterial interactions. In other contexts, it can be referred as a fuel cell in which catalytic reactions of microorganisms assist to convert the chemical energy of the substrate to electrical energy. The name “bio-electrochemical” owes to the involvement of microorganisms, usually bacteria, and their chemical reaction(s) (Kim et al., 2017; Rahimnejadet al., 2015).

The phenomena involved in MFC dates back billions of years as microorganisms present in nature were degrading organics. During the study of this natural phenomenon of microbial degradation, Prof. Potter of Durham University-UK explored for the first time that microbes can produce voltage. He was striving to discover the agents that contribute to the oxidation of insoluble substances (for example, charcoal) in soil, and revealed the phenomenon of bioelectricity during

the same study in 1908. Foregoing in view, in the year of 1911, by utilizing the electrical potential of microbes (*Saccharomyces*), the first microbial cell was discovered by him.

During the last 30 years, environmental issues, especially concerning the chemical and biological contamination of water, have become a major concern for both society and public authorities, but more importantly, for the whole industrial world. Any activities whether domestic or agricultural but also industrial, produce wastewaters or effluents containing undesirable contaminants which can also be toxic. In this context, a constant effort must be made to protect water resources. In general, conventional wastewater treatment consists of a combination of physical, chemical, and biological processes and operations to remove insoluble particles and soluble contaminants from effluents.It used to be said that "the solution to pollution is dilution." When small amounts of sewage are discharged into a flowing body of water, a natural process of stream self-purification occurs. Densely populated communities generate such large quantities of sewage, however, that dilution alone does not prevent pollution.

This makes it necessary to treat or purify wastewater to some degree before disposal. The construction of centralized sewage treatment plants began in the late 19^{th} and early 20^{th} centuries, principally in the United Kingdom and the United States. Instead of discharging sewage directly into a nearby body of water, it was first passed through a combination of physical, biological, and chemical processes that removed some or most of the pollutants. Also beginning in the 1900's, new sewage-collection systems were designed to separate storm water from domestic wastewater, so that treatment plants did not become overloaded during periods of wet weather.

After the middle of the 20^{th} century, increasing public concern for environmental quality led to broader and more stringent regulation of wastewater disposal practices. Higher levels of treatment were required. For example, pre-treatment of industrial wastewater, with the aim of preventing toxic chemicals from interfering with the biological processes used at sewage treatment plants,

often became a necessity. In fact, wastewater treatment technology advanced to the point where it became possible to remove virtually all pollutants from sewage. This was so expensive, however, that such high levels of treatment were not usually justified. Wastewater treatment plants became large, complex facilities that required considerable amounts of energy for their operation. After the rise of oil prices in the 1970's, concern for energy conservation became a more important factor in the design of new pollution control systems. Consequently, land disposal and subsurface disposal of sewage began to receive increased attention where feasible. Such "low-tech" pollution control methods not only might help to conserve energy but also might serve to recycle nutrients and replenish groundwater supplies.

1.2 Problem Statement

Energy production and waste management are two crucial problems facing developing countries, and any scientific resolution and innovative ideas that will solve these burning issues simultaneously will be a welcomed development. In line with this, any technology that promotes the conversion of waste to energy should be encouraged.

1.3 Aim

The main aim of this study is to investigate the simultaneous wastewater treatment and energy harvesting using microbial fuel cells**.**

1.4 Objectives

1. To optimize the effects of selected factors on the voltage generation by MFCs
2. To compare the physicochemical parameters of piggery wastewaters, before and after treatment using MFCs.
3. To determine the bacterial diversities and load in piggery wastewater, before and after treatment.

4. To determine the potentials of MFCs in treatment of wastewater.

1.5 Justification

According to Khan et al., (2012), large volumes of high-strength wastewaters are produced from various domestic, agricultural and industrial processes in the world on daily basis. In some developing countries like Nigeria, some of the wastewaters are treated before disposal on both land and in water bodies, while others are discharged without proper treatment.

Although anaerobic treatment technologies are often employed to reduce cost of treatment, however, these are generally only suitable for high-strength wastewaters typically produced by industries only. Those generated agriculturally and domestically are most times discharged into the environment without treatment. In addition, conventional anaerobic treatment produces high level of methane gas which when released into the atmosphere contributes to global warming. One way to reduce treatment cost while producing useful products from wastewater is the microbial fuel cell (MFC) technology (Liu and Logan, 2004), which provides a means of treating wastewater with simultaneous production of energy.

Regrettably, in Nigeria as well as in most developing countries, some pig farms operate without necessary wastewater treatment systems. Consequently, untreated wastewaters are directly discharged into the environment thereby causing pollution. Besides, mild, non-therapeutic doses of antibiotics are often used as supplements in piggery feed. When untreated wastewaters from such farms are discharged into the environment, it would heighten cases of antibiotic resistance in human beings if infected with pathogenic microorganisms in such untreated wastewater. Moreover, since generation and supply of power which is very essential to the growth of every industry, including pig farms which generate large volumes of wastewaters, have remained inadequate in Nigeria, coupled with the grave impacts of fossil fuels, which is presently the chief

source of electricity, on the environment, there is urgent need for intensive search for more environmentally friendly and sustainable sources.

Finally, despite the potentials of microbial fuel cell, literatures on studies using pig waste water which causes major environment nuisance are lacking. Therefore, it becomes imperative to embark on this study to ascertain the suitability of piggery wastewater in electricity generation, as well as wastewater management capability of microbial fuel cell.

1.6 Scope of Study

1. To recognize MFCs as an excellent choice for simultaneous application of bioenergy generation and waste water treatment.
2. To further investigate the reported suitability of water as a cheaper electron acceptor in microbial fuel cell.
3. To determine the effects of using aluminium electrodes on the generation of electricity in microbial fuel cell.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Development of Microbial Fuel Cell

A Microbial Fuel Cell (MFC) is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms. Microbial fuel cell is one choice that has received attention as alternative energy in directly generating electricity from organic matters. A microbial fuel cell is a bioreactor that converts chemical energy in the chemical bonds in organic compounds to electrical energy through catalytic reactions of micro-organisms under anaerobic conditions. Energy has been the major aspects in the evolution of civilization, as fossil fuels have taken care of industrial revolution part. The energy needs cannot be sustained by fossil fuels only at the end of 21st century as they are not substantial enough because of their limited availability. So, the need for renewable alternative source of energy generation is need of the day. Microbial fuel cells (MFCs) have emerged in recent years as a promising and challenging technology. In a MFC, microorganisms interact with electrodes using electrons, which are either removed or supplied through an electrical circuit. MFCs are the major type of bioelectrochemical systems (BESs), which convert biomass spontaneously into electricity through the metabolic activity of the microorganisms. Microbial fuel cells have become an interesting and promising area of research. There are many applications of MFCs that help to reduce the use of fossil fuels and allow for energy gain from wastes. MFC technology does not have the power to change the world single-handedly; microbial fuel cells will never be able to produce enough electricity to take the place of a coal-fired power plant. They will, however, help to bring the world to becoming a sustainable and more environmentally-friendly place. It is now known that electricity can be produced directly from the degradation of organic matter in a microbial fuel cell. Like a normal fuel cell, an MFC has both an anode and cathode chamber. The anoxic anode chamber is connected internally to the cathode chamber via an ion exchange membrane with the circuit completed by an external wire.

MFCs have various practical applications such as in breweries, domestic wastewater treatment, desalination plants, hydrogen production, remote sensing, and pollution remediation, and they can be used as a remote power source. Widespread use of MFCs in these areas can take our waste products and transform them into energy.

The idea of obtaining energy from bacteria began in 1911 with M. C. Potter, a professor of botany at the University of Durham. In his studies of how microorganisms degrade organic compounds, he discovered that electrical energy was also produced. Potter had the idea of trying to harvest this new found source of energy for human use. He was able to construct a primitive microbial fuel cell, but not enough was known about the metabolism of bacteria for the design to be improved upon.

In recent, researchers are working to optimize electrode materials, types and combinations of bacteria, and electron transfer in microbial fuel cells. Even though the idea of harnessing the energy produced by bacteria has been around for almost 100 years, researchers have just begun to fully understand the MFC and how to bring out its true potential.

2.2 Microbes used in MFCs

Firstly, in order to understand the fundamental function of the MFC, it is important to have a grasp in some of the basic functions of the bacteria. In essence, bacteria breakdown organic matter and release energy in the process. Extra attention will be paid to certain bacteria which have the ability to generate electricity and to transfer electron effectively in the anode.

This type of bacteria is called Exo-electrogens, "exo- "for exocellular and "electrogens" based on the ability to directly transfer electrons to a chemical or material that is not the immediate electron acceptor. There are many anaerobic bacteria that can only transfer electrons to soluble compounds such as nitrate or sulphate (not cell synthesised) that can diffuse across the cell membrane and into the cell. Exo-electrogenic bacteria are the most suited to function within an MFC due to their

ability to transport electrons outside of the cell. This type of bacteria is useful in mediator-less MFC, an MFC system which does not require a 'mediator' to assist in electron transfer. Some mediators according to Du et al. (2007), include, thionin, sulphate/sulphide methylene blue, pyocyanin etc., as well as others. These exoelectrogens can be sourced in a number of places, they are found in soil, marine sediment, waste water, fresh water sediment and activated sludge, which are rich with these microorganisms (Du et al., 2007).A list of tested mediator-less bacteria and their associated substrates are listed in Table 2.1.

Table 2.1: Microbes used in Mediator-less MFCs

Microbes	Substrate	Applications
Aeromonashydrophila	Acetate	Mediator-less MFC
Geobactermetallireducens	Acetate	Mediator-less MFC
Geobactersulfurreducens	Acetate	Mediator-less MFC
Shewanellaputrifaciens	Lactate, pyruvate	Mediator-less MFC
Rhodoferaxferrireducens	Glucose, xylose	Mediator-less MFC

An interesting relationship is found between exoelectrogens and fungi in recent studies which potentially increases stability in electron transfer as fungi act as a natural organic mediator. This can be a significant step toward scaling up MFC systems as fungi and bacteria can be found naturally (Fernándezet al., 2013).

2.3 Principles of Electricity Generation in Microbial Fuel Cell

"Microbial fuel cells (MFCs) are electrochemical devices that use the metabolic activity of microorganisms to oxidise fuels, generating current by direct or mediated electron transfer to electrodes." (Rabaey&Verstraete., 2005; Cercado-Quezada, Delia&Bergel., 2010). The device comprises of an anode chamber, a cathode chamber, electrodes, proton exchange membrane and an external circuit. According to Logan (2008) the MFC convert a biodegradable substrate directly

into electricity. The anode holds the bacteria and the organic material in an anaerobic environment. The cathode holds a conductive saltwater solution in a double chamber type MFC or air if it's the single chamber. Mansoorianet al. (2013), also reported that the bacteria generate protons and electrons as the organic substrate is being converted into energy. This energy is used and stored by the microbes for growth. The electrons are transferred directly to the anode electrode (in a mediator-less set-up) and to the cathode electrode via a copper wire or a conductive material. Protons pass through the ion exchange membrane to the cathode chamber to produce water as a result of the reduction process which is in terms of hydrogen transfer.

$4H^+ + 4e^- + O_2 \rightarrow H_2O$

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

Not all bacteria species are able to transfer electrons directly, therefore use of artificial chemicals such as "thionine, humic acid, neutral red, methyl blue and methyl viologen" is required. These are called redox mediators.

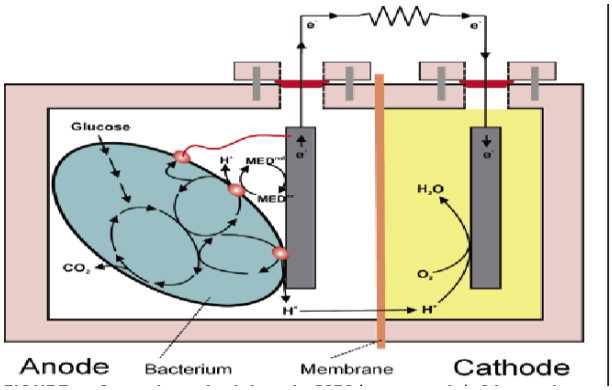


Figure 2.1: Schematic of the basic components of an MFC (source Alzate-Gaviria, 2013)

According to Alzate-Gaviria (2013)., the bacteria grow in the anode, oxidising matter and releasing electrons as they break down the substrate. Some bacteria require exo-electrogenic biofilms in order to effectively transfer the electrons to the electron accepter whereas some transfer electrons directly without the need of a mediator. The cathode is supplied with air or other

inoculum to provide dissolved oxygen for the reaction of electrons via an external circuit, protons and oxygen at the cathode, completing the circuit and producing power.

Chemical energy is converted into electricity by the microbes which releases electrons and hydrogen ions which from water. The oxygen is supplied in the cathode chamber by air or other oxygen source. The material used in the electrodes significantly influences the overall efficiency.

2.4 Components of Microbial Fuel Cell

A typical microbial fuel cell consists of anode and cathode compartments separated by a cation (positively charged ion) specific membrane. In the anode compartment, fuel is oxidized by microorganisms, generating CO_2, electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, while protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

The microbial fuel cell consists of simple yet vital components to effectively harness the energy, which are as follows:Electrodes –both in the anode and cathode chambers, Proton Exchange Membrane –(widely used Nafion as the least resistive membrane),Substrate –any organic matter used as source of energy for microorganisms i.e. wastewater,Bacteria –exoelectrogens, most suited for MFC applications.Latest MFC developments focus on optimising each aspect in order to increase the overall efficiency of the electrochemical device.

Table 2.2: Basic Components of microbial fuel cells

Items	Materials
Anode	Graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black, reticulated vitreous carbon (RVC)
Cathode	Graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black, RVC
Anodic Chamber	Glass, polycarbonate, Plexiglass
Cathodic chamber	Glass, polycarbonate, Plexiglass
Proton Exchange Membrane (PEM)	Nafion, Ultrex, polyethylene.poly (styrene-co-divinylbenzene); salt bridge, porcelain septum or solely electrolyte
Electrode Catalyst	Pt, Pt black, MnO2, Fe3+, polyaniline, electron mediator immobilized on anode

2.5 Factors affecting the MFCs efficiency

There are several factors that affect the performance of MFCs and its energy production in wastewater treatment. In order to have a highly efficient MFC, recognizing and considering these factors are essential. Microorganisms in the anodic chamber are important due to their metabolism and the mediators which are used by them for transferring electron to the anode. There are various substrates which can be used as the source of electron donors in the MFC and oxidized by microorganisms. Operating conditions such as pH, temperature, ionic strength of the mediums, material and construction of the anode, cathode and membrane could have a considerable impact on electricity generation.

A. Electrodes

The efficiency of a MFC is dependent on a number of factors and one is the material of the electrodes. Type of material used in electrode preparation will show vital effect on MFCs

efficiency. Better performing electrode materials usage will always improve the performance of MFC because different anode materials result in different activation polarization losses.

There have been many MFC designs and configuration that have been tested and developed in recent years to improve the performance and efficiency of MFCs. However, the challenge to find the balance between performance and material cost remains difficult. This paper will explore the advantages and drawbacks of some of the electrodes that are widely used in today's MFCs, in terms of their conductivity, surface properties, biocompatibility and cost (Wei, Liang& Huang., 2011).The electrodes have a certain resistance hence the most effective ones are the least resistive. "The anodic resistance contribute to the overall cell resistance in MFC operation."(Liu et al., 2013). However, use of highly efficient electrode materials (i.e. platinum) is not economically feasible for large-scale applications thus investment on more cost-effective alternatives is priority in MFC research. The material characteristics which are critical for an effective electron transfer are high conductivity and mechanical strength. There is no requirement for bacteria adhesion. The scalability and cost-effectiveness are also taken into consideration. (Wei, Liang& Huang., 2011). The most common material used for MFC anodes are carbonaceous materials due to their "good biocompatibility, good chemical stability, high conductivity and relatively low cost." (Wei, Liang& Huang., 2011).

Figure 2.2: Photographs of electrode materials used for MFC: (A) carbon paper; (B) carbon cloth; (C) graphite plate; (D) graphite rod (E) carbon mesh (source: Wei, Liang& Huang 2011)

The materials used in MFC experiments mostly include carbon material and other metals like platinum. It is widely recognized that type and concentration of bacteria on anode electrodes

greatly affect the power/current density in MFCs. Photographs of some of these materials are shown in Figure 2.2. Most commonly used mixed cultures in studies for anode materials are activated sludge or domestic wastewater. In order to maximise bacteria adhesion, the surface area of the electrodes is increased and are divided into a number of configurations: a plane structure, a packed structure, and a brush structure.

(I) Plane structure

Carbon paper, graphite sheets or plates are the most commonly used for plain electrodes in laboratory use. Carbon paper is very thin, and stiff but brittle material. According to Wei et al, (2011) roughened graphite plate electrodes yield a higher power density than their smooth plain counterparts due to the increased surface area in roughened graphite electrodes. These material, however, are not feasible for large-scale application due to its overall low specific area and high cost. A substantially inexpensive carbon mesh is used as a better alternative (Zhang et al., 2009). Besides the common plain and plate electrodes types, some fibrous materials are also reported to yield higher power generation such as graphite foil and carbon cloth due to their high specific surface and absorption capacity.

(II) Packed structure

For a significant increase of surface area available for the bacteria attach to, use of carbon materials in packing forms is becoming increasingly common (Aeltermanet al., 2008).The anode chamber is filled with granular or irregularly shaped packing which has a high specific area as its main advantage. Granular activated carbon yielded the highest maximum power output according to Wei et al, (2011) compared to graphite and carbon felt counterparts (Wei, Liang& Huang., 2011).

Figure 2.2.1: Granular form electrodes for maximum surface area (F) granular graphite, (G) granular activated carbon (source: Wei, Liang& Huang 2011)

(III) Brush structure

The graphite brush anode in an ideal electrode that can achieve high surface area and efficient current collection, which is first reported by Logan et al, (2008). According to their studies, "the brushes were made of carbon fibres with a set length and wound into a twisted core consisting of two conductive but noncorrosive titanium wires" (Wei, Liang& Huang., 2011). However, some clumping were discovered in the brush configuration which prevented the bacteria access and limited the contact between the microbes and the material, thus resulted in low power generation. A photograph of brush anodes are shown in Figure 2.2.2

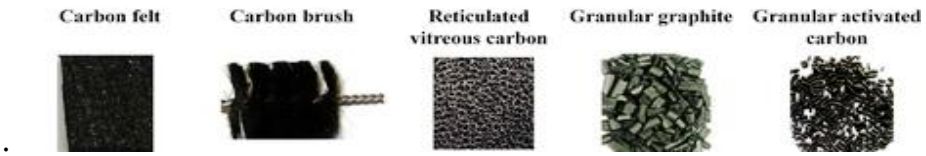


Figure 2.2.2: A photograph of brush anode (H) carbon felt; (I) carbon brush; (J) reticulated vitrified carbon; (K) granular graphite (L) granular activated carbon (source: Wei, Liang& Huang 2011)

B. pH Buffer and Electrolyte

If no buffer solution is used in a working MFC, there will be an obvious pH difference between the anodic and cathodic chambers, though theoretically there will be no pH shift when the reaction rate of protons, electrons and oxygen at the cathode equals the production rate of protons at the anode. The pH difference increases the driving force of the proton diffusion from the anode to the cathode chamber and finally a dynamic equilibrium forms.

C. Proton Exchange Membrane (PEM)

The proton exchange membrane is a core component that greatly influences electrochemical performance in MFCs. The PEM has a structure which enables only hydrogen ions or protons to pass through as illustrated in Figure 5 "Hydrogen with proton exchange membrane fuel cells (PEMFCs) is currently considered as a potential next generation alternative energy technology

because of the high energy density and high abundance of hydrogen in nature." (Dewan, Beyenal& Lewandowski., 2008). The most widely used polyelectrolyte for proton exchange membrane is the Nafion ionomer which increases the three-dimensional zone of catalytic activity. Proton exchange system can affect an MFC system's internal resistance and concentration polarization loss and they in turn influence the power output of the MFC. Nafion (DuPont, Wilmington, Delaware) is most popular because of its highly selective permeability of protons.

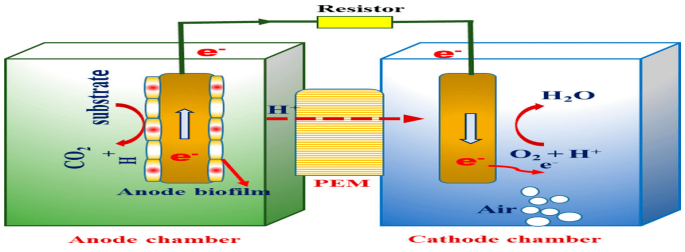


Figure 2.2.3: PEM structure which allows hydrogen ions to pass through (source : Won et al., 2014)

As the hydrogen ions passes through the membrane, it forms water and carbon dioxide with the electrons in the reduction process and completing the circuit.

D. Electron transfer mechanism

Electrons that are produced in the anodic chamber should be transferred to the anode by shuttles or electron mediators. Some microorganisms, such as species and *Saccharomyces*, *E. coli* used in some MFCs, have an outer layer of non-conductive lipid membrane, including peptidoglycans and lipopolysaccharides that slow down the direct electron transfer to the anode. The mediators are typically dyes such as methylene blue, neutral red, thionine, methyl viologen or humic acid.

E. Microbial metabolism and cell potential

Microorganism metabolic pathway and the consequent potential of the anode is the main parameter in determining the cell potential. Bacterial catabolism is the rate limiting step in MFCs. Heterotrophic organisms gain their energy from oxidation of organic compounds.

Due to the involvement of exogenous oxidants, that is, external terminal electron acceptors, two major metabolic pathway staking place in the anodic chamber are respiratory chain and fermentation. The MFC electrical potential depends on the potential differences between the cathode and the anode.

F. Microorganisms

Either microorganisms or enzymes can be used in biofuel cells, however applying microorganism in biofuel cells allows multiple enzymes and, therefore, multiple substrates (or mixed substrates) to be used. While purified enzymes can be used in specific and defined reaction(s), this type of biofuel cells has been developed in biosensor technology in parallel (Bullen et al., 2006). MFC can be inoculated by pure or mixed culture of bacteria. Mixed cultures are more beneficial in comparison with pure cultures due to their nutrient adaptability and stress resistance (Mathuriya, 2014). Application of rich and diverse source of bacteria, such as wastewater, activated sludge, soil or sediments in mediator-less MFCs is more advantageous in wastewater treatment due to the presence of different kinds of bacteria including electronics and giving a high power density (Logan, 2009; Mathuriya., 2014).

G. Substrate

Great varieties of substrates which have been applied in MFCs vary from simple compounds to complex mixtures of organic matters. In some cases, pure substrates such as glucose, (Cheng et al., 2006) acetate, (Cheng & Logan, 2007) butyrate, (Liu et al., 2005) lactate, (Futamata et al., 2013) proteins, cellulose, cysteine, glycine (Chen et al., 2014) and glycerol were used. Acetate is anon fermentable substrate and a suitable electron donor for dissimilatory iron-reducing bacterium which generate power up to 66% higher than butyrate (Liu et al., 2005). Among different substrates, wastewater is a sustainable rich medium which can be treated by MFCs. There are several reports on electricity generation directly from complex organic wastewater such as municipal, (Liu et al., 2020) swine

wastewater, (Min et al., 2005) dairy wastewater, (Mardanpour et al., (2012);Venkata Mohan et al., 2010) slaughter house wastewater, (Katuri et al., 2012) rice mill wastewater, (Behera et al., 2010) tannery wastewater, (Mathuriya, 2014) cassava mill wastewater, (Kaewkannetra et al., 2011) molasses wastewater, (Zhang et al., 2015) refinery wastewater, (Zhang et al., 2014) brewery wastewater, (Mshoperi et al., 2014) winery wastewater, (Sciarria et al., 2015) chemical wastewater, [Mohan et al., 2008; Raghavulu et al., 2009; Velvizhi et al., 2014) sulphide-rich wastewater. Substrate concentration is another factor which must be considered. Jiang and Li (2009) showed that increasing the substrate concentrations from 100 to 850 mg/L, boosted the power output from 0.2 up to 1.2 W/m3. However, at high concentrations of 10001500 mg/L, the power output didn't change.

H. Operating conditions in the anodic chamber

The anode can be defined as the electrode at which electrons leave the cell and oxidation occurs and the cathode as the electrode at which electrons enter the cell and reduction occurs. Conductivity, stability, biocompatibility, non-corrosive manner and surface area are the important characteristics of the anode material. Substrate type, concentration and feed rate are important factors that impact the performance of an MFC. Power density varies greatly with different substrates using same a given microbe or microbial consortium. Electricity generation is dependent on substrate concentration both in batch and continuous flow mode MFCs. In addition, the construction method of the electrodes and architecture of the MFC also affect the performance of MFC. In spite of good conductivity, copper is not a suitable material for anode due to its toxicity for bacteria. Carbon is a more suitable electrode material for bacterial adhesion; however, it has low conductivity for transferring electrons. It is available in forms of carbon felt, cloth, foam, paper and fibres. Anode materials play an important role in the performance of MFCs by affecting the performance and cost of MFCs significantly. The desired properties of an

anode in an MFC should include excellent electrical conductivity, large surface area and high bio- compatibility for bacteria colonization. At the anode chamber, where anaerobic conditions are present, the microorganisms generate gas that should migrate easily out of the system, in order to avoid an increase of pressure in the anode chamber. Hence the need of high bed void fraction is of paramount importance. Another aspect the mechanism involving direct electron transfer is between anode and bacteria which mainly contributes to the power output. Therefore improving the physical contact between anode and bacteria favours the power generation of MFCs.

I. Modification of Anode Materials

The most versatile electrode material is carbon as they are relatively inexpensive, easy to handle, and have a defined surface area. To render MFCs a cost effective and energy sustainable technology, low cost materials can be employed as support for bacteria growth and proliferation. Currently, carbon-based materials, such as graphite fiber brush, graphite rod, graphite felt, graphite plate, carbon paper, and carbon cloth, are the most widely used anode materials due to their high electrical conductivity, strong biocompatibility, and low cost. Furthermore, the modification of electrode surfaces has proven to be an effective way to improve the performance of an MFC because it changes the physicochemical properties of electrodes to facilitate microbial attachment and electron transfer. The modification of electrode materials can be done either by introducing new materials or by treatment of anode surface.

J. Modification by introducing new materials

The introduction of new materials like natural polymers, Melamine sponges, Berl saddles, Carbon nanotube and high capacitance electrode materials have been successful as anode materials in MFC.

The modification of the anode system with natural polymers polyacrylamide with neutral red (PA + NR) and conductive polymer poly pyrrole with positive functional groups poly(pyrrole-alkyl ammonium) can improve voltage output, start up, current production, stability and recovery rate of the sensor's response (Amandeep et al., 2014). Melamine sponges coated with reduced grapheme oxide/carbon nanotube (rGOCNT –sponges) provides a large electrical conductive surface for Escherichia coli growth and high porosity for efficient mass transport & electron transfer in microbial fuel cell. The rGO-CNT sponges showed higher durability and performed better electrochemical properties than traditional carbon based and metal based anodes (Chou et al., 2014).Carbon-coated Berl saddles have become an innovative low-cost anode material which favours an optimal bacteria adhesion and efficiently recover the electrons released by bacteria metabolisms. It provides relatively high useable contact surface area with high void bed which also showed a maximum power density of about 2-3 times higher than the values obtained by using commercial anode materials (Diana et al., 2014).The novel Carbon Nano Tube (CNT) based anode materials show unique biofilm morphology and its performance is better than bare gold electrodes (Hao et al., 2018). Interlacing carbon yarn with stainless steel resulted in high surface anode treating distillery wastewater (Jayesh et al., 2014). The Nickel Oxide (NiO)/carbon cloth anode delivers around 3-fold higher maximum power density higher than that of the plain carbon cloth anode due to the unique nanostructure for high electro catalytic activity contributed from improved active surface area of electrode, strengthened adhesion of bacteria and enhanced interfacial charge transfer between bacteria cells and electrode. The untreated cloth has a hydrophobic surface whereas after modification with NiOnanoflakes, the surface becomes highly hydrophilic one (Yan et al., 2014). The modification of anode with Nano composite materials are more advantageous resulting in enhanced electricity generation of MFC. SnO2 represented approving properties for the anode materials of MFCs. Nano composite of Reduced graphene oxide

and tin oxide (RGO/SnO2) (Ali Mehdinia et al., 2014) and multi-walled carbon nanotubes and tin oxide (MWCNTs/SnO2) were successful as anode materials in MFC. MWCNTs-SnO2/Glassy Carbon Electrode(GCE), MWCNT/GCE and bare GCE anodes showed maximum power densities of 1421mWm−2, 699 mW m−2 and 457 mW m−2, respectively. The improved performance of the MFC was ascribed to the large specific area of the graphene& MWCNTs and synergic effects of SnO2 with these materials. The high conductivity and large specific surface of the Nano composite were greatly improved the biofilm formation and increased the electron transfer.

K. Modification by treatment

The power output of MFC depends on the morphology rather than the oxygen-containing group concentration of the anode surface. The intensive attention should be paid to the design of surface morphology in order to improve power generation of MFC. The surface modification of anode materials has been carried out by oxidation, electrochemical and acidic treatments. The carbon felt treated through oxidation has its surface with increased roughness, exhibits improved biocompatibility and provides MFC with improved power output (Christina et al., 2014). Electrochemical treatment of the surface of graphite felt anode facilitated biofilm formation on the electrode resulting in rapid and enhance current production. Biofilm formation on the treated anode was mainly due to the strong hydrogen or peptide bonds between the amide groups of bacterial materials (including cytochrome c) and carboxyl groups formed on the electrodes (Baitao et al., 2014).

The acidic modification of the carbon cloth anode material increased in the ratio of saturated/unsaturated carbon on the surface and consequently, a decrease in electrode resistance and start-up period were observed Carbon cloth anodes were modified by electrochemical oxidation by electrolytes like Nitric acid+sulfuric acid (-NS), Ammonium CCnitrate (CC-AN), and Ammonium Sulfate (-AS) electrolytes. The results showed the

CCpositive effect of the surface modification on the MFCs output due to increases in the amounts of Nitrogen, Sulfur, Oxygen as well as unsaturated and oxidized carbon. Electrochemical Accessible Surface Area (ECSA) and bacterial attachment have also been increased (Jiseon et al., 2014). Carbon veil (CV) and carbon cloth (CC) anodes were modified with a micro-porous layer (MPL) which resulted in higher bacterial population, increased power performance (2.2 and 1.8 times of unmodified CV and CC) and stability of MFC.

L. Operating conditions in the cathodic chamber:

Oxygen is the most commonly used electron acceptor in MFCs for the cathodic reaction. Power output of an MFC strongly depends on the concentration level of electron acceptors. The design of the cathode is the single greatest challenge for making an MFC a useful and scalable technology. The Oxygen reduction reaction (ORR) at the cathode interface consumes oxygen and generates water or hydrogen peroxide. The ORR of a working MFC in comparison to chemical fuel cells is limited due to the MFC neutral pH and ambient operating temperature. The cathode materials also have a great influence on the performance of a MFC which should have a high redox potential to accept the electrons. The most commonly used materials for a cathode is commercially available carbon paper, carbon cloth and graphite. However it is difficult to obtain high cathodic potentials unless metal catalysts are used. Temperature, pH, ionic strength and salinity are the most important factors in the growth of bacteria which affect the performance of MFCs. Overall; MFCs have mild reaction conditions such as ambient temperature, normal pressure and neutral pH.

2.6 Types of Microbial Fuel Cell

A microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms. Broadly, there are two types of microbial fuel cell: mediator and mediator-less microbial fuel cells.

I. Mediator microbial fuel cell

Most of the microbial cells are electrochemically inactive. The electron transfer from microbial cells to the electrode is facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid, neutral red and so on. Most of the mediators available are expensive and toxic.

II. Mediator-less microbial fuel cell

Mediator-less microbial fuel cells do not require a mediator but use electrochemically active bacteria to transfer electrons to the electrode (electrons are carried directly from the bacterial respiratory enzyme to the electrode). Among the electrochemically active bacteria are, *Shewanellaputrefaciens, Aeromonashydrophila*, and others. Some bacteria, which have pili on their external membrane, are able to transfer their electron production via these pili. Mediator-less MFCs are a more recent area of research and, due to this, factors that affect optimum efficiency, such as the strain of bacteria used in the system, type of ion-exchange membrane, and system conditions (temperature, pH, etc.) are not particularly well understood. Mediator-less microbial fuel cells can, besides running on wastewater, also derive energy directly from certain plants. This configuration is known as a plant microbial fuel cell. Possible plants include reed sweet grass, cord grass, rice, tomatoes, lupines and algae The mediator free microbial fuel cells are of designed in various types, like- Microbial electrolysis cell , Soil-based microbial fuel cell and Phototrophic biofilm microbial fuel cell.

2.7 Microbial Fuel Cell Design

There are different designs of the microbial fuel cell ranging from Single chamber MFCs, Double chamber MFCs, Stacked MFCs to Tubular MFCs.

(a) Single-Chamber MFCs

A typical one-compartment MFC eliminates the need for the cathodic chamber by exposing the cathode directly to the air. The most common is the cube reactor design.The one-compartment MFC consists of an anode in a rectangular anode chamber coupled with air-cathode. Protons are transferred from the anolyte solution to the porous air –cathode. The cube is usually made of perspex plastic (Du, Li&Gu., 2007).

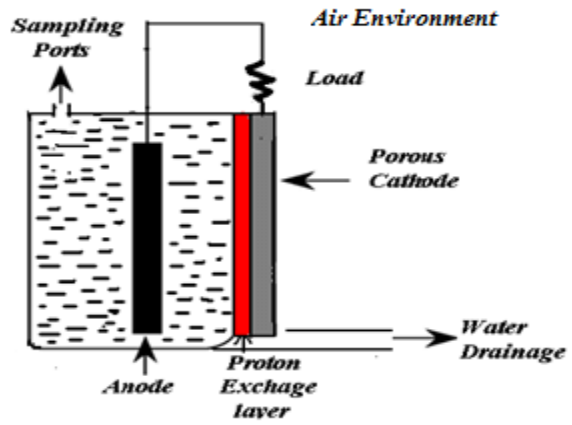


Figure 2.3: Schematic design of single chambered microbial fuel cell (Source:Park&Zeikus, 2003).

(b) Double-Chamber MFCs

This MFC configuration is the most widely used consisting of two compartments with the anode and cathode separated by the proton exchange membrane (Figure 1). The anode chamber is kept oxygen free for anaerobic breakdown process to occur, which is usually purged with nitrogen. Although the H-type or dual-chambered microbial fuel cells is the most common in laboratory use, it is the most challenging to scale up due to the impractical configuration. The single-chamber is the easiest to scale up as it uses air directly as the oxygen source and also due to less material is

required thus less overall cost .

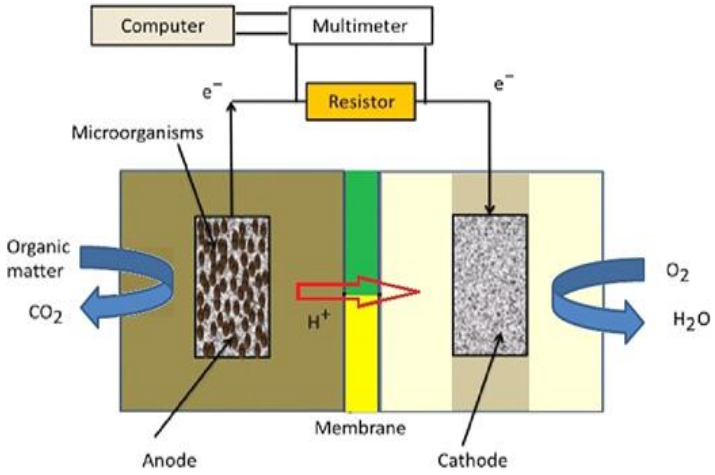


Figure 2.3.1: Simple design of Double chambered Microbial Fuel Cell (source Alzate-Gaviria, 2013)

This set up can accommodate various electrode shapes, i.e. plane, granular and brush as it has a dedicated chambers for the anode and cathode. It can also use other catholyte besides air, which is any source of oxygen. According to a recent research document by González del Campo et al. (2013), use of algae (seaweed) enhances the oxygen production due to photosynthetic process in the plant which can be facilitated by this type of MFC configuration.

(c) Stacked MFC

A stacked microbial fuel is a collection of MFCs connected with each other in series or in parallel connection (Aeltermanet al., 2006). MFC can be stacked by achieving different configurations of both electrode as well as hydraulic flow. These can be of four types such as (i) series electrode connections in parallel flow mode, (ii) parallel electrode connections in parallel flow mode, (iii) series electrode connections in series flow mode and (iv) parallel electrode connections in series flow mode (Choi &Ahn., 2013). Choi andAhn (2013) obtained an overall increase in chemical oxygen demand (COD) removal, Coulombic efficiencies and maximal power densities in parallel electrode connection (series flow mode) while treating wastewater which was attributed to a higher stability of the oxidation–reduction potentials in overall cells. Aeltermanet *al*. (2006) reported a six times higher voltage and Current output when connected in parallel as com-pared to series

thereby implying an overall higher biochemical reaction rate. Thus, these studies imply a possible innovative modification in MFC technology which could assist in increasing the power output thereby contributing toward one

of the application of MFCs.

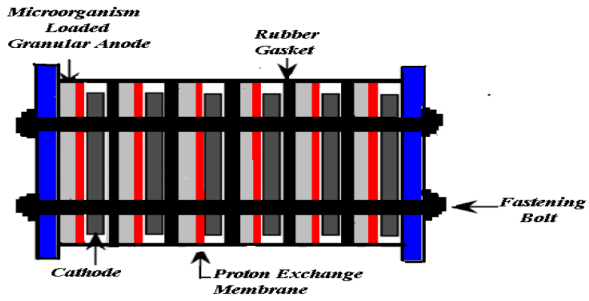


Figure 2.3.2: Schematic diagram of the stacked type microbial fuel cell Source: Aeltermanet al., (2006).

(d) Tubular MFCs

The single-chambered, tubular, continuous microbial fuel cell uses granular graphite matrix as the node which generates high power outputs. This type of MFC have proven to be most effective in continuous flow operation, however, there is about 57% change in the chemical oxygen demand concentration across the reactor and a significant decrease in current density.

In an experiment conducted by Scott et al,(2007) using manure as the organic fuel source, carbon cloth as the electrodes and with no proton exchange membrane. This set up did not require a strictly controlled anaerobic environment and adapted the form of a helix which allows the fuel to flow through at a certain flow rate(Scott, Murano&Rimbu, 2007).This MFC configuration is most applicable in commercial use as it yields high power densities with minimum cost in terms of the materials used. The coiled helix tubular MFC concept might be the next step to realising practical applications. Figure 9 shows the tubular model MFC set up.

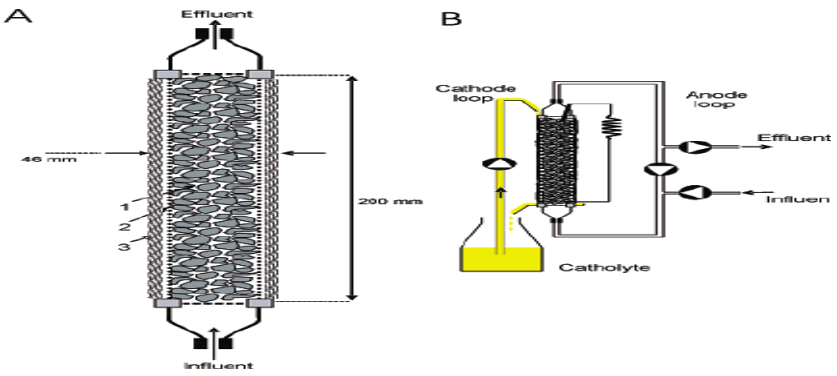


Figure 2.3.3:Schematic diagram of a Tubular MFC (source Alzate-Gaviria, 2013)

2.8 Power Generation and Evaluation of MFC Performance

Anaerobic environment is required at the electroactive biofilm containing anode chamber to generate current through digestion of organic waste. The closest analogues to anodes for microbial metabolism in natural environments are probably Fe^{3+} or Mn^{4+} oxides. The resemblance lies in the fact that all are basically insoluble exocellular electron acceptors. The key difference in microbial electricity production compared to usual biogeochemical processes, such as Mn^{4+} reduction, is that the electron released during microbial respiration reduces anode instead of a natural electron acceptor. In the sedimentary environments, the oxidation of organic matter is coupled to metal (Fe^{3+}) reduction by a consortia of fermentative microorganisms and metal-reducing organisms (Erableet al., 2010). Some nitrate- and sulfate-reducing bacteria have electron donating property in the anode of MFC. The electron donation in anode takes place in the absence of their natural electron acceptor in the MFC. Electricity is generated in an MFC since the overall reaction is thermodynamically favourable. The energy output can be evaluated depending on power generation and time required for bio-electrochemical reaction:

$$V - I \times R \quad (1)$$

$$E - P \times T \quad (2)$$

$$P - V \times I \quad (3)$$

Where P and T indicate the power (Watts) and time (s), respectively. The output operating voltage (V) and corresponding current influence power generation. The operating voltage V is associated

with MFC resistance, by Ohm's law in which R represents the resistance (Ohm). The operating or actual voltage due to cell resistance can be calculated by Eq.4 (V):

$$V - E^{o} - \eta_a - \eta_b - I \times R \quad (4)$$

With E^{o} the theoretical cell voltage which can be calculated using Nernst equation. The η_a and η_b,indicated over-potential or voltage losses at the electrodes, the "I" is current, and "R" signifies ohmic loss due to both anolyte and catholyte resistances. Owing to these losses, the actual voltage output is always lower than theoretical voltage. The measured open circuit voltage is around 750–800 mV,which is lower than theoretical one of 1.1 V. Under close circuit condition, the operating voltage will reduce significantly.

1. Potential Losses in MFC

In reality, MFC always produces low operating voltage (V_{op}) compared to the cell electromotive force (E_{thermo}) that is the thermodynamically predicted potential. These potential losses are irreversible in nature (Chandrasekhar &VenkataMohan ., 2012, 2014a,b). Energy loss can occur in different ways in MFCs: activation loss (maybe for initiating the reactions on both electrodes and extracellularelectron transfer to the anode), bacterial metabolism loss (due to bacteria acquiring energy by oxidizing the substrate), mass transfer loss (due to limited flux of the reactants to the electrode), and ohmic losses (due to proton diffusion resistance and charge transfer resistance (Du et al., 2007). The irreversible losses in an actual MFC are presented in the following equation (Rismani-Yazdiet al., 2008):

$$V_{op} - E_{thermo} - [(\eta_{act} + \eta_{ohmic} + \eta_{conc})_{cathode} + (\eta_{act} + \eta_{ohmic} + \eta_{conc})_{anode}] \quad (5)$$

Where η_{act}, η_{ohmic}, and η_{conc} are the activation loss, ohmic loss, and concentration loss, respectively. These voltage losses occur due to slow electrochemical reaction kinetics,

development of ionic and electronic resistances, and mass transport limitations. The over-potentials of the electrodes are generally current dependent, which can be represented in a polarization curve.

2. Activation Over-potential

Activation energy is required in order to initiate the oxidation and reduction reaction at anode and cathode, respectively. The activation over-potential is the potential loss due to the activation energy required to either oxidize substrate/fuel on the anode surface or and occur during the initial phase of electron transfer from (or) to a mediator involved in chemical reaction on electrode. Various strategieswere employed to reduce the activation over-potential (Rabaeyet al., 2005; Chan-drasekhar&VenkataMohan., 2012). It can be minimized by increasing electrode catalysis, adding mediators to facilitate efficient electron transport from microbial cell membrane to anode surface, by increasing the electrode surface area, and enriching electrogenic biofilm on anode and operational conditions inside anode and cathode compartments.

3. Ohmic Over-potential

The resistance developed owing to the migration of ions in both anolyte and catholyte and the flow of electrons between the anode and cathode causes ohmic over-potential. Therefore, by reducing the gap of anode and cathode, it is possible to minimize the ohmic loss. The current generation is proportional to ohmic over potential. This will ensure the migration of counterion and availability of proton oncathode surface for reduction. Increasing the ionic conductivity of electrolyte is another solution to reduce ohmic over-potential (Chandrasekhar &Venkata Mohan, 2012).

4. Concentration Over-potential

Limitation in transport of reactant or sluggish mass transfer rates in MFC are responsible for the concentration over-potential. The concentration loss occurs at high current densities, where rapid

fall of voltage occurs with increase in current. Unavailability of substrate to the biofilm on the anode is due to mass transfer limitation which contributes toward concentration losses (Qiaoetal., 2010; Chandrasekhar &Venkata Mohan., 2012). Concentration over-potential can be reduced by increasing the substrate concentration of anolyte in anode chamber and terminal electron receiver like O_2 or proton in cathode chamber. However, substrate concentration in anode requires optimization as high concentration may inhibit the normal metabolic activity of biofilm on anode.

2.9 Performance Evaluation for MFC

MFC performance and efficiency is generally measured in terms of energetic parameters (volumetric power density, current, potential difference, open circuit voltage, cell internal resistance) and biological treatment efficiency (COD removal) (Logan., 2012). Coulombic efficiency (CE) is an interesting factor that combines the two previous ones: the amount of electrons that feed the current, collected from the conversion of the organic substrates.

a. Polarization Studies

Polarization or voltage–current curves have become the standard method of presenting MFC performance as is the case with chemical fuel cells (Zhao et al., 2009). A polarization curve generator is a powerful experiment to analyze and characterize quality of fuel cells in terms of power generation. The polarization curve provides lot of information. Polarization curves can be obtained by varying external resistances using a resistance box or using programmed liner sweep Voltammetry. The volumetric current and power density were calculated using Eq.6:

i. $P - V \times I;\ i_d - V / Rv_{and};\ P_d - V^2 / Rv_{and}$

(6)

Where P, V, R_{ext}, and I denote volumetric power, operating cell voltage, applied external resistance, and current, respectively. Volumetric current (i_d) and power density (P_d) were evaluated by normalizing to anolyte volume (v_{and}).

b. **The Current Interruption Method**

The current interrupt technique is commonly used experimental method for the evaluation of total ohmic resistance of electrochemical systems like storage cell, batteries, and fuel cells. In the current interrupt method, cell is initially kept in close circuit with a certain magnitude of external resistance. While it is observed that the MFC generated a stable current output across the terminal of applied external resistance, the circuit is opened suddenly which causes a shoot-up of the cell voltage (VR) followed by gradual further increment (Logan, 2012). The immediate sharp lift of voltage is attributed to ohmic internal over-potential (R_{int}) of the MFC. The total internal resistance of the MFC cell can be measured using following equation (Zhao et al., 2009).

a. $R_{int} — \frac{VR}{I}$ (7)

c. Cyclic Voltammetry

Cyclic voltammetry (CV) is a useful method to investigate the mechanisms of oxidation or reduction reactions on the electrode surface. In CV, external specific range of applied voltage was applied and output current is monitored. From the current–voltage graph, different information can be explained (Fricke et al., 2008; Chandrasekhar &Venkata Mohan 2012). In MFC, CV experiments were carried out extensively to (1) elucidate the different type of exocellular electron transfer mechanisms of biofilm containing anode, (2) to find out the redox potentials of the mediators present in anolyte for electron transfer on anode, and (3) to evaluate the quality of the cathode catalysts for ORR (Zhao et al., 2009).

d. Coulombic Efficiency and Energy Efficiency

The Coulombic efficiency (CE) is basically the ratio of the total Coulombs transferred to the anode after bioelectro-oxidation of the substrate to maximum charge available or stored if all the substrate can be converted to current theoretically, i.e., the total electron charge stored in the substrate (Logan et al., 2006). One of the major useful indexes to evaluate the performance of an MFC is to find out coulombic as well as energy efficiency in terms of the energy recovery (Rabaey et al., 2005). The energy efficiency of an MFC can be defined as the energy recovered from the organic matter to the total energy content of the organic material. In MFCs, energy efficiencies range from 2% to more than 10% depending on the type of substrate (Logan., 2008). CE can be evaluated as per Eq.8.

i. $$CE - \frac{M \int_0^t 1 dt}{Fbv\Delta COD} \qquad (8)$$

Where v is the volume of the anode chamber of MFC, M is 32 g/mol (MW of O_2), Faraday's constant (F) is 96485 C/mol, and b is 4, i.e., the number of electrons exchanged per mole of oxygen; ΔCOD is the subtraction of the initial to final anolyte concentration in terms of COD (g/L)

e. Open Circuit Voltage (OCV)

The cell electromotive force (E.M.F.) is a thermodynamic value that does not take into account internal losses. The open circuit voltage (OCV) is the cell voltage that can be measured after some time in the absence of current. Theoretically, the OCV should approach the cell E.M.F. In practice, however, the OCV is substantially lower than the cell E.M.F., due to various potential losses. This energy loss is often referred to as over-potential, or the difference between the potential under

equilibrium conditions and the actual potential. The main application of thermodynamic calculations is to identify the size and nature of energy losses (Logan et al., 2006b).

2.10 Applications of Microbial Fuel Cell

Microbial fuels cells is an important application in wastewater treatment. The organic carbon waste can be removed, and electricity is produced. Industries that produce wastewaters high in easily degradable organic carbon are good candidates for this application. Examples are food industry, dairies, breweries, the bio-products industry, and the bio-fuels industry, such as bio-refineries.

a) Brewery Wastewater Treatment

Brewery and food manufacturing wastewater can be treated by microbial fuel cells because their wastewater is rich in organic compounds that can serve as food for the microorganisms. Breweries are ideal for the implementation of microbial fuel cells, as their wastewater composition is always the same; these constant conditions allow bacteria to adapt and become more efficient. The power generated from cleaning the brewery wastewater is expected to pay for the initial cost of the MFC in ten years.

b) Sewage Treatment

Sewage wastewater can also be converted via microbial fuel cells to decompose the waste organic material. Microorganisms can perform the dual duty of degrading effluents and generating power. MFCs are presently under serious consideration as devices to produce electrical power in the course of treatment of industrial, agricultural, and municipal wastewater. When micro-organisms oxidize organic compounds present in waste water, electrons are released yielding a steady source of electrical current. If power generation in these systems can be increased, MFCs

may provide a new method to offset operating costs of waste water treatment plants, making advanced waste water treatment more affordable in both developing and industrialized nations. In addition, MFCs are also known to generate less excess sludge as compared to the aerobic treatment process.

c) Desalination

Desalination of sea water and brackish water for use as drinking water has always presented significant problems because of the amount of energy required to remove the dissolved salts from the water. By using an adapted microbial fuel cell, this process could proceed with no external electrical energy input. By adding a third chamber in between the two electrodes of a standard MFC and filling it with sea water, the cell's positive and negative electrodes attract the positive and negative salt ions in the water and, using semi-permeable membranes, filters out the salt from the sea water.

d) Hydrogen Production

Hydrogen production by MFCs operating on organic waste may be an interesting alternative. In such devices, anaerobic conditions are maintained in the cathode chamber and additional voltage of around 0.25 V is applied to the cathode. Under such conditions, protons are reduced to hydrogen on the cathode. Such modified MFCs are termed bio-electrochemically assisted microbial reactors (BEAMR).

e) Remote Sensors

MFCs can run low-power sensors that collect data from remote areas. A simple microbial fuel cell consisting of a cathode attached to an anode by a metal wire. By placing the anode in the anaerobic sediment of a river or ocean and placing the cathode in the aerobic water right above the sediment, a current is generated. Anaerobic bacteria that naturally grow in the sediment produce the small current

that can be used to charge a capacitor to store energy for whenever the sensor needs it. One major advantage of using a microbial fuel cell in remote sensing rather than a traditional battery is that the bacteria reproduce, giving the MFC a significantly longer lifetime than traditional batteries. The sensor can thus be left alone in a remote area for many years without maintenance.

f) Cleansing Polluted Lakes and Rivers

Microbial fuel cells can also be used in the bioremediation of water containing organic pollutants such as toluene and benzene, compounds found in gasoline. The MFC design is altered so that the fuel cell floats on top of polluted water. The anode is submerged in the water where organic pollutants feed the bacteria while the cathode floats on top of the water. The organic pollutants are decomposed to carbon dioxide and water, cleansing the polluted lake or stream. The MFC can be left alone in remote natural bodies of water, just like the remote sensor.

g) Remote Power Source

The advancement of microbial fuel cell technologies provides cheap, accessible power to remote regions of Africa, where 74% of the population lives without electricity. The electrical current produced by a simple homemade MFC is enough to recharge a cell phone battery, an important communication and lighting tool to rural African communities. The materials required to construct this simple MFC are soil, manure, copper wire, buckets, and graphite cloth.

h) Generation of Energy out of Bio-waste/ Organic Matter

Electricity is being generated in a direct way from bio-wastes and organic matter. This energy can be used for operation of the waste treatment plant, or sold to the energy market. Furthermore, the generated current can be used to produce

hydrogen gas. Since waste flows are often variable, a temporary storage of the energy in the form of hydrogen, as a buffer, can be desirable.

i) Direct Conversion of Substrate Energy to Electricity

Application of microbial fuel cells (MFCs) to wastewater treatment for direct recovery of electric energy appears to provide a potentially attractive alternative to traditional treatment processes, in an optic of costs reduction, and tapping of sustainable energy sources that characterizes current trends in technology.

j) Sludge production

A two-chambered microbial fuel cell (MFC) with potassium ferricyanide as its electron acceptor was utilized to degrade excess sewage sludge and to generate electricity. This study demonstrates that this MFC can generate electricity from sewage sludge over a wide range of process parameters.

k) Omission of gas treatment

Generally, off-gases of anaerobic processes contain high concentrations of nitrogen gas, hydrogen sulphide and carbon dioxide next to the desired hydrogen or methane gas. The off gases of MFCs have generally no economic value, since the energy contained in the substrate was prior directed towards the anode. The separation has been done by the bacteria, draining off the energy of the compounds towards the anode in the form of electrons. The gas generated by the anode compartment can hence be discharged, provided that no large quantities of H_2S or other odorous compounds are present in the gas, and no aerosols with undesired bacteria are liberated into the environment.

l) Microbial Fuel Cells for Robotics

For a robot to behave truly autonomously it will need not only to use its energy in an effective way but also extract this energy from its environment. This requires

the robot to convert energy from natural raw materials and also deal with replenishing reserves and waste management. Based on this technology the EcoBot-I, EcoBot-II and EcoBot-III have been developed, which to some extent exhibit this type of behaviour.

EcoBot-I, which was developed in 2002, employed *E. coli* and was fed with sugar. EcoBot-II, which was developed in 2004 used sludge microbes and was fed (amongst other substrates) with dead insects and food waste. EcoBot III developed in 2007 is a lightweight(6kg) self-sustaining robot designed to clean wastewater. Powered by MFCs, the robot runs off human waste, using it to produce electricity necessary for performing its cleaning task. The robot is consists of the ingestion, artificial digestion and solid waste excretion mechanism. The project was funded by Engineering and Physical Sciences Research Council (EPSRC) and developed in collaboration between Wessex Water and the Bristol Robotics Laboratory in England.

2.11 Pig Farming and Waste Management

Pig farming is highly profitable and thus a popular choice of livestock production in some parts of Nigeria. Pig meat (pork) is a good source of animal protein, skin, fat and provides materials used for clothing, ingredients for processed foods, cosmetics and other medical uses (Abiolaet al., 2015). Ajala and Osuhor (2004) reported pig farming as a means to generate the country's GDP and also combat malnutrition of animal protein. With this and many other publications enlightening people on the benefits and profitability of pig farming, the State has experienced a major hike in the production of pigs, over the past five years. Despite its profitability, there is a major problem associated with pig farming, that is waste management, Industrial pig farming however poses numerous threats to the environment and human health as pig wastes and feaces often spread to surrounding neighbourhoods, polluting the air and water with toxic waste particles

(FAO, 2013; Wendee, 2013). Attempts have been made by industrial pig farmers to device means of managing these wastes but apart from using the waste as manure and burying the waste, small scale pig farmers have little choice but to locate their farms in remote areas far from urban residence in an effort to reduce the felt effects of the waste on the environment and human health while taking on considerable risk themselves.

The most effective way to manage waste is by recycling. Countries like Britain and Singapore make use of methane gas generated from swine waste to power farm machineries, but that is not the norm in Nigeria. Pig waste in rivers state is stored in ditches and lagoons, it is then covered and left to decompose and integrate with the soil, while this may seem to be an effective method of pig waste disposal, a lot of dangers are associated with this system. Waste from these farms have the potential to carry pathogens, bacteria and heavy metals that are toxic when ingested (Wendee, 2017). Pig wastes also contribute to ground water pollution in form of ground water seepage and waste spray, the contents of this spray causes mucosal irritation, respiratory ailment, increased stress and also high blood pressure (Hortonet al., 2009).Swine waste also has effects on water quality as well as air quality, toxic elements like nitrates and ammonia seep into the water table close to the base of the ditches and lagoons and contaminates the ground water (Warrick&Stith,1995). More so, studies have shown that people living close to pig farms suffer a variety of adverse health effects, including respiratory diseases, infections, increased risk of cancer and other health risks due to air pollution from pig farms (Environmental Health Perspectives 2016). Adequate management of these wastes would contribute to the creation of piggery resources and the improvement of commercial pork production, thus increasing the profitability of pig farming. Most piggery farms have developed a series of management strategies to dispose of their waste in a way that reduces their environmental impact. A study showed that about 65% of farms sell, dispose of or burn piggery waste while 14.17% recycled waste (as manure) for crop production. However, this could become an environmental problem when manure is applied to

land beyond the threshold of the host crop and its ability to use nutrients (Charles, 2008). The results also revealed that about 5.0% of the farms buried the waste in the ground, which could lead to groundwater contamination (Carr, 1994). While the burning of piggery waste causes air pollution that may pose a risk to humans and livestock (Anon, 2005), flushing of piggery wastes in form of slurry into nearby pits, streams and rivers may have adverse effects on human and aquatic life and the environment. Flushing can also result in a reduction in the amount of dissolved oxygen and high water turbidity. This often threatens the natural habitats of many organisms in nearby water bodies. Huge amounts of organic and inorganic nutrients released as slurry are capable of permanently distorting the aquatic ecosystem. The results also showed that none of the farms had an environmentally friendly piggery waste management system that could completely limit the effect of the smell generated by the waste. This implies widespread pollution of the air, water and soil, as well as risks to the health of human and animal life in the localities. However, in order to provide a timely and inexpensive piggery waste management option, farms should incorporate crop farms such as vegetable farms, tree farms and / or orchards where they could occasionally spread pig waste as manure to keep the soil fertile. Moreover, this will equally help to prevent accumulation of waste in order to reduce the degree of environmental pollution in the neighbourhood.

2.12 Environmental Impact of Pig Farming

The environmental impact of pig farming is mainly driven by the spread of faeces and waste to surrounding neighbourhoods, polluting air and water with toxic waste particles. *Nicole.*,(*2017)* reported that waste from pig farms can carry pathogens, bacteria (often antibiotic resistant), and heavy metals that can be toxic when ingested. Pig waste also contributes to groundwater pollution in the forms of groundwater seepage and waste spray into neighbouring areas with sprinklers. The contents in the spray and waste drift have been shown to cause mucosal irritation (Wing & Wolf., 2017), respiratory ailment (Thorne, 2017*)*, increased stress (*Schiffmanet al., 1995)*, decreased

quality of life (*Bullers, 2005),* and higher blood pressure (*Horton et al., 2009).* This form of waste disposal is an attempt for factory farms to be cost efficient. The environmental degradation resulting from pig farming according to *Edwards,(2005).,* presents an environmental injustice problem, since the communities do not receive any benefit from the operations, and instead, suffer negative externalities, such as pollution and health problems. The United States Agriculture and Consumer Health Department has stated that the "main direct environmental impact of pig production is related to the manure produced (FAO's, *2017).*

Burkholder et al,(2007) reported that many intensive pig farms store the swine waste in vats often referred to as lagoons. These lagoons often contain pathogens such as salmonella, pharmaceuticals like antibiotics and antimicrobials, as well as nitrogen and phosphorus. This can lead to widespread pollution within the watershed the farm is located within, if the water from these lagoons leaches out into the soil and trickles down into the water table beneath. Unlike human sewage, which is always treated with chemical and mechanical filtration, the waste from these lagoons is untreated when it is released back to the environment. Spills according to WarrickandStith,(1995) are the most common contributor to pollution, but regardless of spills, toxic nutrients like nitrates and ammonia can seep into the water table located just below the surface, infecting the groundwater that nearby communities drink. It has been estimated that 35,000 miles of river across over 20 states has been contaminated by manure leakage. Some of the causes for the environmental problems are inadequate sewage treatment and lack of developing technologies. Many farms lack adequate wastewater treatment systems, which release untreated wastewater into the environment in the form of contamination (*Johnson.,1991).* Some spills and leakage of contaminated waste are not accidental. In 2014, Mark Devries used spy drones to expose pig farms in North Carolina that were spraying untreated faecal waste into the surrounding areas, allowing the waste to dissipate into far-off communities (Devries, 2017). Smithfield Foods, the company responsible for one such factory, claimed this was a tactic used to fertilize its fields. It is true that historically hog faeces have been used as fertilizer and can be done safely and without

runoff, but the magnitude was described by Dan Whittle, a former senior policy associate at the North Carolina Department of Environment and Natural Resources, as a "mass imbalance", with far too great a magnitude of faecal matter being sprayed for the crops being generated to not have significant spill off into neighbouring plots of land (Devries, 2017). Many residents of the surrounding areas of such farms complain that the industrially concentrated faecal matter creates an unbearable odour of a different magnitude than typical farm manure.

Communities located near factory pig farms experience negative health and environmental effects due to several factors associated with industrial pig farming. One main issue that arises out of intensive animal agriculture is the waste that the huge number of animals produce. Pig waste is similar to human waste; filled with bacteria and high amounts of ammonia. At most intensive pig farms, hog waste is kept in large open-air pits called lagoons where waste is broken down by anaerobic bacteria and then sprayed onto crops as fertilizer. This is called the lagoon and sprayfield system and remains legal in the United States, including in states like North Carolina, where there have been on-going efforts in the NC legislature to ban open-air lagoon and sprayfield system practices in the state and replace these with more environmentally sound waste management practices (*Buford, 2018).* The waste then reaches neighbouring towns, resulting in civilians not being able to even leave their house in order to avoid pig waste filled air. People living in nearby towns have suffered a variety of adverse health effects including respiratory diseases, infections, increased risk of cancer, and other health risks (*Wing et al., 2013).* The nitrogen from pig waste can also contribute to acid rain in the local areas; team of scientists from the US Agricultural Research Service and the US Department of the Environment has examined and noted that within wastewater lagoons in North and South Carolina, there are a host of genes involved in the process of turning ammonia into nitrogen (*Evans, 2017).*

One case study, conducted by Environmental Health Perspectives, sought to prove that malodour and pollutant concentrations from swine operations are associated with stress, altered mood, and

increased blood pressure. For two weeks, adult volunteers living near swine operations in North Carolina sat outside for ten minutes twice a day. They reported levels of hog odour, and recorded their blood pressure. The study found that like noise and other similar environmental stressors, the malodours from the swine operations were likely associated with an increase in blood pressure, which could contribute to an increase in chronic hypertension (*Wing et al., 2013).*

There are many documented incidences of disease outbreaks occurring due to the presence of pig farms in a given community, particularly industrial pig farms. MRSA (Methicillin-resistant *Staphylococcus aureus*, a type of antibiotic resistant bacteria) outbreaks have been correlated to an individual working in a pig farm, likely attributed to the strong antibiotics often used in industrialized pig farms (*Huijsdenset al., 2006).* Other diseases can also spread in pig farms such as *Salmonella*,Toxoplasma, and *Campylobacter* (*Jensen et al., 2006).* Many of these diseases are preventable given proper safety precautions such as washing hands and clothes, wearing face masks, and covering any open wounds when coming into contact with pigs. Improvements in farmer education about diseases are often cited as the reason for the lack of increase in disease outbreaks in North Carolina despite an increase in pig population by a factor of four in the years leading up to 1998 (*Chagan., 2016).*

2.13 Public Health and Environmental Challenges of Pig Production

Paluszaket al.,(2012) reported that pathogenic microorganisms are found in considerable amounts in slurry used for agricultural purposes and wastewater emanating from pig farms. The microbial pathogens most often isolated from slurry are bacteria of the genus Salmonella which still remains one of the main causes of food poisonings in humans. The malodorous nature of pig effluent did not prevent it from finding appreciable applications in agricultural practices, especially fish farming. Soil and fish farms intensively fertilized with improperly treated slurry may constitute a highly significant link in the transmission of Salmonella bacilli to water and field crops. Getting

into surface and ground waters, they can pose a direct ethiological factor of salmonelloses in humans, particularly if this phenomenon applies to household intakes of drinking water.

However, large quantities of diverse veterinary antibiotics are widely used as additives in food or water at concentrated animal feeding operations to prevent and treat animal disease outbreaks due to their prophylactic and therapeutic qualities, as well as to promote animal growth (Carlson &Fangman, 2000; Union of Concerned Scientists, 2001; Dipeolu&Alonge, 2002). In some cases, antibiotics are used to make up for poor production practices. Most of the antibiotics used in animal feeds are the same as or belong to the same classes as those used in humans, including, tetracyclines, macrolides, bacitracin, penicillins and sulphonamides (APUA, 2010). Bacteria in animals (as in humans) are able to develop antibiotic resistance when exposed to low doses of drugs over a long period of time, hence contributing to rise of pathogens that are able to defeat our shared antibiotic arsenal (Union of Concerned Scientists, 2001; Schneider & Garrett, 2009; APUA, 2010). Antimicrobials can be administered to pigs through feed, water, and injection. Sows and suckling piglets are more often treated with injectable antimicrobials while nursery and grow-finish pigs more often are administered antimicrobials as groups through feed or water (Union of Concerned Scientists, 2001; Schneider & Garrett, 2009).

According to Union of Concerned Scientists (2001)., an estimated 70 percent of all antibiotics sold in the United States are used on healthy livestock for non –therapeutic purposes, which could increase to about 84 percent when all agricultural uses of antibiotics are considered. In many developing countries, the scenario is expected to soar above this figure. Moreover, use of antibiotics in the treatment of swine and other livestock in most farms in Nigeria, according to Erskine et al., 2002; Nwiyi, 2014), is done by the farmers, hence there is consistent over-dosage of antimicrobials to the pigs as the manufacture's guide was not complied with. However, these antibiotics pass through animal bodies and are commonly excreted in urine, faeces and manure as parent compounds, conjugates, or oxidation and hydrolysis by-products (Tolls, 2001). The animal wastes are discharged to anaerobic lagoons for biological treatment and temporary storage.

However, DaughtonandTernes (1999) recorded that many antibiotics are not amenable to biodegradation and accumulate in the lagoons. In places where it is practiced, the lagoons can act as reservoirs of various antibiotics and subsequently, a portion of lagoon bacteria may develop strong resistance to these antibiotics. Unfortunately, some farms in Nigeria do not treat their wastes and wastewaters, but discharge them directly on land or into nearby water bodies. Seepage and runoff of the lagoon wastewater and farm application of the lagoon sediments as fertilizer may lead to the contamination of both surface and ground water with antibiotics and antibiotic resistant bacteria, thus posing a severe threat to public health (Chee-Sanford et al., 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of sample locations

The piggery wastewater that was used for this study was collected from a commercial pig farm at UmualumNekede, Owerri West Local Government Area, Imo State, Nigeria with coordinates, 5^{o}26'48.5"N 7^{o}01'24.5"E. There are about 25 such farms at this location. Each of these farms produce an average of 125 sows, 75 hogs and 200 boars annually. Regrettably, these farms operate without suitable wastewater treatment systems, and this made them to channel the wastewaters directly into a nearby river through cemented gutters, which also serves as source of water for some domestic activities to residents downstream.

3.2 Sterilization of Sample Containers

All the plastic containers used for collection of sample and construction of MFC chambers were purchased and surface sterilized with sodium hypochlorite, absolute ethanol and distilled water. Sterilization of materials was carried out based on the methods by Yee et al (1998), which was done by adding a litre of sodium hypochlorite solution to the containers and shaking them for 30 minutes, followed by subsequent washing with absolute ethanol. The containers were further rinsed three times by shaking with a quantity of sterilized distilled water for 10 minutes in each occasion.

3.3 Collection of Samples

The procedure for collection of samples for physicochemical, microbial fuel cell and microbiological analysis was as described by Ikotunet al (2012) and Singh et al (2012). The surface sterilized 20 litre plastic container, was first rinsed thrice with the piggery wastewater, and the sample was collected from the drain pipes as it flushed out from the pig farm into the cemented gutters through which it enters into the river. Two sterile sample bottles were rinsed with the collected piggery wastewater in the 20 litre container and then filled to the brim. The samples were labelled properly and within an hour transported to the laboratory for physicochemical and microbial analyses.

Similarly, at the end of the 25 days period of treatment using microbial fuel cells, treated samples from each of the thirty MFCs, as well as an untreated sample that was used as control were carefully collected using sterile sample bottles and analyzedphysicochemically. However, the samples for subsequent microbial analysis were collected by aseptically removing the anode of each MFC and using a sterile swap stick to scrape the biofilm on their surfaces into sterilized peptone water contained in different sterile sample bottles. This was done to determine the physiochemical parameters of the treated wastewater and persistent microbes at the end of the treatment.

3.4 Measurement of Physicochemical Parameters of Samples

The physicochemical parameters of the wastewater samples before and after treatment, were determined at Anthony Van Leeuwenhoek Research Centre, UmuomaNekede inOwerri West Local Government Area, Imo State, Nigeria, following procedures as prescribed by standard methods.

pH, and total dissolved solid (TDS), were measured using Hanna Instrument for pH, TDS and Temperature (Model No.: HI9811-5). Dissolved oxygen (DO), was measured using Dissolved

Oxygen meter by LT. Luton (Model No.: DO-5509). Concentrations of ammonia (NH_4^+), phosphate (PO_4^{2-}) and nitrate (NO_3^+) were determined using Hanna COD and multiparameter photometer (Model No.: HI83099).

Plate 3.1: Pictures showing the channels through which untreated pig wastewater are discharged from the farm to a nearby river

(A) Chemical Oxygen Demand (COD)

The method used was the dichromate method where oxidizable organic matter in the wastewater reduced the dichromate (ion) present in the chromic ion (green) reagent. The quantity of organic matter oxidized was determined by the amount of chromic ions formed. 0.2ml of the wastewater sample was added to a vial containing the dichromate reagent, on the other hand, 0.2ml of distilled water was added to another reagent vial which was the blank. The caps were replaced and the vials gently inverted for a couple of times and were placed into the reactor and heated for 2 hours at 150^{o}C. At the end of the digestion period, the reactor was switched off and allowed to cool to about 120^{o}C. The vials were gently inverted several times while still warm and then allowed to cool to room temperature. The blank vial was then placed in the cuvette holder of the Hanna COD-Multiparameter photometer(Model No.: HI83099) to zero the instrument. After which the sample vial was placed in the cuvette and measurement immediately recorded in mg/l.

(B) Biochemical Oxygen Demand (BOD_5)

BOD_5 was determined by dilution method. Also dissolved oxygen (DO) concentration of the wastewater sample was measured before and after an incubation period of 5 days following the correct dilution. 3ml of wastewater sample was placed into a 300ml of incubation bottle and then diluted with dilution water to fill the bottle.

The incubation bottle was covered and then sealed with masking tape and stored in the dark at 20^{o}C for 5 days to prevent the production of oxygen through photosynthesis. BOD_5 was calculated using the formular;

BOD_5 (mg/L) = $(DO_0 – DO_5)P$,

where DO_0 is the dissolved oxygen on day zero (0), DO_5 is the dissolved oxygen on day 5 and P is the decimal dilution factor.

3.5 Preparation of Media and Diluents

Nutrient Agar (NA), Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA) Eosin Methylene Blue Agar (EMBA) were prepared according to manufacturer’s guide. Physiological saline used as diluents was prepared by dissolving 9.8 g of sodium chloride in 1000 ml of distilled

water and dispensed in 9 ml portions. Both diluents and media were sterilized in an autoclave at 121^0C for 15mins (Cheesbrough, 2000).

3.6 Preparation of Samples and Inoculation

Ten millilitre of pig wastes (slurry) were serially diluted in 90 ml of sterile physiological saline and swirled to mix thoroughly. Further dilution was done by transferring I ml into 9 ml diluent until the desired dilution was obtained. Aliquot portion (0.1ml) of appropriate dilution was inoculated into the pre-sterilized and surface dried media. Inocula were spread evenly using an L-shaped glass spreader to ensure uniform and countable colonies. Plates were incubated at ambient temperature for 24-48 hours for visible colonies.

3.7 Determination of microbial population

In order to understand if piggery wastewater was treated, the microbial load of the water was determined. Colony counts that were obtained on the media were counted and expressed as colony forming units per mil (CFU/ml) of the total population. CFU/ml was calculated as:

$$\text{CFU/ml} = \frac{N}{V} \text{ x } \frac{D}{1}$$

Where N is the number of colonies counted, D is the dilution factor and V is the volume plated (0.1 ml).

3.8 Characterization and Identification of Microbial Isolates

Microbial isolates were characterized based on cultural / colonial, microscopic and biochemical methods with reference to standard manuals. The identities of the isolates were cross-matched with reference to standard manuals for the identification of bacteria (Harrigan&McCance, 1990; Buchanan & Gibbon, 2000).

A. Gram Staining Test

The Gram staining technique was used for the bacterial isolates as described by Cheesbrough (2000). A smear of the isolate was made on grease free glass slide with a drop of water and allowed to dry. The smear was fixed by mild heating, flooded with crystal violet and allowed to stand for 30 seconds. The crystal violet was rinsed off with water; Lugol's iodine was added and allowed to stand for 30 seconds. This was washed off with water and acid alcohol, till discoloration. It was counter stained with Safranin for 10 seconds and rinsed with water. The wet slide was allowed to air dry. A drop of oil immersion was added on the slide and viewed using X100 objective lens of the microscope.

3.9Biochemical Characterization of Bacteria Isolates

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to few biochemical tests described by Cheesbrough (2000) and Beishir (1987).

A. Catalase Test

The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria. Catalase has one of the highest turnover numbers of all enzymes such that one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second. Catalase activity can be detected by adding the substrate H_2O_2 to an appropriately incubated (18-24 hours) tryptic soy agar slant culture. Organisms which produce the enzyme breakdown the hydrogen and the resulting O_2 production produces bubbles in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to breakdown peroxide into O_2 and water and are catalase negative.

B. **Coagulase Test**

Coagulase is enzymes that clot blood plasma by a mechanism that is similar to normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease causing bacteria of humans that produce coagulase are *Staphylococcus aureus.* Thus this enzyme is a good indicator of *S. aureus.* In the test, the sample was added to rabbit plasma and held at 37^0C for a specified period of time. Formation of clot within four hours is indicated as positive result and indicative of a virulent *Staphylococcus aureus*strain. The absence of coagulation after 24 hours of incubation is a negative result indicative of an avirulent strain.

C. Oxidase Test

Oxidase test is an important differential procedure that should b performed on all gram negative bacteria for their rapid identification. The test depends on the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and ∞-naphthol. This method uses N, N-dimethyl-p-phenylenediamine oxalate in which all Staphlococci are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram negative bacteria) the N, N-dimethhyl-p-phenylenediamine oxalate and ∞-naphthol react to indophenol blue. *Pseudomonas aeruginosa*is an oxidase positive organism.

D. Sugar Fermentation/Oxidation

This test is used to differentiate between bacteria groups that oxidize carbohydrate such as members of Enterobacteriaceae. One milliliter (1ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes containing 9ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two uninoculated tubes serve as control. Vaseline was used to cover one set of the duplicate tubes, one control to discourage

oxidative utilization of sugar. All tubes were incubated at 37^{o}C for 48h. After the incubation, they were observed for acid production in the culture. Yellow colouration indicates acid production in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction.

E. Hydrogen Sulphide Production (H_2s) Test

The test isolates were aseptically inoculated into a tube containing triple sugar iron agar started by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37^{o}C for 72 hrs and was examined daily. Black precipitation and yellow colouration was checked for. Black precipitate indicates H_2S production and yellow colouration for sucrose, lactose and glucose fermentation.

F. Urease Test

Urease Agar slant in McCartney bottle was inoculated with the bacteria isolate at 30^{o}C for 4 hrs and then overnight. A pink colour in the medium indicated a positive result.

G. ImvicTEST

This test consists of four different test; they are Indole production, Methyl-Red test, Voges-Proskaeur test and Citrate utilization test. This test is specifically designed to determine the physiological properties of microorganism. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

H. Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 hrs.

At the end of incubation period, 3 drops of kovac'sreagents was added and then shaken. A red colour ring at the interface of the medium denotes a positive result.

Methyl red and Voges-Proskaeur test must be considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+.

Methyl red test was preformed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red positive organisms produce a red colouration when five drops of methyl-red indicator is added into 48 h old MR-VP broth culture.

The Voges-Proskaeur test demonstrates the ability of organisms to produce acetoin from glucose metabolism. Some organisms metabolize glucose to produce pyruvic acid which is further broken down to yield Butane-diol and acetyl-methyl carbinol as an intermediate product.

Into one milliliter of the culture add one milliliter of six percent alcoholic solution of alpha-naphtol and one milliliter of 16% KOH and stand for 15-20 minutes. Development of red to pink colour is a positive test.

I. Citrate Utilization Test

This is one of the several techniques used to assist in the identification of enterobacteria. Principle of the test is based on the ability of an organism to use citrate as its only source of carbon. The test was carried out using Simmons citrate agar.

The slopes of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to the slope with a saline suspension of the test organisms before stabbing the butt. The bottles are incubated at 35^{o}C for 48 h. Bright blue colours in the medium

means positive test while no change in colour of medium indicates negative citrate test (Cheesbrough, 2000).

3.10 Construction of Microbial Fuel Cell

A total of thirty units of microbial fuel cells were constructed, each consisting of separate cathode and anode chambers made of plastic material with tight lids. Out of a total of 60 electrodes that were used to set up the 30 units of two chambers MFCs, 16 pieces (8 cathodes and 8 anodes) corresponded to surface area of $0.005m^2$, 28 pieces (14 cathodes and 14 anodes) corresponded to surface area of $0.010m^2$ and 16 pieces (8 cathodes and 8 anodes) corresponded to surface area of $0.015m^2$.

Table 3.1: Experimental design using Box Behnken Design (Minitab 17)®

Runs Setups	Anode Surface Area (m^2)	Cathode Surface Area (m^2)	Anode Volume (ml)
1	0.005	0.005	1125
2	0.015	0.005	1125
3	0.005	0.015	1125
4	0.015	0.015	1125
5	0.005	0.010	750
6	0.015	0.010	750
7	0.005	0.010	1500
8	0.015	0.010	1500
9	0.010	0.005	750
10	0.010	0.015	750
11	0.010	0.005	1500
12	0.010	0.015	1500

13	0.010	0.010	1125
14	0.010	0.010	1125
15	0.010	0.010	1125
16	0.005	0.005	1125
17	0.015	0.005	1125
18	0.005	0.015	1125
19	0.015	0.015	1125
20	0.005	0.010	750
21	0.015	0.010	750
22	0.005	0.010	1500
23	0.015	0.010	1500
24	0.010	0.005	750
25	0.010	0.015	750
26	0.010	0.005	1500
27	0.010	0.015	1500
28	0.010	0.010	1125
29	0.010	0.010	1125
30	0.010	0.010	1125

All the carbon brush electrodes were pre-treated by soaking in 100% ethanol for 30 min and then in 1m HCl for 1hr before storage in distilled water until used. The H –type double chamber configuration was adopted as described by JambeckandDamiano (2010).

Plate 3.2: Plastic materials used in the construction of the MFCs

a. Cathode and Anode Chambers

Sixty plastic containers, purchased from the market were used as anode and cathode chambers. On the lids of the containers, provision was also made for the passage of 1mm wire connecting the electrodes in both chambers to the multimeter outside. After passage of the connector, the hole was sealed with Zuma PVC gum.

b. Proton Exchange Membrane

Nafion N117 was used as the proton exchange membrane. The measurement is 10cm by 10cm (small) and 15cm by 15cm (big). It was cut 3.5cm each and pre-treated by boiling in 6 mls hydrogen peroxide (H_2O_2, 3% v/v) for 1 hr at 80 ^{O}C and it was further rinsed with deionized water. This was followed by heating it in 1 M of H_2SO_4, at 80 ^{O}C for 1hr. After this, it was rinsed again by heating with deionized water for 1 hr, then it was decanted. The membranes were preserved

finally in deionized water prior to use, this was done in other to prevent membrane swelling when placed in the MFC compartments.

c. Electrodes and Electron Acceptors

Aluminium with different surface areas of 0.005, 0.0015 and 0.01sqm, lengths of 0.062, 0.09 and 0.09m^2, and width of 0.005, and height of 0.04, 0.083 and 0.055m^2 respectively each were used as the electrodes. Water was used as the electron acceptor to further investigate its reported suitability as a cheaper electron acceptor in microbial fuel cell.

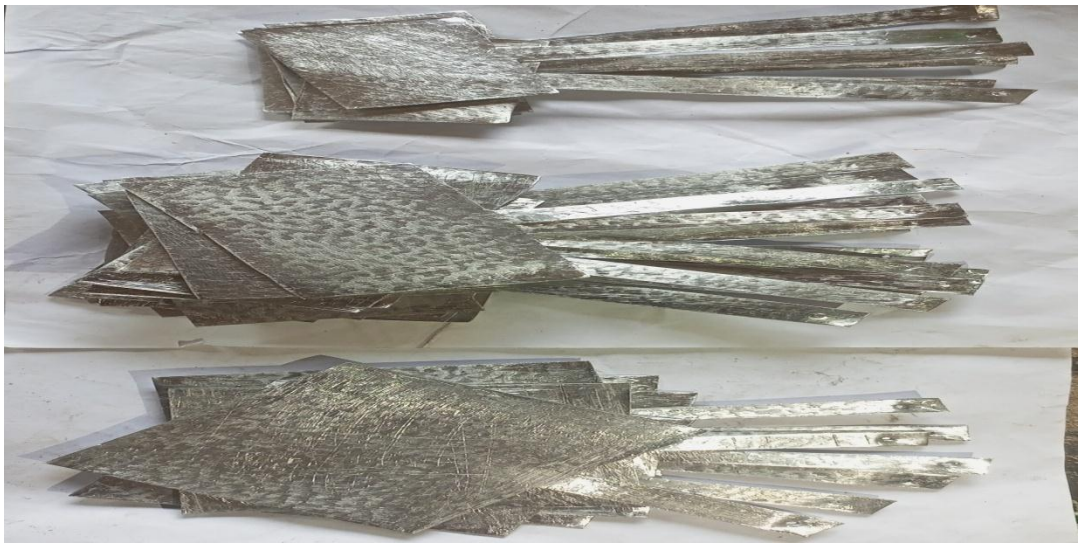


Plate 3.3: Electrodes used for the MFCs

3.11 Loading of MFCs and Measurement of Voltage

After surface sterilization of the plastic containers that was used as MFC chambers, they were connected to each other with 3cm Pvc pipe and adaptor with a diameter of 3cm and Nafion N117 placed in between and then tightly corked to prevent leakage. It was also further sealed with zumapvc gum. Using a measuring cylinder, different volumes 750ml, 1125ml and 1500ml of piggery wastewater sample was introduced into the various anode Chambers of the MFCs, while 1000ml of water which was the electron acceptor was dispensed into each of the cathode chambers of the MFCs respectively. The chambers were tightly corked and the circuits were completed by connecting 1mm copper wires of length 20cm each to the electrodes and then connected to the

digital multimeter. The setups were allowed to stabilize for 24 hours before the voltage generated was read as shown on the digital multimeters. In taking the readings, the open circuit voltage (OCV), was recorded first without an external resistor connected to the circuit, and then 1000Ω and 5000Ω resistors were in turn connected in parallel to the digital multimeters and voltage was recorded when the readings stabilized (Momoh&Neayor,2010). Readings were taken at 6.00 am and 6.00 pm; and the MFCs were allowed to run for 25days.

Plate 3.4: Voltage recorded on H-type, double chamber microbial fuel cell

3.12: Optimization of MFC

At the end of 25 days, average voltage recorded across 10kΩ resistors was calculated and used to determine the optimum factors. These factors were then used to set up another batch of MFCs, in

quadruplicates. In taking the readings, the open circuit voltage (OCV), was recorded first without an external resistor connected to the circuit, and then 100kΩ, 50kΩ and 25kΩ resistors were in turn connected in parallel to the digital multimeters and voltage was recorded when the readings stabilized (Momoh & Neayor,2010). Readings were taken at 6.00 am and 6.00 pm; and the MFCs were allowed to run for 25days.

Average voltage generated was recorded, and compared to estimated and that of un-optimized MFCs.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Physicochemical Analysis

The physicochemical analysis was done to understand the effects of different physicochemical factors on electricity generation. The result of the physicochemical analysis of the samples before and after 25 days period of treatment using microbial fuel cell constructed with aluminium electrodes, and using water as catholyte are as shown in Figure 4.1. Generally, there was an increase in two (pH and TDS) parameters of all the wastewater samples analyzed after treatment using MFCs. However, marked decline in Phosphate, Ammonia and Nitrate concentrations were recorded due to the fact that microorganisms used up the nitrogen and phosphorus sources as nutrient to grow. Biochemical Oxygen Demand, and Chemical Oxygen Demand components of the wastewater samples also recorded a decline because not much oxygen was utilized anymore, and for some of the organisms, the environment was not condusive for them to grow. The Total Dissolved Solids generally recorded slight increase as a result of more debris in the wastewater caused by dead microorganisms. Moreover, when the values of the parameters for the treated wastewaters were compared to those of the untreated (control) sample, it was evident that the MFC configurations used were effective in treating the sample due to the marked differences observed.

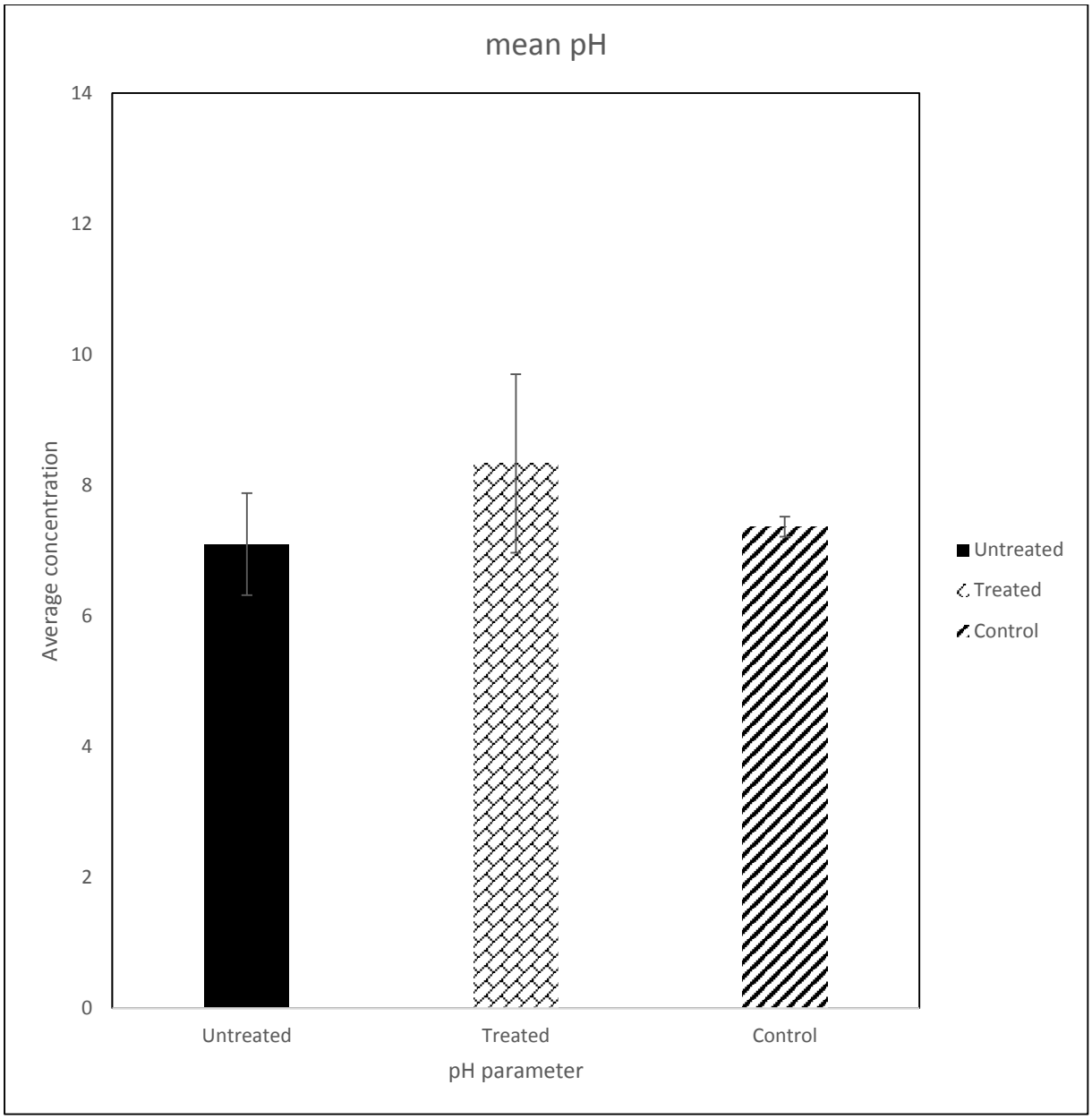


Figure. 4.1: Physicochemical analysis of samples pH before and after treatment

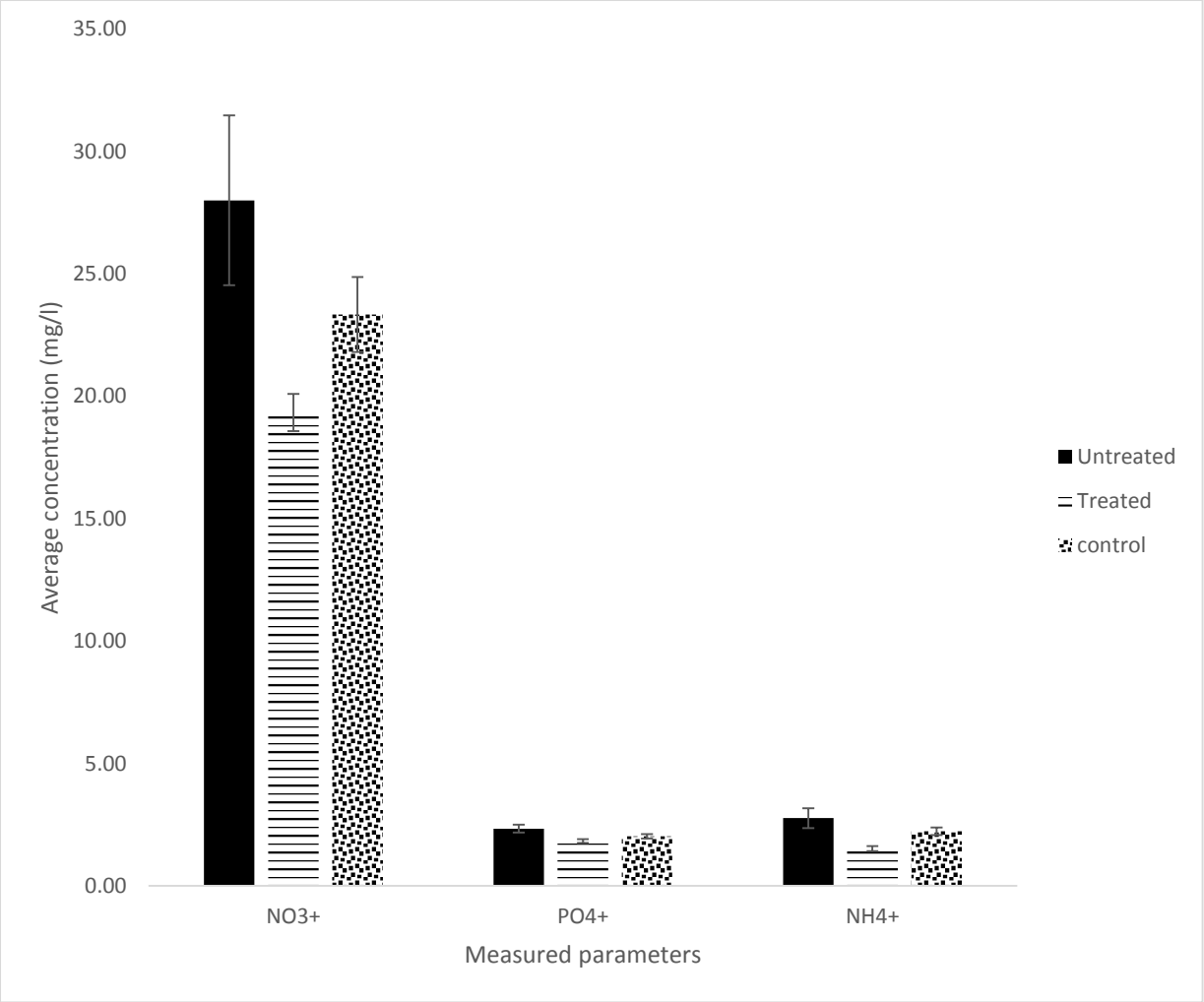


Figure. 4.2: Physicochemical analysis of samples NO_3^+, PO_4^+ and NH_4^+ before and after treatment

4.1.2: Morphological and Microscopic Description of Isolates

Results of the morphological and microscopic description of isolates from the piggery wastewater sample that was done before and after treatment with MFCs using Mannitol salt agar (MSA), Nutrient Agar (NA), Salmonella-Shigella Agar (SSA)and Eosil methylene blue Agar (EMBA) as obtained after 24 –72 hours incubation are as shown in Table 4.1. The biochemical tests employed include Gram stain, Catalase test, Methyl Red test, Oxidase test, Citrate test, Indole test and VogesProskauer test as shown in Table 4.2

Table 4.1: Morphological and Microscopic Description of Isolates

Colonial Characteristics	Gram Morphology	Most Probable bacteria
Flat wavy dull and dry cream colonies	Gram positive short rods and chains	*Bacillus* sp
Small circular moist and shiny bluish green colonies	Gram negative rods predominantly in singles, few in short chains	*Pseudomonas* sp
Round shiny small cream colonies	Gram positive cocci predominantly in chains	*Enterococcus* sp
Moist and shiny golden yellow colonies	Gram positive cocci predominantly in clusters, few in pairs and tetrads	*Staphylococcus* sp
Purple metallic sheen	Gram negative rods in singles and chains	*Escherichia coli*
Smooth circular dull and dry umbonate cream colonies	Gram positive pleomorphic rods arranged like a Chinese letter	*Corynebacterium*sp
Mucoid slimy domed pink colonies	Gram negative rods in singles and in pairs or short chains	*Klebsiella*sp
Small shiny light pink colonies	Gram negative rods in chains and singles	*Enterobacter*sp

Table 4.2: Carbohydrate and Biochemical Characteristics of Bacterial isolates

Coag	IN	MR	VP	Cit	NO_3	Ure	Cat	Glu	Suc	Lac	Mal	Fru	Mann	Oxi	Identity of isolates
-	-	+	-	+	+	-	+	+	+	+	-	+	-	-	*Bacillus* sp
-	-	+	-	+	+	-	+	+	-	+	+	-	-	-	*Bacillus* sp
+	-	+	-	-	+	-	-	+	+	+	+	+	-	-	*Staphylococcus* sp
-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	*Pseudomonas* sp
-	-	+	-	+	+	-	+	+	+	+	+	+	+	-	*Corynebacterium*sp
-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	*Enterococcus* sp
-	+	+	-	-	+	-	+	+	+	+	+	-	-	-	*Escherichia coli*
-	-	+	-	-	_	-	+	+	+	+	-	-	+	-	*Staphylococcus* sp

Cit= Citrate test; NO3= Nitrate reduction test; Ure= Urease production test; Glu= glucose; Lac= lactose; Suc= sucrose; Mal= maltose, Mann= mannitol, Fru= fructose, Oxi= Oxidase. Coag= coagulase; IN= Indole; MR= Methyl Red; VP= VogesProskaeur

4.1.3 Total counts and colonial characteristics of Bacteria Isolates

Results of the total counts and colonial characteristics of bacteria isolated from piggery waste water done in triplicates using Eosil methylene blue Agar (EMBA), Mannitol salt agar (MSA), Nutrient Agar (NA)and Salmonella-Shigella Agar (SSA)as obtained after 24 –72 hours incubation are as shown in Table 4.3 - 4.3.3 respectively. The biochemical characteristics of bacterial isolates after treatment are as shown in table 4.4.

Table 4.3: Total counts and colonial characteristics of Bacteria isolated from piggery wastes/water samples

On EMBA

Sample code	Total counts (Cfu/ml)	Colonial Characteristics	Most Probable Identity
1	No Growth	-	-
2	1.1×10^7	Purple metallic sheen	*Escherichia coli*
		Small smooth light pink colonies	*Enterobactersp*
3	No Growth	-	-

Table 4.3.1: Total counts and colonial characteristics of Bacteria isolated from piggery wastes/water samples

On MSA

Sample code	Total counts (Cfu/ml)	Colonial Characteristics	Most Probable Identity
1	5.0 x 10^4	Small circular light yellow colonies	*Staphylococcus* sp
2	2.0 x 10^4	Small circular light yellow colonies	*Staphylococcus* sp
3	No Growth	-	-

Table 4.3.2: Total counts and colonial characteristics of Bacteria isolated from piggery wastes/water samples

On NA

Sample code	Total counts (Cfu/ml)	Colonial Characteristics	Most Probable Identity
1	3.9×10^9	Mucoid slimy raised colonies	*Bacillus* sp
		Small smooth shiny cream colonies	*Enterococcus* sp
		Small circular yellow colonies	*Micrococcus* sp
2	3.4×10^9	Dull dry serrated cream colonies	*Bacillus* sp
		Small smooth shiny cream colonies	*Enterococcus* sp
		Small circular yellow colonies	*Micrococcus* sp
3	2.9×10^9	Mucoid slimy raised colonies	*Bacillus* sp
		Small smooth shiny cream colonies	*Enterococcus* sp
		Small circular golden yellow colonies	*staphylococcus* sp

Table 4.3.3: Total counts and colonial characteristics of Bacteria isolated from piggery wastes/water samples

On SSA

Sample Code	Total counts (Cfu/ml)	Colonial Characteristics	Most Probable Identity
1	No Growth	-	-
2	3.0×10^6	Small shiny fish eye colonies	*Salmonella* sp
3	No Growth	-	-

EMBA: Eosil methylene blue agar, MSA: Mannitol salt agar, NA: Nutrient agar, SSA: Salmonella shigella agar

Table 4.4: Biochemical Characteristics of Bacterial Isolates after treatment

Bacterial isolates	Cat	Oxi	Coag	In	MR	Vp	Cit	H_2S	G	S	L	M
Staphylococcus sp	+	-	+	-	-	+	-	-	+	+	+	+
Escherichia coli	+	-	-	+	+	-	-	-	+	+	+	+
*Shigella*sp	-	-	-	-	+	-	-	-	-	-	-	+
Salmonella sp	+	-	-	-	+	-	+	+	+	-	-	+
Bacillus subtilis	+	-	-	-	-	+	+	-	+	-	-	-
Enterococcus sp	-	-	-	-	+	-	+	-	+	+	+	+
Bacillus cereus	+	-	-	-	+	-	-	-	+	+	+	-

Cat= catalase; Oxi=oxidase; Coag=coagulase; In= indole; MR= methyl red; VP= VogesProskaeur; Cit= citrate, G= glucose; S= sucrose; L= lactose; M= maltose; H2S= hydrogen sulfide reduction

4.1.4: Bacterial Analysis of Wastewater and Anode Surface

Results of the bacterial analysis of untreated waste water and the swab of the surface of the electrode in the anode chamber using Eosil methylene blue Agar (EMBA), Mannitol salt agar (MSA), Nutrient Agar (NA) and Salmonella-Shigella Agar (SSA)as obtained after 24 –72 hours incubation are as shown in Table 4.5.

Table 4.5: Bacterial Analysis of Untreated Wastewater and Swab of Surfaces of Anode

Sample	Media used	Average total counts (Cfu/ml)	Most Probable Identity
Original wastewater	EMBA	$1.27x10^8$	*Escherichia coli*
			*Enterobacter*sp
			*Klebsiella*sp
	MSA	$9.75x10^7$	*Staphylococcus* sp
	NA	$1.75x10^{11}$	*Bacillus sp*
			Enterococcus sp
			Serratiasp
			Staphylococcus sp
			Pseudomonas sp
			*Corynebacterium*sp
	SSA	$3.0x10^5$	*Salmonella* sp
			*Shigella*sp
Surface of anode	EMBA	$3.67x10^6$	*Escherichia coli*
			*Enterobacter*sp
	MSA	$2.33x10^4$	*Staphylococcus* sp
	NA	3.4×10^9	*Bacillus* sp
			Enterococcus sp
			Micrococcus sp
			Staphylococcus sp
	SSA	$1.0x10^6$	*Salmonella* sp

EMBA: Eosil methylene blue agar, MSA: Mannitol salt agar, NA: Nutrient agar, SSA: Salmonella shigella agar

4.1.5 Generation of Voltage

The average open circuit voltage (OCV) generated daily across each MFC are shown on the appendix and the graphs are presented in Fig.4.4. Generally, the highest open circuit voltage observed in this study was 974 mV generated on day 11, while the lowest was 3 mV generated on day 25 of treatment. The average highest open circuit voltage generated was 360.25mV while the lowest average OCV was 226 mV.

(a)

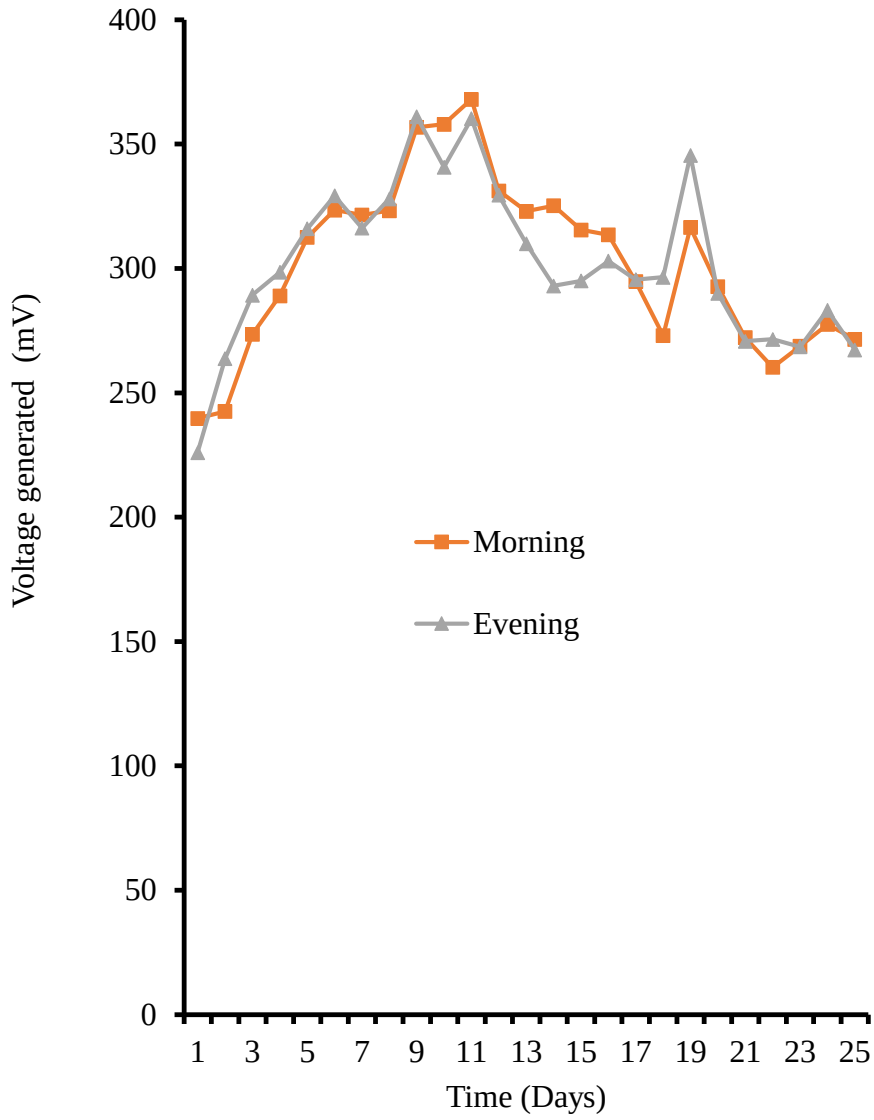


Figure 4.3: Voltage produced across open circuit voltage (OCV) by different MFCs per time.

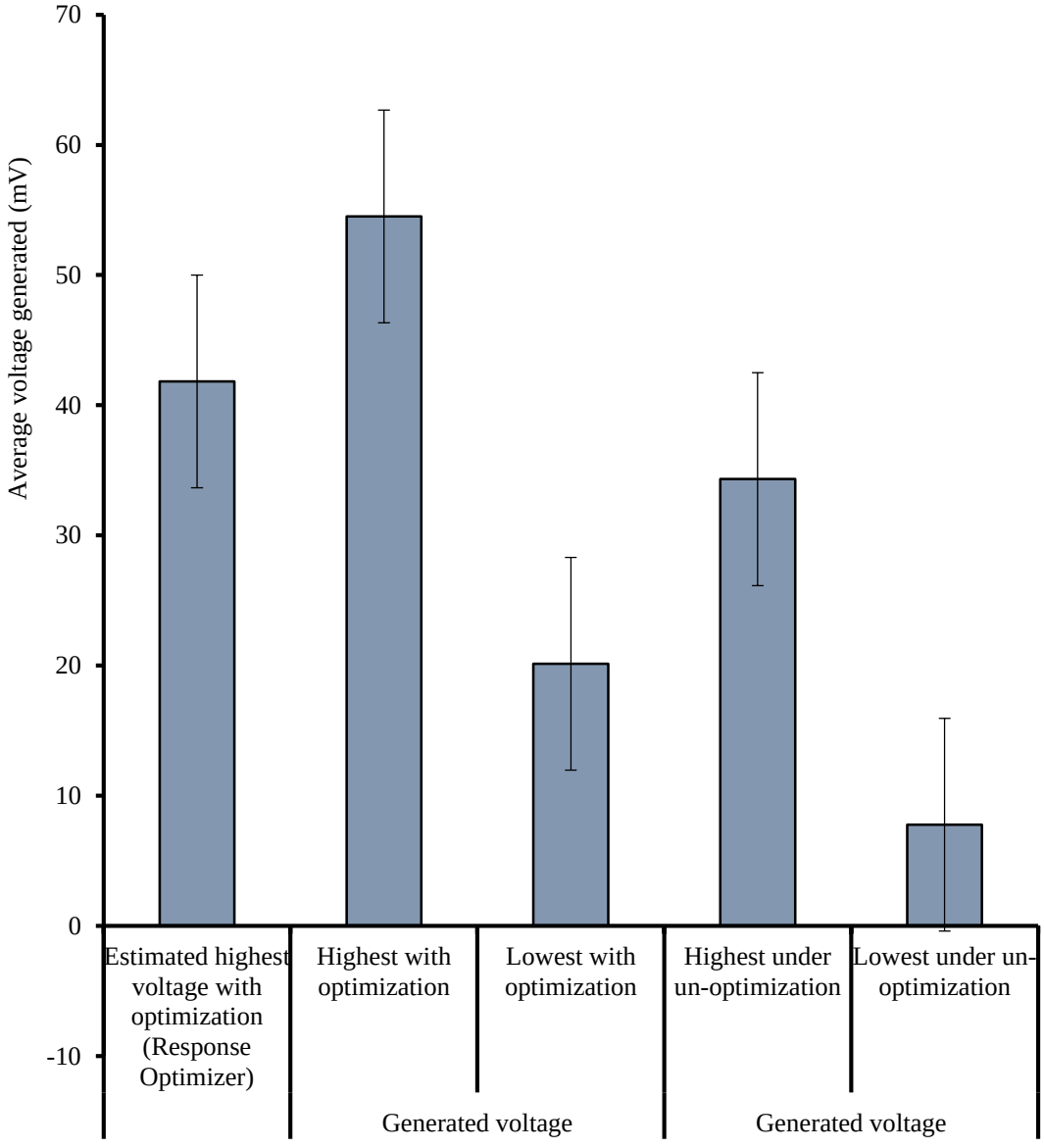
From the graph above, it was observed that the MFCs demonstrated a gradual rise in OCV in the mornings from day 1 to day 11 with 239.7mV and 368mV respectively then it took a plunge from day 12 with 331.25mV down to day 18 with 273mV. The movement is a snakelike movement where the readings rises and falls with every passing day.

External resistance in addition to reactor configuration, substrate characteristics, planktonic microorganisms, and flow rate in continuous operation are factors that affect the performance of microbial fuel cells. External resistance particularly regulates the availability of the MFC anode as the electron acceptor for microbial electron transfers, and therefore affects the anode biocatalyst activity and electrochemical performance. Low resistances lead to more positive potentials, which provide more free energy to the microorganisms and enable a higher flux of electrons through exoelectrogenic metabolisms, imparting a selective advantage to exoelectrogens over competing functional groups. Subsequently, the various voltages recorded across 100kΩ, 25kΩ, and 5kΩ resistors are presented in appendices.

4.1.6 Comparison of Estimated and Different Generated Voltages

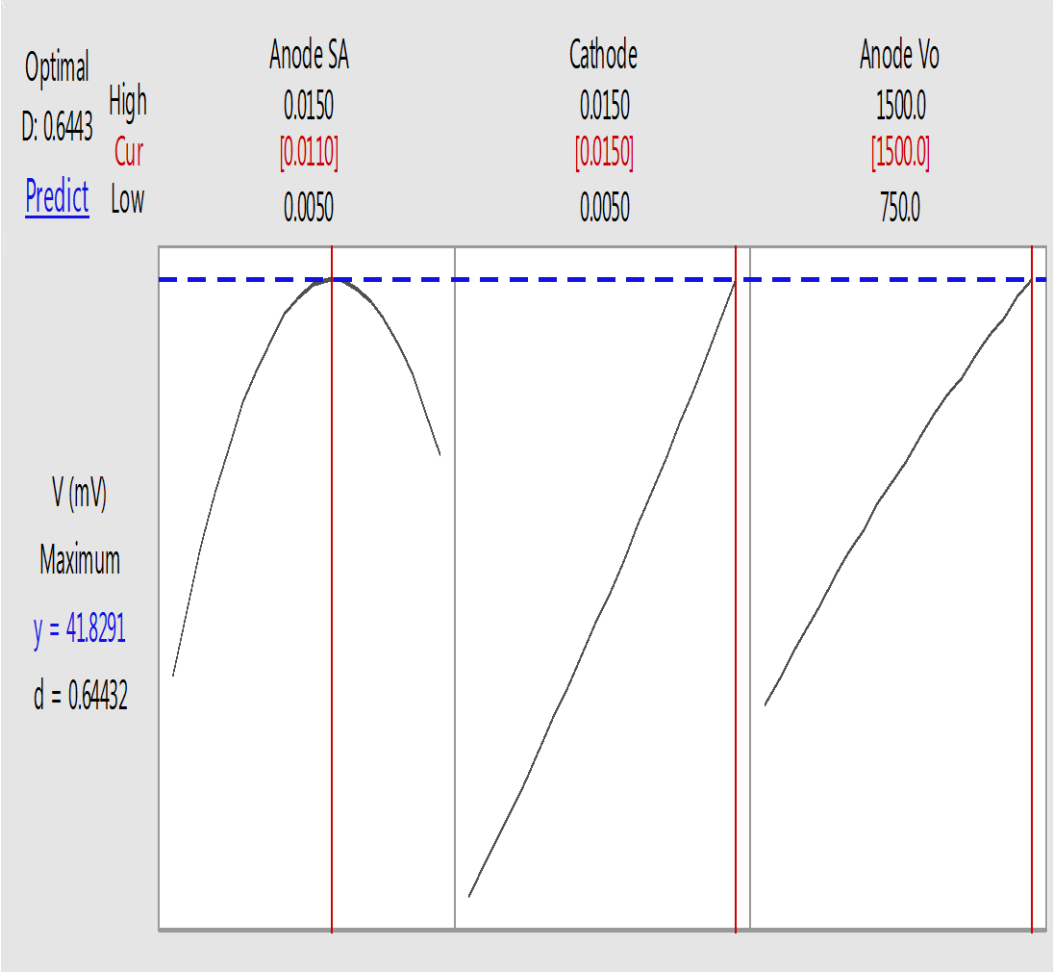
The comparison of the generated voltages with estimated and un-optimized voltages are as shown in Fig 4.2. The Estimated highest average voltage generated was 41.83 mV, highest optimized average voltage generated was 54.5 mV, lowest optimized voltage generated was 20.13 mV, Highest un-optimized voltage was 34.32 mV and lowest un-optimized voltage generated was 7.76 mV.

Percentage increase from expected maximum is 30.29% and percentage increase from expected maximum of un-optimized is 58.80%.



4.1.7 Optimum parameters obtained using Response Optimizer

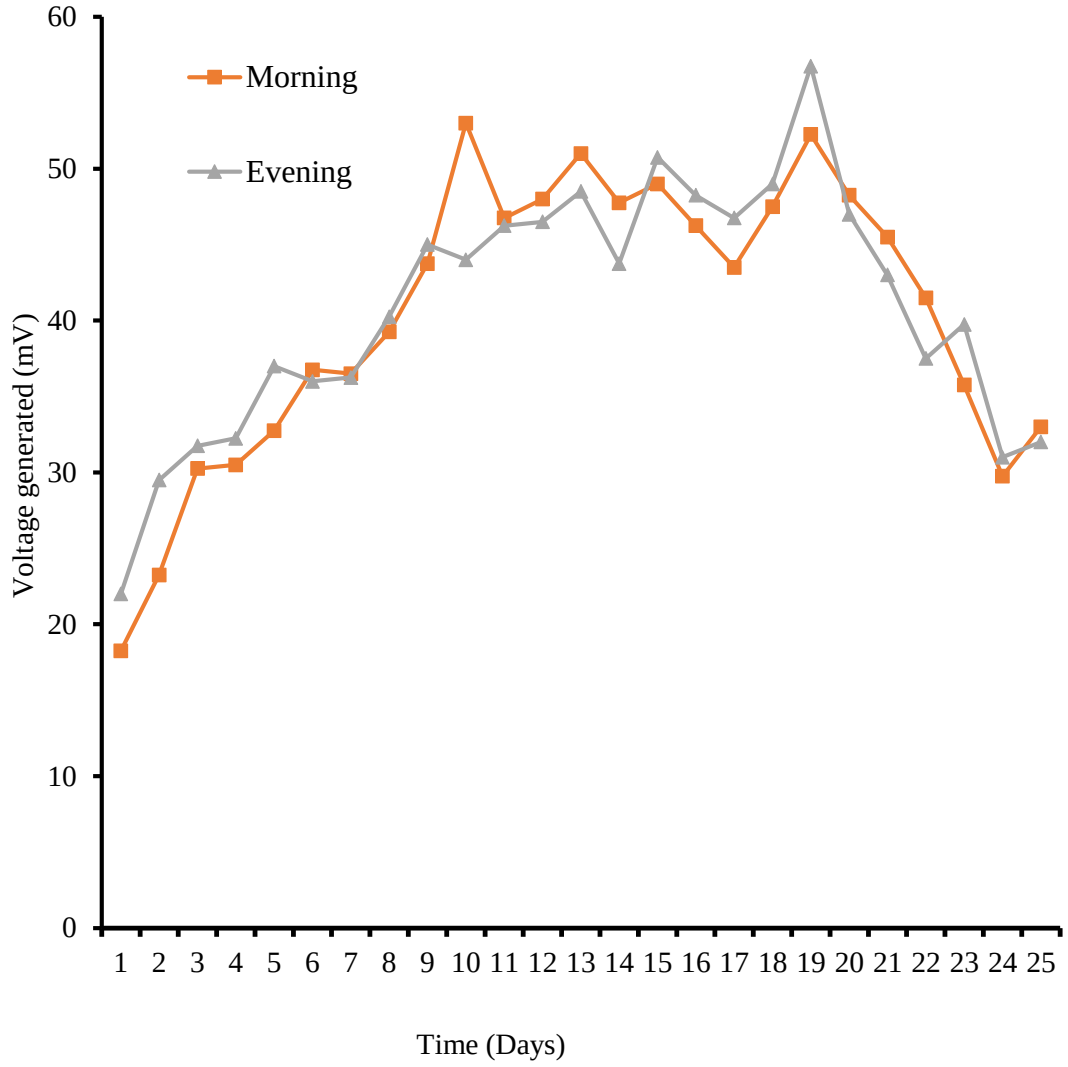
Following 25 days running of the MFCs and daily recording of voltage production (morning and evening), the average voltages, taken across 10,000Ω resistance, as produced by the 30 MFCs were optimized with Minitab® 17. Results showed that 0.011m^2, 0.015m^2 and 1500ml were the optimum surface area of anode, cathode and volume of anode respectively as shown in Fig 4.3, with estimated highest average voltage production of 41.83mV. When these optimums were used to set MFCs, the highest average voltage obtained 54.5mV, which is 25% higher than estimated highest average voltage, while the lowest was 20.13mV. These were higher than highest average voltage of 34.32mV and lowest of 7.76mV obtained without optimization.


Optimal
D: 0.6443
Predict
High
Cur
Low
Anode SA
0.0150
[0.0110]
0.0050
Cathode
0.0150
[0.0150]
0.0050
Anode Vo
1500.0
[1500.0]
750.0
V (mV)
Maximum
y = 41.8291
d = 0.64432

4.1.8 Voltage Generated Across 10kΩ

Result showing the voltage generated across 10 kΩ resistor for 25 days period of treatment usingmicrobial fuel cell. Result was recorded every morning and evening by 6.00 as shown in Fig 4.4.

(b)


Morning
Evening
Voltage generated (mV)
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Time (Days)

In graph A, the voltage of the MFCs initially showed high voltage and then continued increasing on day 3, and after wards. They then consistently followed a rise and fall trend until the end of the treatment. However, graph A maintained a sharp increase from day 1 until day 6 when it began to slow down. Although the highest voltage was recorded in the MFC on day 11, this trend however didn't last long before significantly declining. Similarly, in graph B, while there was a sharp rise from day 1 to day 3, day 10 gave the highest voltage, also it abruptly declined at day 20 and continuously lagged behind until end of the treatment. MFC rose to a highest steady value at day 19and then recorded minor fluctuations until the end of the period of treatment.

4.1.9 Main Effects Plot for Voltage

Other plots gotten during generation of voltage include the main effect plot for voltage (mV) as shown in Fig 4.5, Surface Plot of V (mV) vs Anode Vol. (mL), Anode SA (m^2)as shown in Fig 4.6, Surface Plot of V (mV) vs Anode Vol. (mL), Cathode SA (m^2)as shown in Fig 4.7, and Surface Plot of V (mV) vs Cathode SA (m^2), Anode SA (m^2) as shown in Fig 4.8.

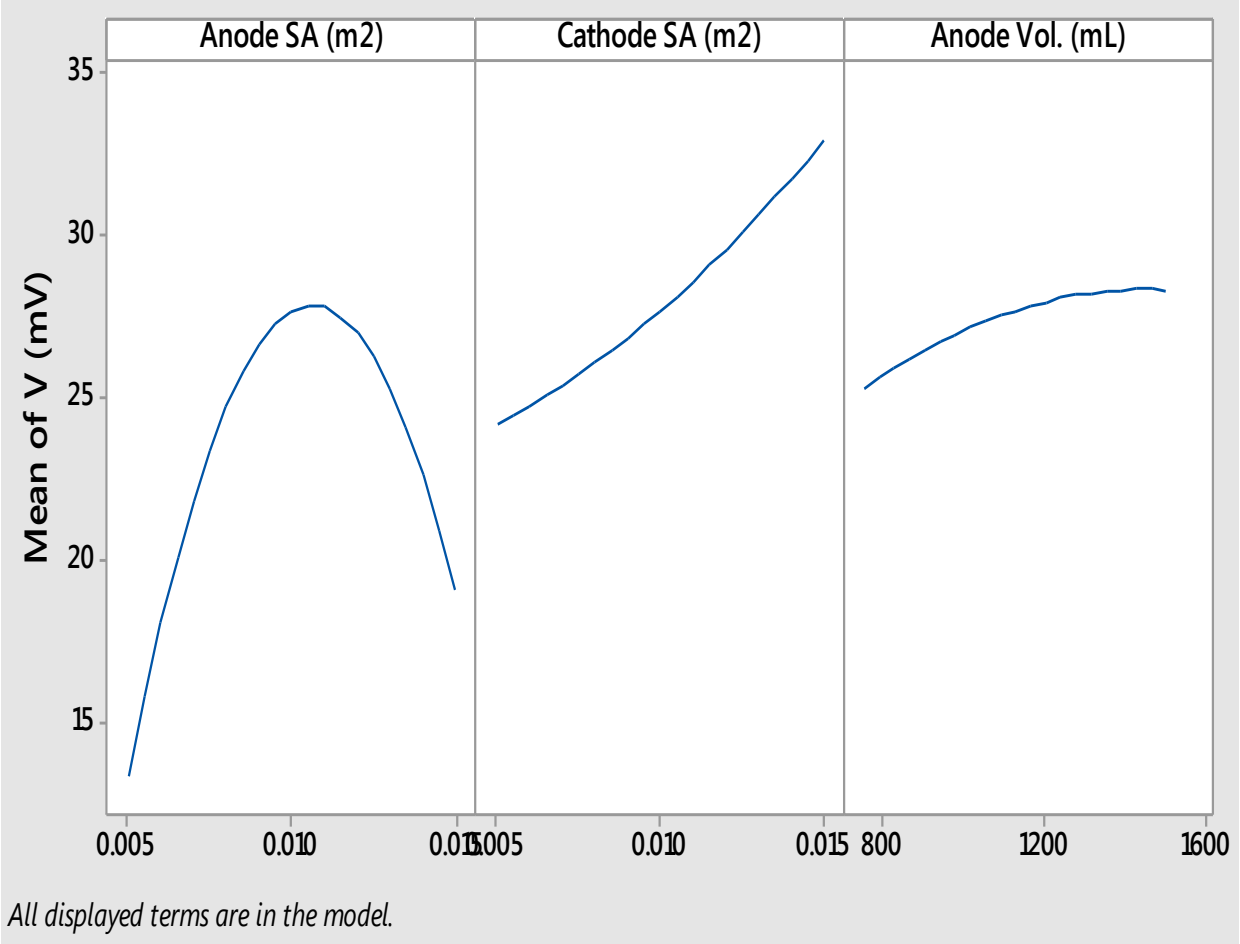


Figure 4.7: Main effects plot for voltage (mV)

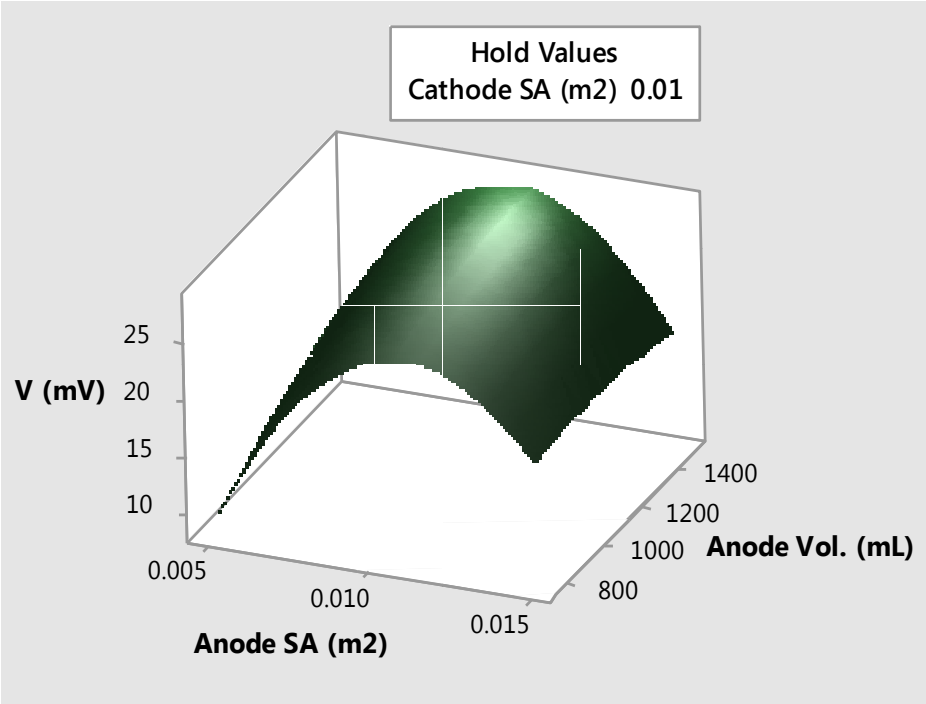


Figure 4.8: Surface Plot of V (mV) vs Anode Vol. (mL), Anode SA (m^2)

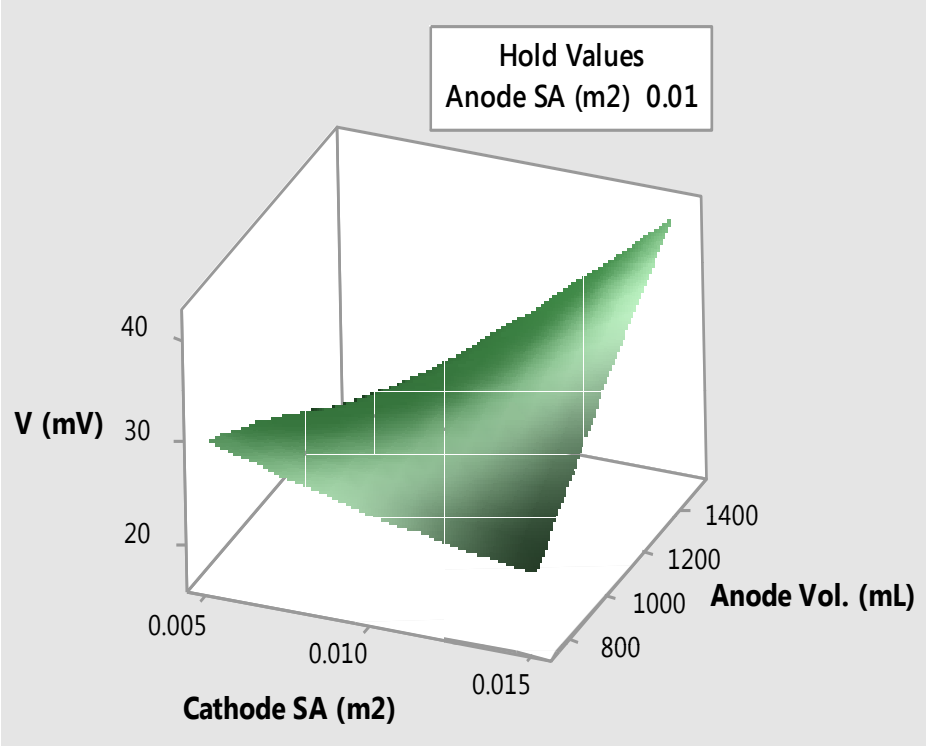


Figure 4.9: Surface Plot of V (mV) vs Anode Vol. (mL), Cathode SA (m^2)

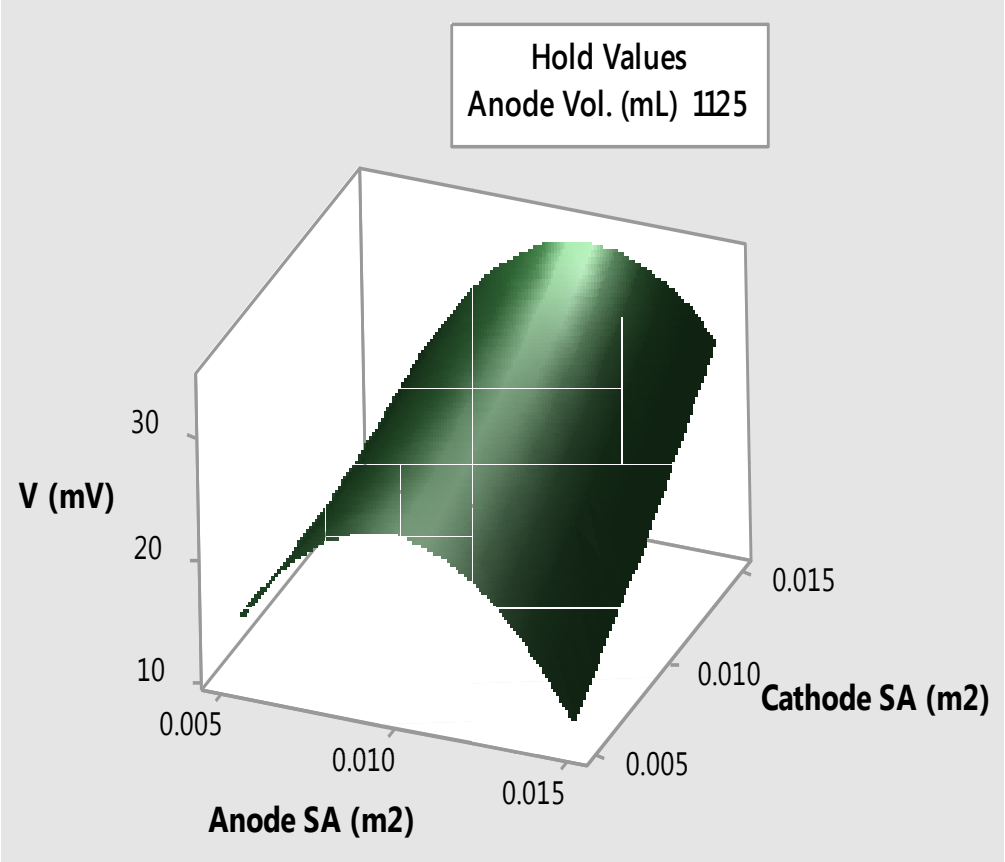


Figure 4.10: Surface Plot of V (mV) vs Cathode SA (m^2), Anode SA (m^2)

4.1.10: Coulombic Efficiency

Coulombic efficiency deals with the electrons that are recovered from the substrate in the form of electric current. It expresses the ratio of amount of actual electrons that is gained from the substrate in the form of electricity against the theoretical amount of electrons which are delivered by the bacteria based on the COD or substrate removal. The coulombic efficiency is one of the most important indexes that are used to describe MFC performance in terms of power generation. It was calculated from the equation,

$$\text{CE} - \frac{M \int_0^t 1 dt}{Fbv\Delta COD}$$

where V is liquid volume (m^3) at the anode chamber, F is Faraday's constant (96485 C/mol of e) and b is mol of electrons produced per mol of O_2 (4 mol/mol), M is the molar mass of O_2 (32 g/mol).

Using average current (I) derived when R_{ext}= 10000Ω, results of the coulombic efficiency of the microbial fuel cells shown in appendix revealed that the MFCs demonstrated an impressive performance in terms of electricity generation from the quantity of waste consumed.

4.1.11: COD and BOD Removal Efficiency

Using the values of COD and BOD of wastewater in the appendix, the efficiency of the MFCs in removal of COD and BOD (expressed in percentage) of the pig wastewater was calculated using the formula below,

$$\frac{\text{Initial COD (BOD) of wastewater (mg/L)} - \text{Final COD (BOD) of wastewater (mg/L)}}{\text{Initial COD(BOD)of wastewater(mg/L)}} \times 100$$

The results obtained showed that the MFC configurations studied demonstrated impressive removal of both COD and BOD of the treated piggery wastewater compared to the original sample. While 18.89% removal of BOD in the microbial fuel cells used was observed, 31.41% of the COD was removed. On the other hand,in the control (untreated) sample, they only reduced to 1583.33mg/l and 4699.67mg/l respectively. Therefore, it was evident that microbial fuel cell significantlyenhanced this reduction. The % COD removed was observed to be higher than % BOD removed.BOD and COD are among major physicochemical parameters that determine the effect of wastewater on the environment. They are key indicators of the environmental health of a surface water supply, and are commonly used in wastewater treatment,but rarely in general water treatment.

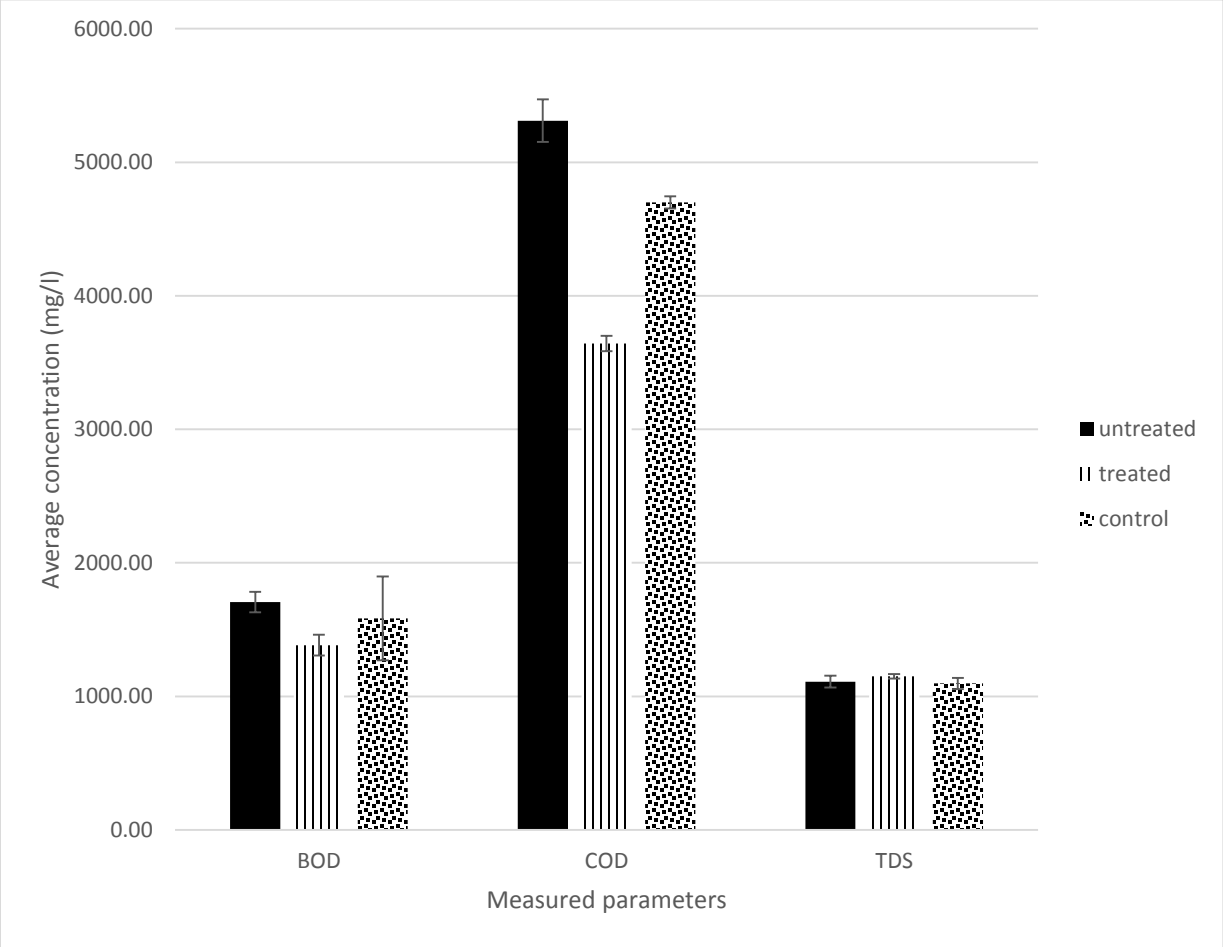


Fig. 4.11: COD, BOD and TDS Removal Efficiency

4.2 Discussion

Generation of electricity from wastewater, using microbial fuel cell (MFC) has emerged as a technology capable of sustainably meeting the dual goals of waste management and energy production. However, it is still at the laboratory stage, thereby requiring more intensive studies for its development and commercial application. To attract more researchers, the overall cost of setting up MFCs should not be prohibitive. Thus research involving use of locally available materials in producing its components, against the imported costly ones, will be of great assistance in crashing the cost. This formed the aim of this study. Plastic containers, PVC pipes, aluminium sheet, copper wire epoxy gum, resistors, water etc. were some of locally available materials used in setting up the dual chambers MFCs used in this study. The MFCs were monitored for 25 days, with both open circuit voltage (OCV) and voltage across 10,000Ω resistance recorded daily, in the morning and evening. Results showed that the highest average OCV of 364mV was recorded on day 11, while the highest average voltage across 10,000Ω resistance was 54.5mV on day 19.Bacterial isolates found in the original piggery wastewater used as substrate and swab of biofilm on anode surface, after treatment, showed a total viable bacterial counts which ranged from 1.0 x 10^6Cfu/ml to 9.75 x 10^7Cfu/ml. identities of the isolates are *Bacillus* sp*, Pseudomonas* sp*, Enterococcus* sp*, Klebsiella*sp*, Serratia*sp*, Staphylococcus* sp*, Enterobacter*sp*, Corynebacterium*sp*, Salmonella* sp*, Shigella*sp*, Micrococcus* sp and *Escherichia coli*.

4.2.1 Cultural Identification of Microorganisms

Culture and biochemical tests employed in the identification of the isolates of the piggery wastewater showed the presence of *Lactobacillus* sp., *Corynebacterium* sp., *Streptococcus* sp., *Bacillus*sp, *Enterobacter*sp.,*Escherichia coli*, *Pseudomonas* sp., *Bacillus* sp.,*Micrococus*sp,*Corynebacterium* sp., *Salmonella* sp, *Serratia*sp and *Shigella*sp. This finding

corroboratesthe reports of Zhu (2000),which similarlyrevealed thatswinefecal bacteria genera listed in order of quantity from high to low includeGram-positive cocci (ca. 39%), *Eubacterium* (ca. 27%), Lactobacillus (ca. 20%), Gram-negative rods (*Escherichia*, ca. 8%), *Clostridium* (ca. 4%), and some other minor groups such as *Propionibacteriumacnes* and *Bacteroides* (<2%). They concluded that among these bacterial genera, *Clostridium* sp., *Lactobacillus* sp., *Peptostreptococcus*, *Eubacterium*, *Peptococcus*, *Propionibacteriumacnes*, *Bacteroides*, and *Megasphaera* are anaerobes; *Streptococcus*, *Staphylococcus*, and *Bacillus* are facultative anaerobes; *Escherichia coli* are aerobes or facultative anaerobes. However, Peuet al(2005)reported that5 of the 9 dominant bacterial phylotypes of pig slurry mostlycorrespondedto uncultured bacteriaclosely related to *Clostridium butyricum*and four othersare close to uncultured *Prevotellaceae, Bacteroidaceae*and*Streptococcaceae.*

4.2.2 Physicochemical Analysis

The significant reduction in BOD and COD contents of the treated wastewater demonstrate the suitability of MFCs in treatment of wastewaters. The decrease in the organic matter content of the treated wastewater is attributable to the metabolic activities of microorganisms which used them as sources of carbon for energy generation. Moreover, the results demonstrate that MFC enhanced this decrease as the extent of reduction of BOD and COD of the treated wastewater is significantly higher than what was obtained in the control sample.

Furthermore, this study demonstrates the ability of a Microbial Fuel Cell to remove some nutrients from the wastewaters. The COD and BOD removal efficiency of 31.41%, and 18.89% was recorded. This can be correlated to 86 % COD removal efficiency reported in a study by (Min et al., 2005). It is also in accordance with the findings of (Egbadonet al., 2016), where theCOD and BOD removal efficiency of 93.31%, and 51.74% was recorded. Ammonia, Nitrateand Phosphate

parameters were also examined to determine the effect of MFC on these parameters present in piggery wastewater and 45.13%, 32.14% and 18.41% decrease was recorded respectively. Total dissolved solids value increased by -21.62% in piggery wastewater. The increase is as a result of more debris in the wastewater caused by dead microorganisms..

4.2.3: Microbial Fuel Cell

4.2.3.1: Generation of Voltage

The observation after 24 hours of setting up the MFCs that the digital multimeters used to monitor voltage generated had started recording increasing values indicated the presence of 108 exoelectrogens in the swine wastewater used in this study. Consequently, no biostimulation may be required prior to bioelectricity generation using swine wastewater. This is in line with the preliminary experiments conducted using a two chambered MFC, which demonstrated that electricity could be generated using swine wastewater, and that the bacteria needed were already present in the wastewater (Min *et al*., 2005). This scenario is quite unlike cases where a substance called "mediator" which enhances the shuttle of electrons from the bacterial cells to the anode is required before bioelectricity can be produced. Therefore, MFCs using piggery wastewater as the substrate will most likely be mediator-less. This is advantageous because mediated microbial fuel cells tend to be inefficient, expensive, and produce low levels of power (Seop*et al*.,2006). However, overtime it was observed that voltage recorded in each of the microbial fuel cells continued to vary; while some maintained gradual increase in voltage. This initial increase in voltages, might have resulted from both biological and chemical factors, based on the differences of the potential between the two chambers (Minet al., 2005). The voltage and currents were seen to decrease with time. This decrease in OCV can be attributed to the rate of utilization of the organic substrate in the medium (wastewater) (Patilet al., 2013). The results from this study

conducted using a dual-chamber MFC demonstrated that electricity can be generated from piggery wastewaters. This is in agreement with previous studies carried out by (Logan et al., 2007) who conducted similar studies using Swine wastewater. The results from this study revealed a maximum Open Circuit Voltage (OCV) of 974 mV. This high Open Circuit Voltage can however be compared to a similar Open Circuit Voltage of 836 mV generated in a study carried out by (Egbadonet al., 2016) using different source of catholyte (K3[Fe(CN)6]). Also, the lowest Open Circuit Voltage of 3mV recorded in this study during the treatment of piggery wastewater is lower when compared to the lowest Open Circuit Voltage (OCV) of 351 mV recorded in a study carried out by (Egbadonet al., 2016) using Swine wastewater.The low open circuit voltage recorded in this study could have resulted from a difference in the Proton exchange membrane used in various studies. The reactor design could also have acted as a factor in the maximum opencircuit voltage generated.

During operation of MFC, the open circuit voltage for piggery wastewater remained at zero (0) throughout day one and from day 2, there was a sharp rise in the morning and a decline toward the evening which continued in this manner for a period of time. However, it is suggested from this study that the fairly uniform nature of the piggery wastewater comprising mostly of urine (45%) and swine faeces (55%) and wash water couldhave played a major role as the bacteria community present in the piggery wastewater were able to easily metabolize the substrate in the wastewater.

The result of this study is in agreement with the report of Min et al (2005) which demonstratedthat animal wastewaters such as swine wastewater can be used for power generation in MFCs while at the same time achieving wastewater treatment. In MFCs, substrate is regarded as one of the most important biological factors affecting electricity generation(Liu et al.,2009).

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CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1: Conclusion

In view of the outcome of this study, piggery wastewater which has constituted nuisance to the environment close to many pig farms has demonstrated to be a useful resource, if appropriately utilized using technologies like microbial fuel cell. This is because it contains both the substrates and appropriate exoelectrogenic consortium needed for its decomposition and generation of electrical energy using microbial fuel cell. Consequently, there may be no need for foreign exoelectrogens when using piggery wastewater in MFCs. This is impressive, especially now that many crude oil-producing, developing nations including Nigeria may be diversifying their economic base into other sectors like agriculture, of which pig farming is expected to have its fare share of the revolution. The implication of this would be increased generation of agricultural wastes and wastewaters like piggery wastewater. Moreover, the cost of treating wastewaters is usually very high and may not be affordable to most farmers in developing nations. However, microbial fuel cell provides a very cheaper alternative method of treating wastewaters and as well generating electricity, which has remained a major challenge to the economic and social development of many developing nations, like Nigeria. A comparison of the physicochemical parameters of the sample before and after treatment has shown that the MFCs used in this study impressively achieved up to 31.41% and 18.89% reduction in chemical oxygen demand (COD) and biochemical oxygen demand (BOD) respectively.

5.2: Recommendations

The present research has not only indicated the suitability of piggery wastewater as substrate for generation of electricity using microbial fuel cell, but it has also revealed that piggery wastewater contains enough exoelectrogens needed for this conversion. Also, a commendable level of treatment of the wastewater was achieved in this study. However, the output recorded in the present study is lower than the reported maximum bioelectricity output of MFCs so far studied, which itself is still far below what is estimated to be necessary for commercial consideration. Therefore, further studies to optimize all the necessary parameters and upscale the technology to industrial level in order to achieve better harvest of energy and wastewater treatment is recommended. Having demonstrated the performance of aluminium as the electrode in the MFCs using piggery wastewater in this study, further studies should delve into using other materials known to enhance electricity generation to identify the most suitable electrodes for this setup. This should equally apply to the catholytes.

Furthermore, this study revealed the presence of diverse microbial consortia in piggery wastewater, including Bacillus spp. which mostly persisted in the treated wastewater. This could imply that they participated in the course of generation of electricity, having been initially reported by other researchers to be exoelectrogens. However, further studies should be separately carried out using pure cultures of these microorganisms from piggery wastewater to establish their capabilities and perhaps eliminate species that may be antagonistic in activities.

Also, further study of environmental conditions, physicochemical parameters and any other requirements that may enhance the performance of the useful microorganisms is recommended. Studies to ascertain the mode of electron transfer in these identified microorganisms should be embarked upon with the aim of enhancing it for better generation of electricity. Genetic studies and modifications of the genes (plasmids) of these organisms may be carried out to increase their wastewater degrading and conversion capabilities and enhance electrons transfer potentials

through their electrochemically active surface proteins, pili etc. Now that the role of renewable energy sources in ensuring sustainable power supply has been globally acknowledged, more effort should be made by the government, co-operate organizations and industries to encourage further research and development of microbial fuel cell, especially in developing countries like Nigeria where there is high burden of environmental contamination and inadequate power supply.

5.3 Contribution to Knowledge

1. Serratia sp was isolated which is one of the bacteria isolates that generated the electricity
2. It has proven that the performance of MFCs can be improved by optimization of effects of factors affecting it.

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APPENDICES

APPENDIX 1: OPEN CIRCUIT VOLTAGE (OCV) RECORDED FOR 25 DAYS

	MFC 1		MFC 2		MFC 3		MFC 4		MFC 5		MFC 6		MFC 7		MFC 8		MFC 9	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	142	137	134	125	208	213	241	232	181	190	143	138	214	208	164	157	212	221
3	145	141	301	256	149	133	198	191	116	126	251	243	171	164	173	174	256	263
4	173	169	125	143	110	132	266	251	207	213	241	230	146	134	230	221	226	238
5	123	115	250	146	154	151	260	232	183	249	272	243	179	156	251	167	279	336
6	91	108	453	433	153	147	187	271	231	254	151	173	151	172	210	251	288	245
7	128	132	415	403	151	138	283	300	275	306	196	328	184	201	263	331	216	200
8	221	208	156	171	184	182	302	334	169	176	207	231	120	132	233	231	220	218
9	192	189	246	259	181	177	462	485	245	258	325	364	146	137	242	232	231	240
10	201	193	380	342	186	191	633	620	350	336	527	509	156	147	261	252	276	261
11	185	178	354	363	197	206	608	591	325	328	501	498	172	203	248	254	267	270
12	179	182	375	391	217	233	597	589	335	342	506	511	206	201	278	285	267	263
13	185	168	429	411	274	257	610	618	356	336	522	525	212	239	301	293	270	273
14	149	135	385	326	231	207	624	635	321	305	527	532	374	301	284	262	276	283
15	66	66	344	344	273	267	341	369	432	445	552	526	325	313	238	231	271	269
16	275	259	355	356	288	232	476	675	461	433	463	544	356	349	243	276	277	285
17	184	166	360	374	267	245	626	614	476	491	525	513	361	354	271	293	292	284
18	147	132	398	381	224	197	681	675	470	443	528	516	388	412	327	341	297	285
19	80	107	465	316	144	163	802	821	380	369	542	428	497	513	290	375	303	281
20	135	182	294	228	187	210	832	854	344	326	496	437	548	617	349	313	225	177
21	179	165	267	256	288	265	831	820	304	286	448	431	582	537	297	281	195	218
22	174	116	312	162	341	144	803	733	231	182	433	419	243	220	255	273	284	263
23	109	97	197	225	168	194	751	782	193	207	437	456	213	208	318	335	231	213
24	85	79	387	365	223	209	820	834	230	222	582	468	229	213	403	403	218	205
25	75	73	399	455	224	218	852	866	251	244	573	550	231	257	525	618	211	207
AVR	**144.9**	**139.9**	**311.2**	**289.2**	**200.9**	**188.4**	**523.4**	**535.7**	**282.6**	**282.7**	**397.9**	**392.5**	**256.2**	**255.5**	**266.2**	**274.0**	**243.5**	**239.9**
Overall AVG		**142.4**		**300.24**		**194.66**		**529.56**		**282.66**		**395.22**		**255.84**		**270.06**		**241.72**

	MFC 10		MFC 11		MFC 12		MFC 13		MFC 14		MFC 15		MFC 16		MFC 17		MFC 18	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	205	214	177	186	191	203	179	186	282	286	534	521	257	249	333	326	382	378
3	275	288	164	173	174	187	303	314	227	232	330	321	273	275	309	297	344	341
4	768	473	211	225	188	205	219	203	508	511	405	397	279	253	300	289	330	330
5	364	526	218	241	201	411	134	143	529	533	302	251	280	283	272	267	321	316
6	802	809	302	297	520	532	185	189	440	457	224	238	344	320	317	272	300	294
7	815	834	271	262	547	694	208	255	569	601	251	422	344	338	243	341	280	285
8	782	775	285	263	737	724	189	193	661	652	285	289	340	337	365	361	371	380
9	791	785	230	193	752	747	221	234	668	654	303	311	346	342	338	334	270	365
10	960	952	177	169	781	776	329	315	687	693	358	345	325	321	390	394	313	318
11	974	981	171	178	781	795	322	327	715	783	356	372	328	324	396	391	344	347
12	962	948	193	189	771	753	321	315	752	738	407	461	425	417	368	362	221	212
13	885	897	295	277	660	638	324	336	603	627	534	515	377	372	348	346	207	209
14	904	921	265	254	595	564	351	380	689	832	493	335	382	376	319	322	188	176
15	945	927	282	275	700	727	472	464	812	818	479	492	386	323	329	318	186	171
16	919	892	269	256	822	703	487	405	838	817	530	395	336	328	319	312	182	186
17	892	874	278	293	681	667	439	405	658	686	393	381	321	326	308	319	158	136
18	851	825	335	357	635	603	394	361	715	736	364	352	314	306	318	311	143	136
19	789	772	460	437	479	507	242	224	862	871	336	304	354	347	338	329	139	127
20	769	787	385	301	538	583	216	209	865	887	272	205	252	247	296	291	128	122
21	741	713	288	274	566	531	199	193	854	801	241	233	245	331	320	314	108	212
22	670	632	246	234	400	346	190	230	716	628	274	307	271	232	284	295	117	202
23	627	612	310	324	314	306	221	213	681	653	301	284	254	247	307	245	115	107
24	623	651	426	406	298	303	184	197	779	753	212	204	249	236	332	316	113	106
25	863	717	387	366	330	354	221	233	767	798	195	187	241	228	314	271	66	92
AVR	**727.0**	**712.2**	**265.0**	**257.2**	**506.4**	**514.4**	**262.0**	**261.0**	**635.1**	**641.9**	**335.2**	**324.9**	**300.9**	**294.3**	**310.5**	**304.9**	**213.0**	**221.9**
Overall AVG		**719.62**		**261.1**		**510.4**		**261.48**		**638.48**		**330.02**		**297.62**		**307.72**		**217.48**

	MFC 19		MFC 20		MFC 21		MFC 22		MFC 23		MFC 24		MFC 25		MFC 26		MFC 27	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	473	465	360	354	339	331	333	321	389	382	348	345	270	263	508	503	502	494
3	444	443	296	291	279	274	305	308	365	359	342	348	222	214	492	487	468	465
4	479	477	306	281	315	371	374	362	336	304	344	360	262	277	528	518	472	467
5	497	492	306	309	328	325	413	417	346	342	314	312	236	235	517	519	455	451
6	531	598	324	292	450	379	332	328	317	289	272	273	210	217	519	500	464	454
7	533	529	304	302	347	343	279	283	271	275	247	246	194	197	501	493	449	442
8	684	677	286	281	270	265	333	329	315	311	242	238	221	225	489	493	427	425
9	570	566	247	343	362	357	285	281	271	268	264	262	240	237	501	507	404	401
10	559	557	248	243	349	353	328	225	272	279	269	273	236	234	477	482	385	389
11	727	731	229	235	359	351	333	327	308	313	257	255	236	231	464	461	377	379
12	671	663	253	251	375	369	316	312	385	379	323	327	229	225	467	472	409	401
13	553	547	198	205	296	292	427	424	252	246	319	315	205	201	444	446	352	346
14	694	691	282	276	353	356	368	363	186	181	433	451	194	186	429	425	340	332
15	673	665	262	265	333	303	376	389	193	207	446	453	195	193	437	420	334	343
16	496	482	252	246	236	239	450	457	271	265	524	528	185	181	414	419	326	331
17	425	446	260	204	371	324	453	447	243	209	518	490	170	174	402	380	320	324
18	421	393	255	273	313	291	445	443	213	205	478	468	167	152	387	384	331	307
19	365	347	261	249	316	332	461	468	216	211	453	447	187	220	376	361	315	298
20	356	349	223	216	327	324	432	425	202	207	478	470	148	134	358	360	270	263
21	369	398	173	264	315	306	320	310	237	252	497	471	129	146	365	366	252	240
22	356	374	188	199	302	295	237	223	248	241	431	390	128	107	336	363	232	253
23	338	394	188	244	287	266	161	213	243	236	367	347	42	32	323	314	243	240
24	351	326	259	275	261	344	226	232	238	224	331	303	28	25	319	316	231	224
25	330	313	314	210	237	229	185	249	229	215	265	339	3	6	264	286	236	215
AVR	**475.8**	**476.9**	**251.0**	**252.3**	**308.8**	**304.8**	**326.9**	**325.4**	**261.8**	**256.0**	**350.5**	**348.4**	**173.5**	**172.5**	**412.7**	**411.0**	**343.8**	**339.4**
Overall AVG		**476.36**		**251.64**		**306.78**		**326.16**		**258.92**		**349.46**		**172.98**		**411.84**		**341.56**

	MFC 28		MFC 29		MFC 30	
Days	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0
2	624	618	315	312	640	625
3	565	569	305	308	396	385
4	591	530	287	301	486	476
5	525	522	314	314	362	301
6	588	488	297	266	268	285
7	490	483	258	255	301	506
8	497	495	212	210	342	346
9	561	556	209	205	363	373
10	547	551	181	188	429	414
11	542	540	179	183	427	446
12	571	562	312	305	488	553
13	529	533	171	167	640	618
14	521	526	158	165	591	402
15	517	503	169	201	574	590
16	440	445	158	153	636	474
17	439	431	194	225	471	457
18	426	419	235	230	436	422
19	423	428	206	149	403	364
20	396	413	150	187	326	246
21	408	397	160	213	289	279
22	386	382	255	268	328	368
23	373	346	253	202	361	340
24	354	366	225	236	254	244
25	326	350	180	193	234	224
AVR	**465.6**	**458.1**	**215.3**	**217.4**	**401.8**	**389.5**
Overall AVG		**461.84**		**216.38**		**395.66**

Appendix 1a: Daily Open Circuit Voltage ACROSS 10,000Ω Resistor

	MFC 1		MFC 2		MFC 3		MFC 4		MFC 5		MFC 6		MFC 7		MFC 8		MFC 9	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	8	7	8	7	21	15	12	14	26	21	23	19	20	18	23	21	19	20
3	8	7	5	4	12	12	18	15	16	18	16	13	13	14	27	30	23	27
4	7	6	4	6	11	13	14	13	22	20	7	11	17	15	35	31	30	33
5	6	7	8	12	16	13	13	15	14	12	14	12	14	11	28	23	38	32
6	8	7	14	13	15	13	19	16	12	14	11	11	7	13	19	26	46	35
7	9	8	14	12	13	12	17	14	19	15	12	10	17	20	24	27	31	33
8	6	4	10	7	18	16	20	22	10	5	13	11	15	10	29	26	24	17
9	13	11	8	12	14	18	25	29	6	9	12	10	9	10	21	19	13	25
10	12	11	10	14	21	24	35	32	11	9	14	11	13	10	15	12	34	38
11	10	8	19	16	28	25	38	37	8	6	13	14	11	9	14	12	54	48
12	9	6	21	18	26	22	36	34	7	5	17	15	10	8	9	5	57	51
13	10	9	23	20	22	20	35	31	9	7	19	16	9	8	7	6	48	44
14	8	7	18	16	23	21	32	36	8	7	17	23	8	16	4	5	35	42
15	6	5	14	11	19	20	38	41	8	9	26	30	28	32	4	3	48	59
16	5	4	17	15	20	17	32	35	8	8	25	22	11	20	18	15	48	35
17	6	4	5	7	20	18	28	31	13	25	18	20	22	27	5	7	37	33
18	5	7	9	11	20	22	35	33	31	35	23	23	28	25	6	8	39	35
19	8	9	13	11	24	22	36	33	43	46	29	25	27	24	9	7	37	34
20	9	11	14	12	25	22	36	32	52	55	27	23	31	27	10	7	31	28
21	13	11	13	11	19	17	34	30	59	56	24	22	29	25	9	9	27	24
22	15	12	12	9	17	15	30	32	58	64	20	18	23	21	7	8	26	28
23	8	6	7	8	18	15	20	23	30	25	11	9	25	23	6	8	24	28
24	7	5	9	7	17	14	27	31	21	17	11	9	22	20	11	9	25	21
25	5	5	8	7	16	15	39	34	11	13	12	10	20	21	10	8	18	15
AVR	**8.04**	**7.08**	**11.32**	**10.64**	**18.2**	**16.84**	**26.76**	**26.52**	**20.08**	**20.04**	**16.56**	**15.48**	**17.16**	**17.08**	**14**	**13.28**	**32.48**	**31.4**
Overall AVG		**7.56**		**10.98**		**17.52**		**26.64**		**20.06**		**16.02**		**17.12**		**13.64**		**31.94**

	MFC 10		MFC 11		MFC 12		MFC 13		MFC 14		MFC 15		MFC 16		MFC 17		MFC 18	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	22	22	18	19	36	32	19	25	32	27	35	24	5	4	17	16	28	25
3	23	31	12	25	28	26	33	30	23	25	26	23	6	4	13	12	23	20
4	36	34	34	33	32	30	33	30	39	36	22	19	7	6	11	11	20	31
5	32	28	31	32	31	54	26	28	39	42	15	12	5	7	9	9	19	19
6	20	22	34	29	85	77	32	30	47	44	7	16	6	13	13	10	18	34
7	25	28	27	24	86	84	29	33	46	49	19	14	8	9	7	12	16	19
8	25	20	22	21	67	43	33	31	35	24	11	6	11	10	19	21	25	21
9	30	29	20	18	80	75	32	34	28	32	7	11	12	11	18	19	16	18
10	32	31	23	21	77	74	36	32	41	44	12	10	10	11	21	20	23	21
11	36	37	17	13	81	84	37	34	51	59	10	11	10	9	23	25	23	19
12	38	40	15	12	91	90	40	38	65	67	15	17	12	11	26	26	15	10
13	35	31	17	19	90	86	41	37	63	61	19	22	11	10	14	13	7	8
14	29	35	21	20	83	89	36	39	55	67	24	22	11	9	15	12	7	6
15	39	44	20	18	85	97	41	43	75	88	20	18	11	13	14	14	6	5
16	33	30	26	29	90	95	42	47	55	51	26	22	14	12	9	12	5	6
17	31	30	18	15	88	84	43	40	54	51	30	33	12	10	14	16	7	5
18	32	32	10	12	95	81	45	38	47	45	31	28	13	11	15	14	9	8
19	36	34	19	22	76	70	41	35	48	52	25	22	10	9	9	8	7	5
20	36	27	38	33	51	48	31	27	66	58	16	21	7	5	8	9	4	3
21	31	25	35	29	43	41	29	27	53	47	25	23	6	5	10	9	3	5
22	23	20	24	17	40	37	29	32	38	36	27	33	6	5	8	9	4	10
23	18	19	6	11	34	37	30	26	24	27	34	28	5	4	11	10	2	2
24	20	18	15	13	38	33	27	25	29	32	27	25	4	3	9	9	3	2
25	18	21	18	16	41	35	30	27	39	34	30	26	3	2	7	5	1	1
AVR	**28**	**27.52**	**20.8**	**20.04**	**61.92**	**60.08**	**32.6**	**31.52**	**43.68**	**43.92**	**20.52**	**19.44**	**8.2**	**7.72**	**12.8**	**12.84**	**11.64**	**12.12**
Overall AVG		**27.76**		**20.42**		**61**		**32.06**		**43.8**		**19.98**		**7.96**		**12.82**		**11.88**

	MFC 19		MFC 20		MFC 21		MFC 22		MFC 23		MFC 24		MFC 25		MFC 26		MFC 27	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	45	43	15	16	24	21	23	22	24	23	26	25	19	20	39	36	37	37
3	41	37	12	15	28	25	33	28	20	22	17	18	13	10	31	29	35	36
4	41	42	11	11	18	24	25	19	18	19	17	17	14	13	33	32	39	37
5	42	42	11	11	20	21	22	22	19	20	14	13	9	9	28	26	30	32
6	50	56	14	12	35	31	18	17	16	13	11	11	8	9	34	33	39	38
7	46	53	11	10	23	21	14	16	8	14	14	13	7	9	30	28	29	27
8	74	67	12	11	21	27	16	14	21	18	12	10	10	11	34	33	34	35
9	60	56	10	8	39	32	12	11	19	17	12	12	10	9	35	33	34	32
10	50	57	8	7	27	27	13	12	18	16	13	11	9	10	32	33	24	26
11	72	75	8	6	28	32	15	14	15	17	12	12	10	9	32	33	29	27
12	79	79	7	6	33	36	17	17	18	20	14	16	11	10	34	35	29	28
13	58	55	5	6	23	21	21	20	11	10	18	19	8	9	28	39	24	23
14	56	52	8	7	22	21	21	19	10	9	26	29	9	8	27	24	22	21
15	50	49	11	9	20	19	18	20	9	10	42	60	7	7	25	25	14	18
16	43	37	9	8	21	18	22	22	8	7	65	68	6	5	21	23	11	13
17	34	32	8	6	20	19	24	24	8	8	71	75	6	7	19	22	15	13
18	27	25	11	10	13	15	27	25	7	6	63	60	6	5	19	20	13	12
19	23	22	8	8	15	14	23	22	5	5	57	55	4	3	19	18	11	10
20	21	19	6	4	16	16	18	15	4	3	54	52	4	4	19	17	9	9
21	18	20	3	3	17	15	10	7	4	3	52	46	3	2	16	15	3	7
22	22	15	5	5	16	13	6	7	3	3	37	31	3	4	17	18	9	10
23	29	26	4	5	13	11	4	6	3	2	26	23	2	2	12	10	10	9
24	25	24	6	8	13	9	5	8	4	2	19	18	1	0	11	12	10	8
25	36	33	15	12	11	7	6	5	3	2	12	13	0	1	9	7	12	10
AVR	**41.68**	**40.64**	**8.72**	**8.16**	**20.64**	**19.8**	**16.52**	**15.68**	**11**	**10.76**	**28.16**	**28.28**	**7.16**	**7.04**	**24.16**	**24.04**	**20.88**	**20.72**
Overall AVG		**41.16**		**8.44**		**20.22**		**16.1**		**10.88**		**28.22**		**7.1**		**24.1**		**20.8**

	MFC 28		MFC 29		MFC 30	
Days	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0
2	56	55	21	23	42	28.8
3	45	44	12	12	31.2	27.6
4	46	42	13	14	26.4	22.8
5	34	33	16	13	18	14.4
6	46	36	17	18	8.4	19.2
7	28	31	14	12	22.8	16.8
8	37	38	11	12	13.2	7.2
9	47	43	11	9	8.4	13.2
10	37	39	8	9	14.4	12
11	43	44	8	10	12	13.2
12	46	45	11	12	18	20.4
13	40	41	9	8	22.8	26.4
14	43	40	9	7	28.8	26.4
15	41	41	8	8	24	21.6
16	36	34	5	4	31.2	26.4
17	36	39	4	6	36	39.6
18	41	43	5	7	37.2	33.6
19	35	33	6	4	30	26.4
20	30	31	5	5	19.2	25.2
21	29	28	5	6	30	27.6
22	31	27	10	12	32.4	39.6
23	31	30	9	11	40.8	33.6
24	38	41	14	15	32.4	30
25	28	27	9	7	36	31.2
AVR	**36.96**	**36.2**	**9.6**	**9.76**	**24.624**	**23.328**
Overall AVG		**36.58**		**9.68**		**23.976**

Appendix 1b: Daily Open Circuit VoltageAcross 5,000Ω Resistor

	MFC 1	MFC 2	MFC 3	MFC 4	MFC 5	MFC 6	MFC 7	MFC 8	MFC 9

Days	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	3	6	5	11	10	20	18	12	10	11	9	10	9	9	9	12	11
3	3	3	2	3	6	4	8	9	8	9	1	2	5	7	11	10	13	15
4	4	2	2	3	5	7	11	10	21	17	2	2	10	9	14	12	19	17
5	3	2	2	3	7	5	12	11	5	4	1	2	7	5	22	8	22	18
6	2	2	4	3	3	4	10	11	3	3	1	1	4	6	4	6	15	21
7	5	6	3	4	5	6	11	12	3	4	2	1	8	11	7	9	28	40
8	6	5	2	2	9	8	13	12	9	8	1	3	9	8	10	7	18	17
9	5	4	3	2	9	10	11	10	8	7	5	7	8	9	6	6	19	17
10	4	3	4	3	11	12	11	12	8	9	6	8	10	9	4	5	23	24
11	4	3	3	3	12	13	13	14	11	13	11	13	10	12	5	6	23	26
12	3	3	3	3	10	11	15	13	15	12	12	13	11	11	7	6	25	24
13	3	2	3	2	9	10	14	15	13	14	14	12	12	13	7	7	25	25
14	2	2	3	4	8	8	17	18	17	19	13	15	13	13	7	8	26	27
15	2	2	2	2	9	8	16	15	25	22	10	9	21	14	7	5	22	19
16	2	1	3	4	9	8	15	13	21	23	8	9	16	17	3	2	21	23
17	2	2	4	3	9	10	15	14	27	26	10	11	19	20	1	3	21	20
18	2	1	5	3	8	7	17	15	26	25	13	15	21	19	2	4	18	16
19	2	2	3	3	7	7	19	18	25	25	16	14	20	21	3	3	17	15
20	3	4	4	7	5	6	20	23	26	24	16	20	22	23	4	5	13	13
21	5	6	5	5	4	3	20	21	21	19	18	17	21	18	5	4	12	12
22	6	8	5	6	3	2	19	17	15	4	13	7	13	7	4	3	11	12
23	1	1	1	1	1	2	19	17	14	11	6	5	8	7	3	4	13	11
24	2	1	1	1	2	3	15	13	9	7	7	6	7	8	5	6	12	10
25	1	1	1	1	5	3	9	7	5	3	8	6	9	7	9	5	10	8
AVR	**3.04**	**2.76**	**2.96**	**3.04**	**6.68**	**6.68**	**14**	**13.52**	**13.88**	**12.72**	**8.2**	**8.28**	**11.76**	**11.32**	**6.36**	**5.72**	**17.52**	**17.64**
Overall AVG		**2.9**		**3**		**6.68**		**13.76**		**13.3**		**8.24**		**11.54**		**6.04**		**17.58**

	MFC 10		MFC 11		MFC 12		MFC 13		MFC 14		MFC 15		MFC 16		MFC 17		MFC 18	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	7	8	9	7	19	17	10	6	20	15	14	12	5	4	8	5	13	11
3	11	13	6	10	14	13	1	2	11	13	10	10	6	4	6	5	10	11

4	15	13	16	15	17	16	3	4	19	18	11	12	7	6	5	5	15	9
5	15	16	15	14	15	18	4	3	19	17	17	16	5	7	4	4	12	13
6	20	16	18	14	39	37	1	1	22	26	18	12	6	13	5	4	18	14
7	13	10	12	11	41	46	2	1	29	35	9	6	8	9	3	7	13	15
8	14	12	9	9	40	42	1	1	11	15	3	3	11	10	9	8	17	13
9	13	14	8	7	44	46	2	1	16	19	4	3	12	11	9	11	8	9
10	17	16	8	8	50	48	2	2	23	25	4	5	10	11	12	10	10	12
11	14	15	8	9	46	45	3	3	27	31	5	6	10	9	9	11	13	9
12	13	12	10	8	44	43	4	3	28	26	6	6	12	11	11	12	6	4
13	13	13	9	10	45	43	4	4	24	22	6	8	11	10	7	7	3	3
14	12	14	9	10	44	48	4	5	21	21	6	7	11	9	6	5	2	2
15	14	13	12	9	45	45	5	5	54	48	16	14	11	13	6	5	2	3
16	12	10	8	7	47	45	6	5	22	20	15	15	14	12	3	4	3	3
17	9	8	6	8	44	42	6	6	19	19	16	18	12	10	6	6	2	2
18	8	8	9	7	38	36	7	5	21	22	17	17	13	11	4	5	3	2
19	8	9	7	5	36	34	5	7	20	22	16	15	10	9	4	3	2	1
20	8	10	3	4	30	26	6	8	23	24	16	18	7	5	3	2	1	1
21	9	7	3	3	23	22	6	6	21	18	16	16	6	5	4	3	1	1
22	6	5	2	3	20	19	5	6	16	16	17	18	6	5	4	4	1	6
23	6	5	2	3	16	15	7	5	17	15	19	18	5	4	4	3	1	1
24	7	6	5	6	17	18	4	3	18	16	16	14	4	3	4	6	1	1
25	7	5	8	8	20	17	4	2	19	15	16	13	3	2	5	3	1	1
AVR	**10.84**	**10.32**	**8.08**	**7.8**	**31.76**	**31.24**	**4.08**	**3.76**	**20.8**	**20.72**	**11.72**	**11.28**	**8.2**	**7.72**	**5.64**	**5.52**	**6.32**	**5.88**
Overall AVG		**10.58**		**7.94**		**31.5**		**3.92**		**20.76**		**11.5**		**7.96**		**5.58**		**6.1**

	MFC 19		**MFC 20**		**MFC 21**		**MFC 22**		**MFC 23**		**MFC 24**		**MFC 25**		**MFC 26**		**MFC 27**	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	20	18	6	5	11	10	10	8	12	11	12	10	12	9	18	14	20	18
3	19	17	7	6	12	11	9	11	11	10	8	7	5	7	15	13	17	17

4	20	20	5	4	12	13	13	11	9	9	8	8	7	5	14	14	18	15
5	19	18	4	3	14	13	12	10	6	5	6	6	5	4	14	12	17	16
6	21	24	5	5	15	15	9	7	7	6	5	5	3	4	13	13	18	18
7	20	24	5	7	11	13	5	6	6	7	5	4	4	3	13	9	11	12
8	30	28	4	3	11	15	7	6	8	5	5	4	3	3	8	7	13	12
9	25	24	4	3	17	13	5	5	3	2	5	5	4	3	6	8	14	12
10	24	26	3	5	11	13	6	8	2	2	6	4	4	3	13	12	11	10
11	31	29	6	5	14	9	9	7	3	5	5	8	4	4	13	12	10	11
12	25	34	5	4	8	4	6	9	4	6	9	11	5	4	13	14	11	12
13	15	16	2	3	9	10	9	7	4	5	9	8	3	3	12	10	13	12
14	16	14	3	3	7	7	9	8	5	4	20	18	3	3	11	10	6	7
15	16	15	3	2	7	8	9	9	4	3	20	26	3	2	11	11	6	8
16	14	12	3	3	7	7	10	11	3	2	30	32	2	3	10	11	10	8
17	11	14	4	3	8	10	10	11	3	3	34	36	2	3	9	7	6	6
18	15	17	4	4	9	8	10	11	3	2	33	30	2	2	8	9	5	5
19	14	13	3	2	5	6	8	7	3	2	27	25	2	2	8	7	5	3
20	17	15	2	2	3	2	8	5	2	2	24	21	2	2	8	6	4	3
21	12	9	1	2	3	2	4	3	2	2	24	21	1	3	7	5	1	2
22	10	20	3	4	3	3	2	2	3	2	18	15	5	3	7	7	4	5
23	15	14	2	3	2	2	3	2	3	2	12	10	1	1	5	4	5	4
24	13	12	3	4	2	2	3	2	3	2	6	8	1	0	4	5	5	4
25	11	10	3	2	3	2	3	2	2	2	6	5	0	1	3	4	8	5
AVR	**17.32**	**17.72**	**3.6**	**3.48**	**8.16**	**7.92**	**7.16**	**6.72**	**4.44**	**4.04**	**13.48**	**13.08**	**3.32**	**3.08**	**9.72**	**8.96**	**9.52**	**9**
Overall AVG		**17.52**		**3.54**		**8.04**		**6.94**		**4.24**		**13.28**		**3.2**		**9.34**		**9.26**

	MFC 19		**MFC 20**		**MFC 21**		**MFC 22**		**MFC 23**		**MFC 24**		**MFC 25**		**MFC 26**		**MFC 27**	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	20	18	6	5	11	10	10	8	12	11	12	10	12	9	18	14	20	18
3	19	17	7	6	12	11	9	11	11	10	8	7	5	7	15	13	17	17
4	20	20	5	4	12	13	13	11	9	9	8	8	7	5	14	14	18	15

5	19	18	4	3	14	13	12	10	6	5	6	6	5	4	14	12	17	16
6	21	24	5	5	15	15	9	7	7	6	5	5	3	4	13	13	18	18
7	20	24	5	7	11	13	5	6	6	7	5	4	4	3	13	9	11	12
8	30	28	4	3	11	15	7	6	8	5	5	4	3	3	8	7	13	12
9	25	24	4	3	17	13	5	5	3	2	5	5	4	3	6	8	14	12
10	24	26	3	5	11	13	6	8	2	2	6	4	4	3	13	12	11	10
11	31	29	6	5	14	9	9	7	3	5	5	8	4	4	13	12	10	11
12	25	34	5	4	8	4	6	9	4	6	9	11	5	4	13	14	11	12
13	15	16	2	3	9	10	9	7	4	5	9	8	3	3	12	10	13	12
14	16	14	3	3	7	7	9	8	5	4	20	18	3	3	11	10	6	7
15	16	15	3	2	7	8	9	9	4	3	20	26	3	2	11	11	6	8
16	14	12	3	3	7	7	10	11	3	2	30	32	2	3	10	11	10	8
17	11	14	4	3	8	10	10	11	3	3	34	36	2	3	9	7	6	6
18	15	17	4	4	9	8	10	11	3	2	33	30	2	2	8	9	5	5
19	14	13	3	2	5	6	8	7	3	2	27	25	2	2	8	7	5	3
20	17	15	2	2	3	2	8	5	2	2	24	21	2	2	8	6	4	3
21	12	9	1	2	3	2	4	3	2	2	24	21	1	3	7	5	1	2
22	10	20	3	4	3	3	2	2	3	2	18	15	5	3	7	7	4	5
23	15	14	2	3	2	2	3	2	3	2	12	10	1	1	5	4	5	4
24	13	12	3	4	2	2	3	2	3	2	6	8	1	0	4	5	5	4
25	11	10	3	2	3	2	3	2	2	2	6	5	0	1	3	4	8	5
AVR	**17.32**	**17.72**	**3.6**	**3.48**	**8.16**	**7.92**	**7.16**	**6.72**	**4.44**	**4.04**	**13.48**	**13.08**	**3.32**	**3.08**	**9.72**	**8.96**	**9.52**	**9**
Overall AVG		**17.52**		**3.54**		**8.04**		**6.94**		**4.24**		**13.28**		**3.2**		**9.34**		**9.26**

	MFC 19		**MFC 20**		**MFC 21**		**MFC 22**		**MFC 23**		**MFC 24**		**MFC 25**		**MFC 26**		**MFC 27**	
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	20	18	6	5	11	10	10	8	12	11	12	10	12	9	18	14	20	18
3	19	17	7	6	12	11	9	11	11	10	8	7	5	7	15	13	17	17
4	20	20	5	4	12	13	13	11	9	9	8	8	7	5	14	14	18	15
5	19	18	4	3	14	13	12	10	6	5	6	6	5	4	14	12	17	16

6	21	24	5	5	15	15	9	7	7	6	5	5	3	4	13	13	18	18
7	20	24	5	7	11	13	5	6	6	7	5	4	4	3	13	9	11	12
8	30	28	4	3	11	15	7	6	8	5	5	4	3	3	8	7	13	12
9	25	24	4	3	17	13	5	5	3	2	5	5	4	3	6	8	14	12
10	24	26	3	5	11	13	6	8	2	2	6	4	4	3	13	12	11	10
11	31	29	6	5	14	9	9	7	3	5	5	8	4	4	13	12	10	11
12	25	34	5	4	8	4	6	9	4	6	9	11	5	4	13	14	11	12
13	15	16	2	3	9	10	9	7	4	5	9	8	3	3	12	10	13	12
14	16	14	3	3	7	7	9	8	5	4	20	18	3	3	11	10	6	7
15	16	15	3	2	7	8	9	9	4	3	20	26	3	2	11	11	6	8
16	14	12	3	3	7	7	10	11	3	2	30	32	2	3	10	11	10	8
17	11	14	4	3	8	10	10	11	3	3	34	36	2	3	9	7	6	6
18	15	17	4	4	9	8	10	11	3	2	33	30	2	2	8	9	5	5
19	14	13	3	2	5	6	8	7	3	2	27	25	2	2	8	7	5	3
20	17	15	2	2	3	2	8	5	2	2	24	21	2	2	8	6	4	3
21	12	9	1	2	3	2	4	3	2	2	24	21	1	3	7	5	1	2
22	10	20	3	4	3	3	2	2	3	2	18	15	5	3	7	7	4	5
23	15	14	2	3	2	2	3	2	3	2	12	10	1	1	5	4	5	4
24	13	12	3	4	2	2	3	2	3	2	6	8	1	0	4	5	5	4
25	11	10	3	2	3	2	3	2	2	2	6	5	0	1	3	4	8	5
AVR	**17.32**	**17.72**	**3.6**	**3.48**	**8.16**	**7.92**	**7.16**	**6.72**	**4.44**	**4.04**	**13.48**	**13.08**	**3.32**	**3.08**	**9.72**	**8.96**	**9.52**	**9**
Overall AVG		**17.52**		**3.54**		**8.04**		**6.94**		**4.24**		**13.28**		**3.2**		**9.34**		**9.26**

	MFC 28		**MFC 29**		**MFC 30**	
Days	**M**	**E**	**M**	**E**	**M**	**E**
1	0	0	0	0	0	0
2	27	25	10	9	16.8	14.4
3	23	22	8	7	12	12
4	21	19	6	3	13.2	14.4
5	19	20	7	5	20.4	19.2
6	21	18	6	7	21.6	14.4
7	24	21	7	6	10.8	7.2

8	15	16	8	6	3.6	3.6
9	18	17	5	4	4.8	3.6
10	17	16	3	3	4.8	6
11	16	15	3	4	6	7.2
12	17	19	6	5	7.2	7.2
13	19	17	4	4	7.2	9.6
14	19	21	3	4	7.2	8.4
15	19	17	3	3	19.2	16.8
16	18	17	2	2	18	18
17	19	19	1	0	19.2	21.6
18	21	22	2	4	20.4	20.4
19	17	15	3	3	19.2	18
20	13	12	2	2	19.2	21.6
21	13	15	2	2	19.2	19.2
22	20	16	4	4	20.4	21.6
23	13	18	5	4	22.8	21.6
24	19	26	4	5	19.2	16.8
25	14	12	4	2	19.2	15.6
AVR	**17.68**	**17.4**	**4.32**	**3.92**	**14.064**	**13.536**
Overall AVG		**17.54**		**4.12**		**13.8**

Appendix 2: Daily Voltage For Optimization With Different Resistors

	OPEN CIRCUIT VOLTAGE (OCV)											
	MFC 1		**MFC 2**		**MFC 3**		**MFC 4**		**AVG**		**Overall**
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**AVG**
1	237	241	218	242	226	223	278	198	**240**	**226**	**232.9**
2	224	246	285	318	271	241	190	250	**243**	**264**	**253.1**
3	218	206	314	309	268	367	294	275	**274**	**289**	**281.4**
4	156	169	315	327	388	393	297	305	**289**	**299**	**293.8**
5	284	218	325	335	392	394	249	317	**313**	**316**	**314.3**

	ACROSS 100kΩ										
	MFC 1		**MFC 2**		**MFC 3**		**MFC 4**		**AVG**		**Overall**
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**AVG**
1	118	126	144	153	125	139	161	178	**137**	**149**	**143**
2	121	162	160	165	146	189	111	146	**135**	**166**	**150**
3	126	113	173	177	205	224	172	170	**169**	**171**	**170**
4	90	102	174	188	219	227	163	179	**162**	**174**	**167.8**
5	102	125	184	206	230	250	178	210	**174**	**198**	**185.6**

6	185	270	384	343	385	383	340	321	**324**	**329**	**326.4**
7	225	195	336	332	379	372	346	366	**322**	**316**	**318.9**
8	204	245	342	335	369	367	378	365	**323**	**328**	**325.6**
9	300	311	332	337	443	435	352	361	**357**	**361**	**358.9**
10	367	294	327	343	413	361	325	365	**358**	**341**	**349.4**
11	386	381	386	383	362	356	338	321	**368**	**360**	**364.1**
12	313	333	384	359	345	341	283	285	**331**	**330**	**330.4**
13	273	275	382	334	333	338	304	293	**323**	**310**	**316.5**
14	262	257	352	327	408	346	279	242	**325**	**293**	**309.1**
15	264	263	344	356	362	342	292	219	**316**	**295**	**305.3**
16	307	345	286	312	377	327	284	228	**314**	**303**	**308.3**
17	308	269	240	284	309	321	322	308	**295**	**296**	**295.1**
18	297	309	256	268	219	293	320	316	**273**	**297**	**284.8**
19	343	355	310	373	299	301	314	353	**317**	**346**	**331**
20	307	286	276	285	266	257	322	332	**293**	**290**	**291.4**
21	286	261	215	235	258	250	330	337	**272**	**271**	**271.5**
22	281	312	195	204	233	229	332	341	**260**	**272**	**265.9**
23	302	272	195	224	244	245	334	333	**269**	**269**	**268.6**
24	304	307	209	215	245	257	352	354	**278**	**283**	**280.4**
25	252	245	245	246	273	264	316	314	**272**	**267**	**269.4**
Max V(mV)									**368**	**361**	**364.1**
Min V(mV)									**240**	**226**	**232.9**

6	123	154	242	226	250	261	217	223	**208**	**216**	**212**
7	140	126	220	222	248	241	234	240	**211**	**207**	**208.9**
8	133	160	220	224	241	243	254	252	**212**	**220**	**215.9**
9	193	198	224	229	290	294	250	253	**239**	**244**	**241.4**
10	233	192	218	227	298	250	246	267	**249**	**234**	**241.4**
11	260	251	252	256	250	252	255	234	**254**	**248**	**251.3**
12	216	228	272	261	254	255	210	221	**238**	**241**	**239.6**
13	194	186	226	237	245	258	215	209	**220**	**223**	**221.3**
14	172	182	249	232	284	250	193	188	**225**	**213**	**218.8**
15	185	185	305	251	256	258	198	142	**236**	**209**	**222.5**
16	206	207	251	317	225	242	164	172	**212**	**235**	**223**
17	217	201	174	185	224	185	240	239	**214**	**203**	**208.1**
18	162	188	190	221	198	218	246	242	**199**	**217**	**208.1**
19	235	370	284	296	247	235	241	260	**252**	**290**	**271**
20	230	213	208	233	221	221	258	272	**229**	**235**	**232**
21	211	203	179	177	208	209	240	272	**210**	**215**	**212.4**
22	211	193	151	132	201	197	270	259	**208**	**195**	**201.8**
23	193	209	141	168	181	189	263	267	**195**	**208**	**201.4**
24	204	216	147	153	175	182	264	238	**198**	**197**	**197.4**
25	223	218	161	165	190	194	225	223	**200**	**200**	**199.9**
Max V(mV)									**254**	**290**	**271**
Min V(mV)									**135**	**149**	**143**

ACROSS 50kΩ

	MFC 1		**MFC 2**		**MFC 3**		**MFC 4**		**AVG**		**Overall**
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**AVG**
1	73	85	86	92	75	88	97	108	**83**	**93.3**	**88**
2	88	108	110	112	102	132	83	111	**96**	**116**	**105.8**
3	83	78	118	122	149	145	125	121	**119**	**117**	**117.6**

ACROSS 25kΩ

	MFC 1		**MFC 2**		**MFC 3**		**MFC 4**		**AVG**		**Overall**
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**AVG**
1	37	42	39	43	29	35	43	46	**37**	**42**	**39.25**
2	39	54	48	58	47	67	39	57	**43.3**	**59**	**51.13**
3	42	37	57	62	74	82	64	65	**59.3**	**62**	**60.38**

4	66	78	126	139	162	175	127	140	**120**	**133**	**126.6**
5	77	85	137	152	173	184	135	161	**131**	**146**	**138**
6	95	107	167	155	184	189	161	167	**152**	**155**	**153.1**
7	94	82	148	142	175	164	173	171	**148**	**140**	**143.6**
8	87	107	146	150	175	165	177	180	**146**	**151**	**148.4**
9	132	138	157	151	195	203	179	173	**166**	**166**	**166**
10	147	151	148	156	224	176	169	186	**172**	**167**	**169.6**
11	145	148	148	159	204	195	183	176	**170**	**170**	**169.8**
12	154	162	190	202	181	190	166	170	**173**	**181**	**176.9**
13	142	138	177	183	199	205	159	146	**169**	**168**	**168.6**
14	134	131	190	185	211	182	142	141	**169**	**160**	**164.5**
15	135	135	199	198	178	194	150	133	**166**	**165**	**165.3**
16	161	139	205	184	184	177	139	137	**172**	**159**	**165.8**
17	163	160	105	140	172	190	184	194	**156**	**171**	**163.5**
18	137	143	147	154	164	166	194	199	**161**	**166**	**163**
19	165	184	158	192	168	169	197	235	**172**	**195**	**183.5**
20	177	167	173	158	160	148	202	227	**178**	**175**	**176.5**
21	179	155	155	122	175	183	205	222	**179**	**171**	**174.5**
22	150	141	113	105	173	152	222	223	**165**	**155**	**159.9**
23	116	138	95	111	129	142	202	217	**136**	**152**	**143.8**
24	140	138	100	104	118	119	199	185	**139**	**137**	**137.9**
25	141	138	112	109	125	121	161	167	**135**	**134**	**134.3**
Max V(mV)									**179**	**195**	**183.5**
Min V(mV)									**83**	**93.3**	**88**

4	34	36	61	65	81	87	66	68	**60.5**	**64**	**62.25**
5	38	40	66	73	92	100	66	86	**65.5**	**75**	**70.13**
6	43	48	83	75	96	93	82	83	**76**	**75**	**75.38**
7	47	39	80	70	89	89	88	90	**76**	**72**	**74**
8	42	49	72	71	89	78	93	99	**74**	**74**	**74.13**
9	57	59	71	73	108	99	97	103	**83.3**	**84**	**83.38**
10	62	70	60	76	98	94	110	99	**82.5**	**85**	**83.63**
11	80	82	85	89	112	114	105	107	**95.5**	**98**	**96.75**
12	77	81	95	99	116	115	105	93	**98.3**	**97**	**97.63**
13	90	87	107	102	113	123	97	86	**102**	**100**	**100.6**
14	85	64	91	98	129	121	75	73	**95**	**89**	**92**
15	73	78	105	101	122	109	88	88	**97**	**94**	**95.5**
16	114	74	108	88	122	110	87	82	**108**	**89**	**98.13**
17	108	90	104	89	84	112	94	118	**97.5**	**102**	**99.88**
18	89	85	103	97	91	93	97	99	**95**	**94**	**94.25**
19	93	85	94	92	105	106	105	130	**99.3**	**103**	**101.3**
20	82	85	85	96	89	86	125	129	**95.3**	**99**	**97.13**
21	92	90	71	74	94	101	120	134	**94.3**	**100**	**97**
22	82	73	58	57	86	84	119	121	**86.3**	**84**	**85**
23	65	72	48	56	72	79	119	130	**76**	**84**	**80.13**
24	76	78	44	47	56	56	112	94	**72**	**69**	**70.38**
25	78	78	52	51	58	58	85	81	**68.3**	**67**	**67.63**
Max V(mV)									**108**	**103**	**101.3**
Min V(mV)									**37**	**42**	**39.25**

ACROSS 10kΩ

	MFC 1		**MFC 2**		**MFC 3**		**MFC4**		**AVR**		**Overall**
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**AVG**

ACROSS 5kΩ

	MFC 1		**MFC 2**		**MFC 3**		**MFC 4**		**AVR**		**Overall**
Days	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**M**	**E**	**AVG**

1	17	19	18	21	16	23	22	25	**18**	**22**	**20.13**
2	21	27	26	28	25	33	21	30	**23**	**29.5**	**26.38**
3	21	18	29	31	38	43	33	35	**30**	**31.8**	**31**
4	17	19	30	34	42	45	33	31	**31**	**32.3**	**31.38**
5	17	18	32	35	50	51	32	44	**33**	**37**	**34.88**
6	20	12	38	37	48	49	41	46	**37**	**36**	**36.38**
7	19	19	37	35	47	43	43	48	**37**	**36.3**	**36.38**
8	21	23	39	36	47	52	50	50	**39**	**40.3**	**39.75**
9	27	32	37	39	57	52	54	57	**44**	**45**	**44.38**
10	38	37	42	38	68	49	64	52	**53**	**44**	**48.5**
11	37	36	40	44	54	52	56	53	**47**	**46.3**	**46.5**
12	39	35	46	49	56	53	51	49	**48**	**46.5**	**47.25**
13	46	39	52	53	54	57	52	45	**51**	**48.5**	**49.75**
14	36	30	53	52	60	55	42	38	**48**	**43.8**	**45.75**
15	38	38	59	57	55	61	44	47	**49**	**50.8**	**49.88**
16	38	38	31	47	73	59	43	49	**46**	**48.3**	**47.25**
17	49	39	33	48	48	50	44	50	**44**	**46.8**	**45.13**
18	43	42	40	42	55	57	52	55	**48**	**49**	**48.25**
19	45	45	47	57	59	60	58	65	**52**	**56.8**	**54.5**
20	43	38	47	41	47	46	56	63	**48**	**47**	**47.63**
21	36	35	37	28	43	51	66	58	**46**	**43**	**44.25**
22	34	27	27	21	45	40	60	62	**42**	**37.5**	**39.5**
23	22	30	21	23	44	46	56	60	**36**	**39.8**	**37.75**
24	32	33	20	22	26	27	41	42	**30**	**31**	**30.38**
25	32	31	25	23	30	31	45	43	**33**	**32**	**32.5**
Max V(mV)									**53**	**56.8**	**54.5**
Min V(mV)									**18**	**22**	**20.13**
									Overall AVG V(mV)		**40.62**

1	10	11	9	11	7	**8**	**11**	10	**9.25**	**10**	**9.625**
2	12	12	13	16	11	14	10	12	**11.5**	**14**	**12.5**
3	10	9	14	14	19	20	16	16	**14.8**	**15**	**14.75**
4	8	9	14	15	20	22	16	17	**14.5**	**16**	**15.13**
5	7	10	16	16	23	26	16	19	**15.5**	**18**	**16.63**
6	9	10	21	20	24	27	20	25	**18.5**	**21**	**19.5**
7	8	9	17	20	23	21	21	24	**17.3**	**19**	**17.88**
8	14	11	20	17	23	25	27	26	**21**	**20**	**20.38**
9	13	16	19	18	27	23	29	28	**22**	**21**	**21.63**
10	22	15	17	17	26	21	28	30	**23.3**	**21**	**22**
11	24	22	20	21	26	25	34	28	**26**	**24**	**25**
12	16	16	19	26	23	24	23	23	**20.3**	**22**	**21.25**
13	22	21	27	26	27	31	31	24	**26.8**	**26**	**26.13**
14	19	13	25	20	32	23	20	18	**24**	**19**	**21.25**
15	16	17	24	16	36	29	22	22	**24.5**	**21**	**22.75**
16	19	19	14	29	36	31	23	28	**23**	**27**	**24.88**
17	23	14	20	17	33	30	32	31	**27**	**23**	**25**
18	19	20	16	18	27	29	23	25	**21.3**	**23**	**22.13**
19	21	23	25	33	33	34	37	39	**29**	**32**	**30.63**
20	18	18	21	18	18	20	31	37	**22**	**23**	**22.63**
21	19	15	18	14	20	16	33	28	**22.5**	**18**	**20.38**
22	74	13	10	12	17	18	31	34	**33**	**19**	**26.13**
23	12	13	10	11	16	18	27	31	**16.3**	**18**	**17.25**
24	12	13	9	11	10	13	23	22	**13.5**	**15**	**14.13**
25	15	13	13	12	16	14	23	23	**16.8**	**16**	**16.13**
Max V(mV)									**33**	**32**	**30.63**
Min V(mV)									**9.25**	**10**	**9.625**

ACROSS 100kΩ

MFC1		MFC 2		MFC 3		MFC 4		AVG		Overal
M	E		E	M	E	M	E	M	E	AVG
118	126	144	153	125	139	161	178	**137**	**149**	**14**
121	162	160	165	146	189	111	146	**135**	**166**	**15**
126	113	173	177	205	224	172	170	**169**	**171**	**17**
90	102	174	188	219	227	163	179	**162**	**174**	**167.**
102	125	184	206	230	250	178	210	**174**	**198**	**185.**
123	154	242	226	250	261	217	223	**208**	**216**	**21**
140	126	220	222	248	241	234	240	**211**	**207**	**208.**
133	160	220	224	241	243	254	252	**212**	**220**	**215.**
193	198	224	229	290	294	250	253	**239**	**244**	**241.**
233	192	218	227	298	250	246	267	**249**	**234**	**241.**
260	251	252	256	250	252	255	234	**254**	**248**	**251.**
216	228	272	261	254	255	210	221	**238**	**241**	**239.**
194	186	226	237	245	258	215	209	**220**	**223**	**221.**
172	182	249	232	284	250	193	188	**225**	**213**	**218.**
185	185	305	251	256	258	198	142	**236**	**209**	**222.**
206	207	251	317	225	242	164	172	**212**	**235**	**22**
217	201	174	185	224	185	240	239	**214**	**203**	**208.**
162	188	190	221	198	218	246	242	**199**	**217**	**208.**
235	370	284	296	247	235	241	260	**252**	**290**	**27**
230	213	208	233	221	221	258	272	**229**	**235**	**23**
211	203	179	177	208	209	240	272	**210**	**215**	**212.**
211	193	151	132	201	197	270	259	**208**	**195**	**201.**
193	209	141	168	181	189	263	267	**195**	**208**	**201.**

204	216	147	153	175	182	264	238	**198**	**197**	**197.**
223	218	161	165	190	194	225	223	**200**	**200**	**199.**

Comparison of estimated and different generated voltages

	Estimated highest average voltage	41.83
	Highest optimized voltage generated	54.5
Actual	Lowest optimized voltage generated	20.13
	Highest un-optimized voltage generated	34.32
Actual	Lowest un-optimized voltage generated	7.76
% Reduction from expected maximum		**2.89%**
% Increase from expected maximum		**30.29%**
% Increase from maximum of un-optimized		**58.80%**

Appendix 2B: Result of physicochemical analysis before and after treatment

Result of physicochemical analysis before and after treatment

S/N	Parameters	Sample before treatment	Sample after treatment	Sample (control)
1	pH	7.10	8.33	7.37
2	NO_3^+ (mg/l)	28.00	19.33	23.33
3	PO_4^{3+} (mg/l)	2.34	1.83	2.02
4	NH_4^+ (mg/l)	2.77	1.52	2.23
5	BOD(mg/l)	1705.33	1383.33	1583.33
6	COD (mg/l)	5311.67	3643.33	1196.67
7	TDS (mg/l)	1110.00	1350.00	4699.67