KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

PERFORMANCE ANALYSIS OF ELECTRODE MATERIALS (ACTIVATED CARBON AND CARBON BUTTS) IN MICROBIAL FUEL CELLS USING DOMESTIC WASTEWATER

BY

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Philosophy degree and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of the degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT

The study investigated the performance of activated carbon from palm kernel shells and carbon butts as electrode materials in microbial fuel cells (MFCs) with faecal sludge and grey water as inoculum sources. It particularly sought to examine the power generation and wastewater treatment potential of the selected electrode materials when applied in the MFC technology. The motivation for this work is as a result of the growing demand for decentralized power generation and wastewater treatment systems especially for rural household and schools. The study was conducted through the examination of performance data from MFCs operating with the selected electrode materials and inoculum through the application of electrical, electrochemical and biochemical techniques. The study established that faecal sludge and grey water formed efficient biofilms containing electrogenic bacteria such as *Geobacter sp.* which initiates substrate oxidation for the release of electrons to the electrode material. It further established that activated carbon from palm kernel shells can be efficiently applied in MFCs since it generated power densities of up to 1.74W/m^3 which is comparable to the carbon paper (standard) by up to 86%. Carbon butts were inefficient in MFCs generating negligible power densities of up to 0.001W/m^3. Also organic substrate removal efficiencies of up to 72% were achieved by MFCs operating with the activated carbon.

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LIST OF ABBREVIATIONS

AC	Activated carbon
AC	Alternating current
AC-FS	Activated carbon with faecal sludge
AC-GW	Activated carbon with grey water
ADP	Adenosine diphosphate
AEM	Anion exchange membrane
ATP	Adenosine triphosphate
BES	Bio-electrochemical system
BPM	Bipolar membrane
CB	Carbon butts
CE	Coulombic efficiency
CEM	Cation exchange membrane
CHP	Combined heat and power
CNTs	Carbon nanotubes
COD	Chemical oxygen demand
CP	Carbon paper
CP-FS	Carbon paper with faecal sludge
CP-GW	Carbon paper with grey water
DC	Direct current
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
ECG	Electricity Company of Ghana
EIS	Electrochemical Impedance Spectroscopy
EMF	Electromotive force
EPA	Environmental Protection Agency
FS	Faecal sludge
GW	Grey water
M	Marker used
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell

MFC	Microfiltration membrane
MW	Megawatts
PCR	Polymerase chain reaction
PEM	Proton exchange membrane
PTFE	Polytetrafluoroethylene
RVC	Reticulated vitreous carbon
SEM	Scanning electron microscopy
UFM	Ultrafiltration membrane
VALCO	Volta Aluminium Company
VRA	Volta River Authority

CHAPTER 1. INTRODUCTION

1.1 Introduction

Electrical energy generation in Ghana

Electrical energy is an indispensable form of energy needed in every home for lighting, heating and operating electronic appliances. In industries it is used in operating most machinery and also for heating and lighting purposes. It is generated using several forms of renewable and non-renewable electricity generation systems.

Ghana has an installed electricity generation capacity of 2846.5MW from 12 Generation plants. This includes 3 Hydro plants namely the Akosombo, Bui and Kpong Power plants. These Hydro plants generate a combined total of 1580MW of electricity. These plants are supported by 8 Thermal plants which use fossil fuel sources in the form of light crude oil, natural gas and distillate fuel oil as fuel for generating power. The Thermal plants deliver a total of 1248MW. A Solar facility is also available which delivers a total of 2.5MW. [1]

The use of Hydro and Solar Power plants by the nation to generate over 56% of its energy is a very commendable effort in the promotion of the use of clean, renewable and sustainable energy sources globally. More is still expected though due to the wealth of options available to the nation still in the renewable energy sector. This is in reference to the abundant all year winds in the coast for wind energy and sunshine for solar energy in the northern sector of the nation. There is also great potential in the development of bioenergy especially from the various forms of domestic and industrial wastes generated. Harnessing these resources efficiently would enable the nation reach a remarkable 100% renewable energy usage in electric power generation.

The need for the replacement of the thermal facilities with renewable sources has been worsened by consistent periods of power rationing in the years 2012, 2013, 2014 and early 2015 due to various difficulties including the high cost in natural gas and light crude oil supply. An energy deficit therefore exists caused: by the use of thermal facilities which rely on unreliable and depleting fossil fuels; the lack of supply to some rural communities; and the current energy demands by consumers as against the supply from providers.

Utility providers in Ghana have stated that their major challenges include; increasing demand by existing customers, high customer population growth, rapid expansion of rural networks, unavailability of natural gas and increases in world market prices of distribution materials. These challenges have become detrimental to the expansion of electricity to more rural communities [2]. People in these communities would have to resort to more expensive off-grid alternatives in the form of diesel generators. Others who cannot afford diesel generators would have to use firewood and lanterns. There is the need therefore to offset the current energy deficits with efficient, low cost and renewable energy generation systems applicable in both homes and schools especially in rural communities.

Such systems would directly respond to the Renewable Energy Policy goals of Ghana. The goals includes: achieving a 10% contribution of modern renewables (excluding large hydro and wood fuels) in the electricity generation mix by 2020; reducing the demand on wood fuels from 72% to 50% by 2020; and promoting the development and use of other biomass technologies including biogas, biofuels and waste-to-energy [3].

Wastewater management in Ghana

Wastewater generated in Ghana is primarily from domestic effluent, industrial effluent and urban run-off. Domestic wastewater can be grouped as grey water and black water. Grey water refers to wastewater from washrooms, kitchens and laundries whiles black water contains urine, faecal matter and flush water from toilets. The total amount of grey and black wastewater produced in urban Ghana is estimated at 280 million m^3 annually. It is estimated that urban wastewater generation in Ghana will increase from 530,346 m^3/day in 2000 to 1,452,383 m^3/day in 2020 [4].

Wastewater treatment in Ghana is very limited with less than 8% of wastewater being treated currently. Majority of available wastewater treatment facilities are used for treating domestic wastewater. The Greater Accra region alone is home to 505 of these facilities most of which are currently under the management of public institutions including hospitals, schools and security services. A faecal treatment plant located in Kumasi could treat only 5% (180 – 500 m^3/day) of the total faecal sludge produced in the city. A biological treatment plant located near the Korle Lagoon, Accra is able to handle only 8% of Accra's inner city wastewater from domestic and industrial sources. It is also estimated that only 10% of Accra's wastewater is collected for some kind of treatment [5].

Most of the known wastewater treatment facilities are either defunct or working below capacity. Efforts have been made by some industries to build their own wastewater treatment facilities but the major issue of wastewater management nationwide still persists. A review of Environmental and sanitation policies in Ghana makes little mention of wastewater treatment after wastewater is generated and collected. Also WASHcost evaluations on the cost of providing decent sanitation have consistently failed to include cost of treatment in their reports on sanitation indicating the enormous

work ahead in the area of wastewater treatment. There is the need therefore to develop simple, cost-effective, sustainable and efficient wastewater treatment systems to prevent the possible outbreak of diseases and other serious environmental hazards.

Recent research and development in bioenergy generation has pointed to a new alternative to decentralised electricity production known as a microbial fuel cell (MFC) which can provide continuous supply of electricity, while at the same time treating wastewater. A successful scale-up application of MFCs will minimize greatly the dependence on fossil fuels and would enhance wastewater treatment especially for homes and schools in rural communities. It would also promote the use of clean and renewable energy in Ghana.

The construction of an MFC will require materials which are available locally. This will enhance its economic feasibility, sustainability and maintenance. The Electrode is the key component in deciding the performance and cost of an MFC. Electrode design is the greatest challenge in making MFCs a cost-effective and scalable technology [6].

This research will look at the use of selected electrode materials available in Ghana for use in MFCs. Emphasis will be made on their properties, application and performance. The findings of this research will aid in the selection of an appropriate electrode material that could be used in the construction of a scale-up MFC unit in Ghana for power generation and wastewater treatment.

1.2 Problem Statement

There is a high demand for decentralized power generation systems especially for rural communities in Ghana. These systems will primarily be used for lighting in homes and schools. There is also the need for economical and efficient wastewater treatment

systems in the nation. In order to recommend the Microbial fuel cell technology as a viable alternative solution, it is imperative to develop simple, low cost and efficient electrode materials which is a critical component in its construction and operation. Evidently none of the electrode materials used in the technology currently is on the Ghanaian market.

1.3 Objectives of the Research

Main Objective

The purpose of the research is to examine the performance of selected local materials to be used as electrodes in Microbial Fuel Cells for power generation and wastewater treatment. This would enhance the development and sustainability of microbial fuel cells to provide continuous supply of electricity, while at the same time treating wastewater especially from rural households and schools.

Specific Objectives

- To assess the feasibility of selected domestic wastewater as inoculum sources.
- To test the feasibility of the use of activated carbon and carbon butts as electrodes in MFCs.
- To analyze the power output of an MFC operating with activated carbon and carbon butts as electrodes.
- To measure the efficiency of organic substrate removal when the selected electrodes are used

1.4 Research Questions

- What are the available electrode materials in Ghana?
- Which of these can be used efficiently in a microbial fuel cell for power generation and wastewater treatment?

- What is the performance of the MFC when these electrode materials are used?
- Are the selected electrode materials comparable with those currently in use?

1.5 Justification of Study

- There is the need to develop cost-effective and sustainable renewable energy generation systems to offset the energy deficit created by; the use of thermal facilities which rely on fossil fuels, the lack of power supply to some rural communities and the current energy demands by consumers as against supply from providers.

- There is a massive need for the development of efficient wastewater treatment methods to curb the huge environmental hazards posed by the release of large volumes of untreated wastewater generated in both rural and urban areas.

- The electrode is the key component in deciding the performance and cost of an MFC, as such the construction of an MFC would require efficient electrode materials available locally to make it more sustainable and cost-effective.

1.6 Limitations of study

- This research is limited to the use of a double chamber MFC configuration only. The use of other configurations will not necessarily yield the same results for parameters to be measured and calculated.
- Wastewater sources to be used would be from selected domestic sources. However major variations may exists in wastewaters from other sources which may not be cited as sample sources for this research. As such variations in results from the sources not cited in this work cannot be quantified.

- Wastewater characterization would be limited to parameters for which equipment and reagents are available or can be acquired with available resources.
- The field of microbiology relative to microbial fuel cells is rapidly expanding and as such various contributions made by microbes and their niche conditions are being discovered. Findings on microbes would be reported relative to samples, materials and conditions used in this research only.
- Electrical circuits vary greatly in their components and outputs. Measurements to be made during experiments would be done with the appropriate equipment and results would be relative only to the electrical circuit constructed for this research and similar circuits.

1.7 Method of the Research

This research would involve the collection of performance data on some selected electrode materials which would be used as electrodes in MFCs. Some parameters would be calculated based on data collected. Measured and calculated parameters would be analysed using electrical, electrochemical and biochemical techniques. Conclusions and derivations would be drawn from data trends, measured and calculated parameters relevant to the study.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

The study of the microbial fuel cell technology has become a major research focus by researchers globally. This is as result of the global call for renewable and sustainable forms of energy generation to replace fossil fuels due to the environmental hazards

they pose. Even though research into the technology has been ongoing since 1911 [7] focus has intensified globally only in the last two decades.

Major breakthroughs have been made in terms of understanding in microbial activity, sources of substrate, unit configurations and operating conditions. More breakthroughs are needed in enhancing the sustainability and construction cost of MFCs. The final major breakthrough still being explored is the development of a sustainable and high performing scale-up model. The succeeding sections seeks to outline the various developments made in the MFC technology with respect to microbes and electron transfer, components and their configurations, operating conditions and limitations in performance.

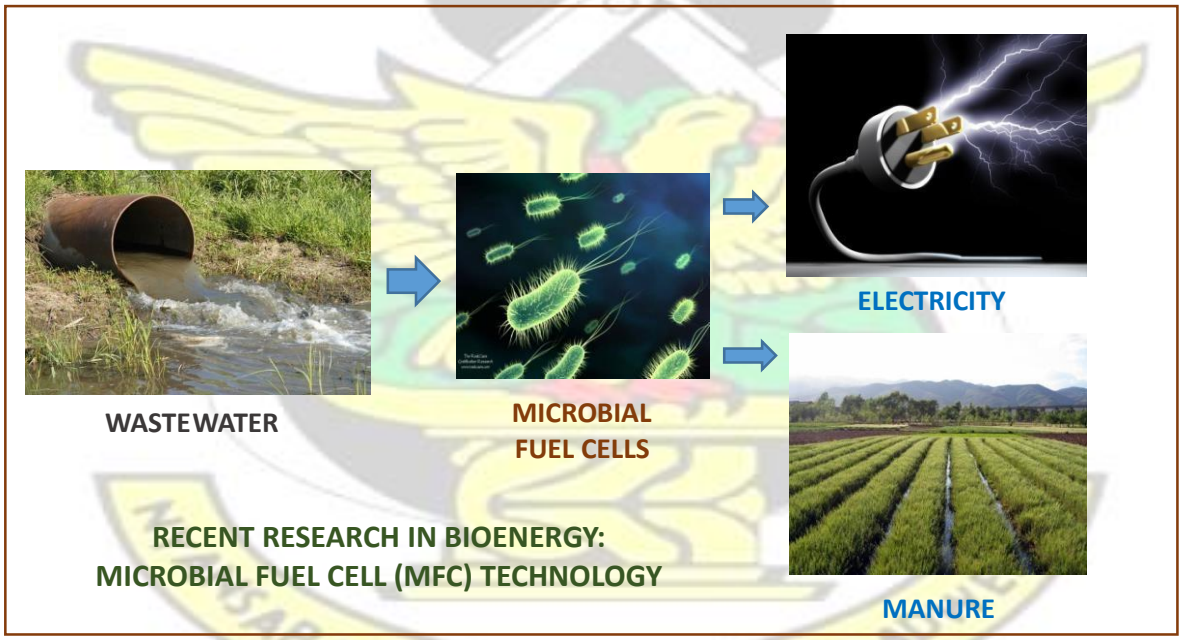


Figure 2-1. Conversion process of wastewater with an MFC

2.2 History of MFC Development

Bio-electrochemical systems (BES) are systems which are able to produce electrical current through microbial activity at their anodes. These systems include a microbial fuel cell (MFC) and microbial electrolysis cell (MEC). The system is referred to as a microbial fuel cell when the current produced is used directly as an electrical energy

source. While when the current produced is used to drive the reaction in the cathode with the addition of some external power to produce a product, the system is referred to as a microbial electrolysis cell [8].

The idea of electron transfer through the use of microbes was discovered by Potter in his research using E. coli [7]. The first actual MFC was constructed by Cohen in 1931. This technology gained prominence in the 1960s through the study of biological corrosion [9]. More focus was shifted to bio-electrochemical systems in the 1990s due to its possibilities in the production of clean energy. Microbial fuel cell technology has received enormous attention in the last two decades due to its potential in clean, renewable energy generation and wastewater treatment.

BES can be compared to conventional energy systems such as chemical fuels cells due to their power generation capabilities and also their similarities in configuration. Chemical fuel cells have higher power densities than BES due to their highly conductive chemical electrolytes. They also have similar operating conditions with respect to their performance in ambient conditions (25– 32°C). Power densities of chemical fuel cells have been found to be in the range of 10-150 kW/m^3 [10, 11]. The non-renewable nature of chemical fuels cells makes BES a likely alternative in the future if power densities are further improved.

BES can also be compared to anaerobic digestion systems due to their similarities in substrate and their capabilities in wastewater treatment along with energy generation. In both systems liquid biomass is converted by microbes to a final product (i.e. current in MFCs and methane in anaerobic digestion systems). BES have the advantage of direct conversion of substrate to electricity and other products while Anaerobic digestion systems require a combined heat and power (CHP) module to convert methane to electrical energy [12].

The capacity of microbes in MFCs under BESs to degrade a wide range of contaminants in wastewater makes it a very important tool for wastewater treatment with the production of electrical energy as a by-product. MFCs also have the potential to desalinate sea water [13], produce hydrogen [14] and hydrogen peroxide [15] and can also be applied in de-nitrification systems [16]. MFCs under BESs are receiving the most extensive attention and will be the focus of this particular research. This is due to the great need for effective wastewater treatment methods and renewable energy by developing countries such as Ghana.

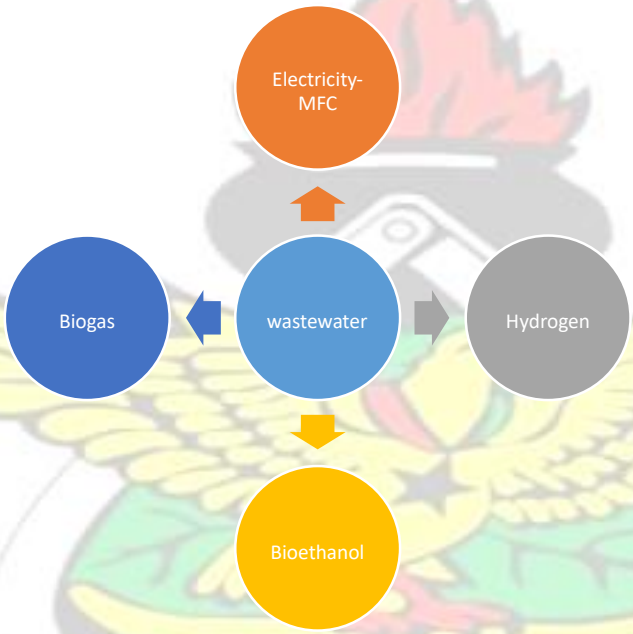


Figure 2-2. Some useful products generated from wastewater

2.3 Theoretical Framework of MFCs

A microbial fuel cell (MFC) is a bio-electrochemical system that converts chemical energy in the chemical bonds in organic compounds to electrical energy through catalytic reactions of microorganisms under anaerobic conditions. Organic matter is oxidized at the anode by bacteria and reduced at the cathode by the catholyte. Bacteria oxidise organic matter to release electrons, protons and carbon dioxide. The electrons are deposited on the anode and transferred through an external circuit through a load

to the cathode. At the cathode electrons and protons are reduced to form water in the presence of oxygen.

It is crucial to note and understand the various fundamental theories and processes that form the foundation and framework of this technology. These theories are found through studies in electrochemistry, biochemistry and electricity. The succeeding sections would seek to outline the basic theories and processes which are most relevant to this technology in these three fields of study.

2.3.1 **Electrochemistry in MFCs**

Electrochemistry deals with the chemical changes produced by electric current and with the production of electricity by chemical reactions. Electric current refers to the transfer of charge. Charge can be conducted through metals and through pure liquid electrolytes or solutions containing electrolytes. Electrodes are surfaces on which oxidation or reduction half reactions occur. They may or may not participate in the reactions. Regardless of the kind of cell, electrolytic or voltaic, the electrodes are identified as anodes and cathodes. The cathode is defined as the electrode at which reduction occurs as electrons are gained by some species. The anode is the electrode at which oxidation occurs as electrons are lost by some species.

MFCs are similar to voltaic or galvanic cells which are electrochemical cells in which spontaneous oxidation-reduction reactions produce electrical energy. The two halves of the redox reaction are separated requiring electron transfer to occur through an external circuit to obtain useful electrical energy. Unlike voltaic cells, the oxidation process in MFCs is facilitated in the anode chamber solely by microbes.

2.3.2 **Biochemistry in MFCs**

In MFCs wastewater serves as the source of bacteria for electron transfer and also the source of substrate to be oxidised. Most organisms in wastewater are microscopic in size but there are some organisms that are macroscopic such as bristle worms and insect larvae. Bacteria are the most important microbes to MFCs and in wastewater treatment in general. It is observed with a light microscope at its highest magnification.

Bacteria are the most numerous organisms with regards to number of species and total biomass. They are small, unicellular prokaryotic organisms. They can be classified by structure (morphology), response to chemical stains, nutrition, and metabolism. Most bacteria can be grouped into three basic shapes: bacillus (rod), coccus (sphere), and spirillum (spiral). The lack of separation in some species during cell division results in the formation of several arrangements of bacterial growth including colonies, tetrads, and chains or filaments.

Bacteria structure can be divided into the following, the cytoplasm and its content, the cell membrane, cell wall and other external structures (Figure 2-3). The cytoplasm is found in the cell membrane and is mostly water with a semi-fluid nature due to a suspension of carbohydrates, enzymes, inorganic ions, lipids, and proteins [17]. Within it also is found the nucleoid (instead of a nucleus), ribosomes, storage products, and endospores (Figure 2-3).

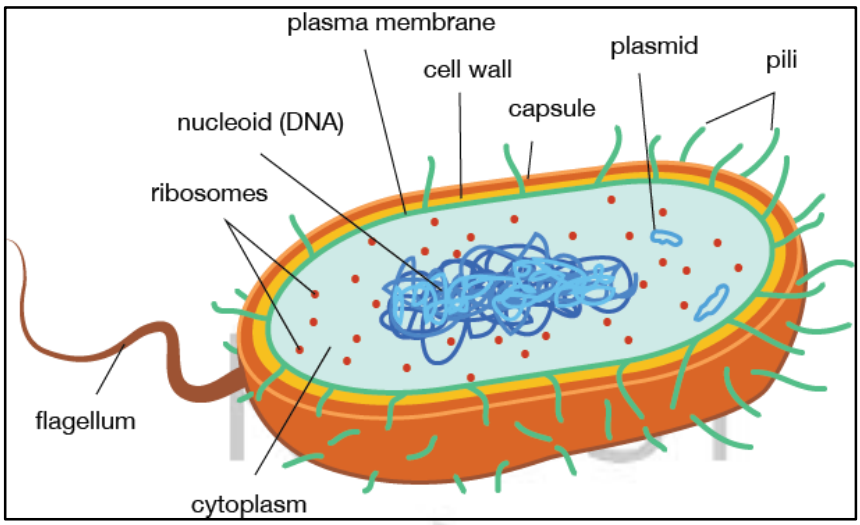


Figure 2-3. Schematic of bacteria cell structure (© 2015 Shmoop University) The nucleoid consists mainly of genetic material in a circular chromosome. The cell membrane or plasma membrane is a flexible semi-permeable membrane that surrounds the cytoplasm. The other external structures include the axial filament, capsule, flagella, glycocalyx, and pili. These structures perform specific functions. Pili or fibrils are short hollow projections of the cell membrane that extend through the cell wall into the surrounding environment (Figure 2-3). The pili are used by some bacteria for attachment to other bacteria (for reproduction) and by many bacteria for attachment to other inert surfaces (floc formation and biofilm development).

Bacteria have an absorptive means of nutrition; that is, substrates and nutrients must be water-soluble or lipid-soluble in order to enter the bacterial cell. Biochemical reactions occur inside the cell using substrates and nutrients in the presence of water. Substrates and nutrients enter the cell and wastes leave the cell by crossing the cell membrane. The cell membrane is semi-permeable and contains a layer of lipids that are hydrophobic and influences the mechanism and rates at which substances move across the membrane. Three basic mechanisms are observed diffusion, facilitated diffusion, and active transport. They are used by bacteria to transport substrates, nutrients, and wastes across the cell membrane.

Most bacteria move by the beating action of flagella. Flagella are used to move (taxis) toward substrate (chemotaxis), light (phototaxis), or oxygen (aerotaxis). Each movement is a positive taxis and is produced by concentration gradients for substrate, dissolved oxygen and light intensity. They are also used to move away from inhibitory and toxic substances. The growth of bacteria in wastewater is influenced by availability of substrates, nutrients, pH, temperature, and response to free molecular oxygen. For most bacteria the optimum pH at which they grow best is usually near neutral (pH 7). Bacteria cannot tolerate pH values below 4 or above 9.5 [18].

Bacteria can be grouped into three based on their ideal temperatures for growth and activity. These groups are psychrophiles, mesophiles, and thermophiles. Psychrophiles can grow in the range from 10 to 30°C but grow best at temperatures from 12 to 18°C. Mesophiles are the largest group of organisms and grow best at temperatures from 25 to 40°C. Some can be found in the gastrointestinal tract of humans (body temperature approximately 37°C is very favourable for growth) and also animals. They enter wastewater in large quantities through human and animal excreta. Thermophiles grow best in hot conditions at temperatures from 50 to 65°C but can also survive in the range of 35 to 75°C [17].

Bacteria can be grouped into three according to their response to free molecular oxygen (presence or absence). These groups are aerobes, anaerobes, and facultative anaerobes. Aerobes require oxygen for substrate degradation. Anaerobic bacteria do not require free molecular oxygen for substrate degradation. Facultative anaerobes have the ability to live under aerobic and anaerobic conditions [18].

Each group of organisms in a biological system has its own habitat and niche. The habitat is where the organism lives, and the niche is the role the organism performs in

the habitat (such as wastewater). Symbiotic relationships are beneficial relationships between organisms and are very critical in wastewater treatment. MFCs rely completely on the activity of bacteria for the oxidation of substrate to electrons.

2.3.3 **Electricity in MFCs**

An electric current is a flow of electrons through a conductive material from a point of negative potential to a positive potential. The flow rate of electrons is expressed in terms of an ampere. An ampere of current is said to flow at a point when one coulomb (6.24×10^{18} electrons) passes through a given point in one second [19]. The force that drives the electrons along a conducting material to cause the flow of current is called an electromotive force (EMF). The EMF or difference in potential is measured in volts. The unit of EMF is responsible for the term voltage being used in place of the terms EMF and potential difference.

Most substances with atoms bearing a number of free electrons are conductors of electricity. These include copper, iron, aluminium, silver and gold. Copper is the strongest of these as such permits the largest amount of current when wires are made of these substances with the same length and diameter. The quality of a conductor which limits the flow of electrons is referred to as resistance. Resistance is expressed in Ohms. The resistance of a conductor can be calculated when the applied voltage and resulting current is known. It can also be measured with an instrument called an Ohmmeter. A relation between the voltage, current and the resistance of a circuit exists. This is expressed in terms of Ohm's law as I=V/R, where I is the current flow, V the applied voltage and R the resistance in the circuit [20].

To form an electrical circuit through which electricity travels, a source of electricity is needed. The electricity source would have a negative terminal and a positive terminal.

The electrons will be pushed at a certain voltage from the negative terminal. The electrons would require a conducting material (e.g. copper wire). A circuit is formed when a pathway from the negative to the positive terminal through which electrons can flow is created. A load is placed in the middle of the circuit to be powered by the source of electricity. Circuits can be very complex but the simplest circuit will always have a source of electricity, a load and two wires carrying electricity between the source and the load. Electrons travel from the source through the load and back to the source.

Generally there are two types of electric current flow. These are the alternating current (AC) and direct current (DC). The AC first flows in one direction then next in the opposite direction in an established pattern. The current builds up to a peak in the forward direction of the first half cycle and then drops back to zero at the end of the same half cycle (Figure 2-4). It then reverses its direction of flow and builds up to the maximum in the reverse direction. It again builds and drops back to zero at the end of the second half cycle which completes one full cycle [19]. DC is that which flows in one direction between the negative and positive terminals rather than changing direction as in AC. Batteries, fuel cells, solar cells and most importantly MFCs produce direct current (DC).

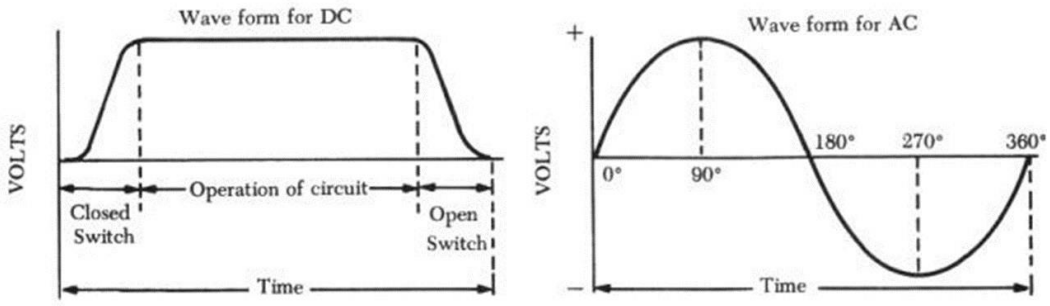


Figure 2-4. Schematic of the motion of AC and DC currents

2.4 Conceptual Framework of MFCs

Based on the theoretical framework the concept of power generation via means of microbial activity under anaerobic conditions is formulated under the following assumptions and hypothesis;

- The oxidative action of bacteria on organic substrate in wastewater under anaerobic conditions with the release of electrons forming a negative terminal.
- The deposition of electrons on to a conducting material by the bacteria and the conduction of the charge to the external circuit.
- The reduction action of electrons and protons in the cathode forming a positive terminal.
- The potential difference created between the negative and positive terminal generates a voltage which drives a resulting current through the external circuit for the generation of power.
- The conducting material (electrodes) becomes indispensable without which electron transfer between the two terminals cannot be created.

2.5 Microbial Fuel cell components

MFC designs and configurations have been modified in many ways by researchers to increase power output and also to minimize losses. The main component in all cases is an anode in an anaerobic compartment, a cathode in an aerobic compartment and in most designs a cation exchange membrane. In all configurations, substrate is oxidized by electrogenic bacteria at the anode with the release of electrons and protons to the cathode. The various configurations developed may run in fed-batch mode or continuous mode. The configurations include a two chambered MFC, single chambered MFC and tubular MFC.

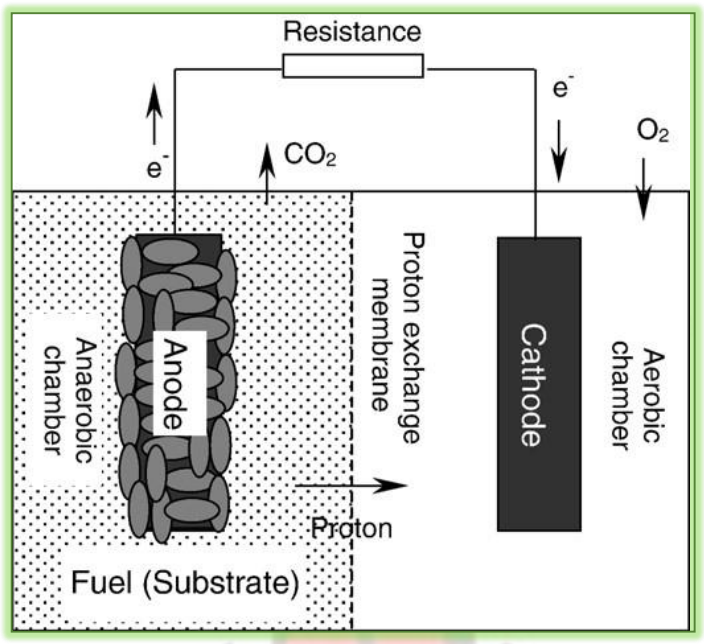


Figure 2-5 Diagram showing the typical components of an MFC [21]

2.5.1 **Anode**

The anode compartment of an MFC is primarily the compartment which contains the anode electrode and the anolyte. The anolyte in most cases contains both the inoculums and substrate. The anode is the site for substrate oxidation and electron transfer. Electron transfer at the anode is either by direct or indirect transfer mechanisms. Direct electron transfer mechanism involves the direct deposition of electrons by bacteria using its external cell structures. Indirect electron transfer mechanism requires mediators also referred to as electron shuttles. These are generated by the associated bacteria or deposited as additives into the anolyte by the operator.

Electron mediators used in MFCs include methylene blue and neutral red. Performance of these two mediators has been compared in a single chambered MFC inoculated with Pseudomonas sp. with the open circuit potential of methylene blue being twice that generated from neutral red [22]. Humic substances which are heterogenous high molecular organic materials found mainly in aquatic environments, have been reported to shuttle electrons to the electrode of MFCs [23].

Phosphate buffer solutions are often used as additives in MFCs in either the anode or cathode to maintain pH suitable for the growth of electrogenic bacteria. In some cases it is used to increase solution conductivity. The use of phosphate buffers in scale-up models would not be sustainable since large concentrations would be required which would mean an increase in the operational cost of the MFC. Other researchers have recommended bicarbonate buffer as a cost effective alternative with comparable power densities to phosphate buffers [24].

2.5.2 **Cathode**

In MFC cathodes, oxygen is generally used as the electron acceptor or catholyte due to its abundance atmospherically and also its low capital cost. Reduction kinetics for oxygen has been reported to be poor and as such catalyst are required to support electrode materials (e.g. platinized graphite) [25, 26]. Metals such as platinum, copper, gold, manganese and tungsten have been explored as catalysts but their high cost makes them less favourable.

Potassium ferricyanide has been reported to be an excellent cathodic electron acceptor for two chambered MFCs with recorded power densities eight times higher than aerated cathodes [27]. Another alternative electron acceptor to it is potassium permanganate. The use of potassium permanganate in a two-chambered MFC yielded a maximum power density of 115.60mW/m^2 which was comparatively higher than ferricyanide (25.62mW/m^2) and oxygen (10.2mW/m^2) as cathodic electron acceptors in the same study [28].

Platinized carbon cathode with oxygen, plain carbon electrode with oxygen and ferricyanide has also been compared as electron acceptors. An open circuit potential of 772mV, 751mV and 572mV was measured for ferricyanide, platinized carbon cathode with oxygen and plain carbon electrode respectively [29]. The use of platinum

coating as a catalyst on the cathode is very dominant with single chambered air cathode MFCs. This catalyst is used to correct the poor reduction rate of electrons by atmospheric oxygen which functions as the electron acceptor in this type of MFC configuration.

Bio-cathodes are associated with MFCs which use organic materials and microbes in the cathode chamber. Bio-cathodes gain oxygen for electron and proton reduction through the photosynthetic activity of some plants used [30]. Advantages of biocathodes include low construction cost due to the avoidance of abiotic substances such as electron mediators and electron acceptors. They also assist in de-nitrification processes [31].

2.5.3 **MFC Separator (membrane)**

Separators are used to physically separate the anodic and cathodic chambers to prevent the mixing of electrolytes and also to regulate the movement of ions in the MFC. An ideal separator should be able to inhibit oxygen and electrolyte transfer while permitting efficient proton transfer. Various materials have been tested with large variations in components, thickness, surface structure, configuration, material conditioning and operating conditions. The separators used in MFCs include cation exchange membrane (CEM), anion exchange membrane (AEM), bipolar membrane (BPM), microfiltration membrane (MFM), ultrafiltration membrane (UFM), salt bridge, glass fibres, porous fabrics and other coarse-pore filter materials.

The cation exchange membrane (CEM) also often referred to as proton exchange membrane (PEM) regulates the movement of protons from anode to the cathode and also prevents the movement of oxygen to the anode from cathode. The membrane is sometimes excluded in some configurations due to its high cost but its absence also

has the risk of oxygen leakage into the anode thereby reducing power output. The high cost of membranes also makes it less practicable for upscale units.

Nafion 117 (Dupont Co., USA) is the most commonly used CEM in MFCs due to its high conductivity to numerous cations. It is a perfluorosulfonic acid membrane consisting of hydrophilic fluorocarbon backbone to which is attached hydrophilic sulfonate groups. The negative charge of the sulfonate groups facilitates the transfer of protons [32]. Other examples of cation exchange membranes used in MFCs include; Ultrex CMI 7000 (Membranes Inc., USA), Selemion (Asahi Glass Co., Japan), PTFE; Polytetrafluoroethylene (Sartorius Stedim, Germany), Isopore (Millipore, USA), Zirfon [33] and Biomax (Millipore Corp., Germany).

2.5.4 **MFC Configurations**

The two chamber MFC consists of an anode and a cathode chamber separated by a cation exchange membrane. The cathode chamber of a two chambered MFC contains an electrolyte in the form of permanganate or ferricyanide which are both strong oxidizing agents. Two chambered MFCs have the advantage of easy application in denitrification systems and bio-hydrogen production. It is often constructed with plastic bottles, plastic tubes, plastic frames or glass bottles. The compartments in two chambered MFCs can be of various functional shapes and sizes. Their two compartment structure makes them less favourable for upscale even though they could be run in both batch and continuous mode [30].

Single chambered MFCs do not have a cathode compartment but have the cathode exposed to air for the reduction of protons and electrons by atmospheric oxygen [15, 34]. They have the advantage of sustainability with air as its catholyte and also a reduced internal resistance because of its single chamber [35]. Some single chambered MFCs are configured without a membrane giving it the advantage of upscale suitability

at a reduced cost [35]. The slow diffusion of oxygen to the cathode for the reduction of electrons and protons is a major concern with single chambered MFCs. Platinum has been used in some units to modify the cathode surface as a catalyst for the cathode reactions.

The continuous up-flow membrane-less tubular MFC consist of an anode at the bottom with the cathode at the top of a column separated by glass wool and glass beads instead of a membrane. This configuration can be easily scaled up for use in wastewater treatment systems but faces the major challenge of oxygen leakage to the anode [36]. Another configuration of the tubular MFC consists of an outer cathode chamber with inner concentric anode chamber. The two chambers are separated by a proton exchange membrane [37].

Another configuration of MFCs is the stacking of several small units to form a larger single unit. Stacked configuration of MFCs is a method used in increasing power output of MFCs. Several individual units are connected electrically in series or parallel depending on which parameter is to be enhanced i.e. current and voltage. Voltage is increased when the units are connected in series and current is also increased when the units are connected in parallel [38].

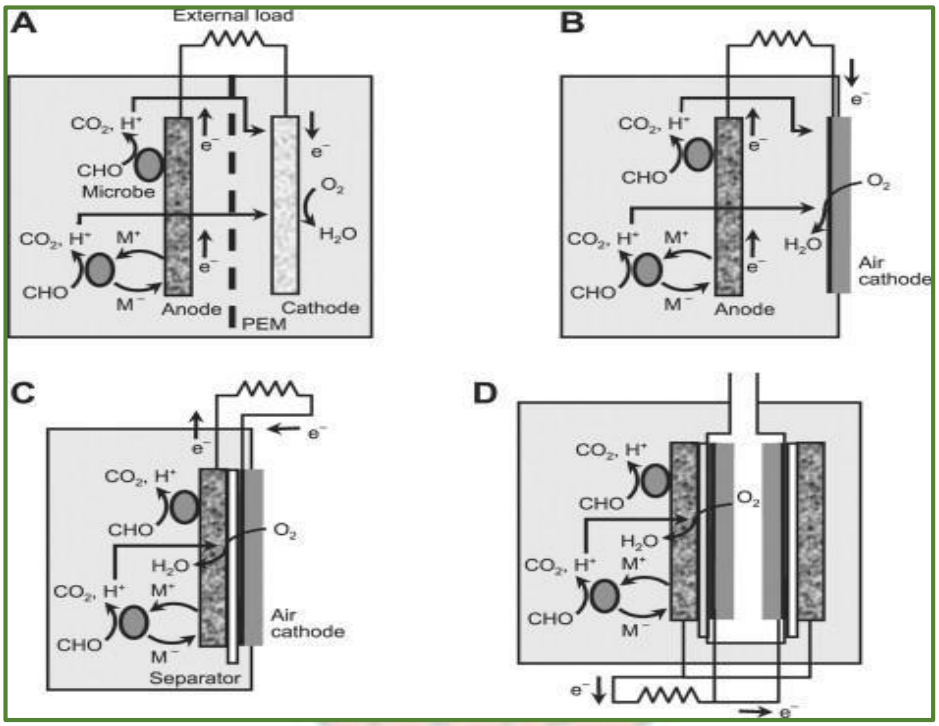


Figure 2-6. Diagram showing different configurations of MFCs [39] (A- typical two chamber MFC, B- air-cathode single chamber MFC, C- air-cathode single chamber MFC with a separator, D – Tubular MFC)

2.5.5 **Mode of Operation**

There are two commonly used operational modes in MFCs namely; batch mode (sometimes referred to as fed-batch) and continuous mode. In the batch mode, a known amount of substrate is fed into the MFC and operated until substrate is completely depleted before beginning a new cycle. In the continuous mode substrate is pumped into the MFCs without any break between cycles. The unit therefore maintains a constant concentration of substrate all throughout its period of operation. These operational modes are unique such that each has conditions specific to its performance. A property often associated with both batch mode and continuous mode include organic loading rate while hydraulic retention time is uniquely associated to the continuous mode. A major condition which affects performance under both operational conditions is substrate concentration.

2.6 Operation of an MFC

Microbes present in MFCs oxidize organic substrates at the anode releasing electrons and protons. Electrons are transferred to the anode via direct and or indirect extracellular transfer then to the cathode through an external circuit [40]. The protons move to the cathode through a cation exchange membrane if any is present. The protons and electrons are reduced at the cathode by a catholyte such as oxygen to form water. Microbes obtain their energy and carbon needed for growth through the oxidation of complex substrates this enhances the sustainability of MFCs [41]. Below are typical electrode reactions in MFCs with acetate as an example of a substrate.

Anodic reaction: $CH_3COO^- + 2H_2O \xrightarrow{microbes} 2CO_2 + 7H^+ + 8e^-$

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

2.6.1 Microbes and Biofilm development

A large range of microbes have been found to operate in MFCs for the oxidation of organic substrates. These microbes are mainly species of bacteria and are often referred to as electrogenic bacteria or electrogenes. MFCs can be operated using pure cultures or mixed cultures. Mixed cultures have the advantage of handling complex substrates such as those present in wastewater [42]. They also have the advantage of high resistance against process disturbances and also a higher power output than pure cultures [43]. The microbes are introduced into the anode of the MFC via inoculation.

The microbes in the MFC form a biofilm on the electrode surface during inoculation. The biofilm is an extracellular polymeric substance with surface adhering microbial community [44]. Anodic biofilm development on the electrode surface involves an initial bacterial attachment phase followed by the production of extracellular polymeric substances to create an environment that can sustain the structural and

organizational needs of the biofilm [45]. The most active microbial cellular activity is found in microbes in close proximity with the anode surface [46]. Bacteria should therefore be in direct contact with the solid electrode to achieve maximum current density.

Bacteria in MFCs do not gain energy from the electrode directly but rather gain energy from the movement of protons across the inner cell membrane to form a proton gradient. This drives the formation of ATP (Adenosine triphosphate) from ADP (Adenosine diphosphate) through ATPase (Adenosine triphosphatase). Energy for bacteria growth and metabolism therefore is derived from the synthesis of ATP [40].

Bacterial analysis has shown that the bacterial population on the anode biofilm are partially different from that of the wastewater used as inoculum [47, 48]. The results from a 16sRNA-DGGE analysis in a study showed that microbes involved in current generation were present in small populations in the wastewater and propagated during the MFC inoculation operation (biofilm development phase) in a selective process dependent on the enrichment conditions present [47, 49]. Gram negative pure cultures (includes Geobacter sulfurreducens, Shewanella oneidensis, Pseudomonas aeruginosa) develop thicker biofilms (40 – 50µm), higher microbial towers and produced higher current. Gram positive pure cultures (includes Clostridium acetobutylicum, Enterococcus faecium) produce thinner biofilms (10 – 20µm), smaller towers and lower current [50]. The table 2-1 is a list of microbes which have been used in MFCs.

Table 2-1. Some microbes in MFCs and their primary substrates [21].

Micro-organism	**Substrate**	**References**
Actinobacillus succinogenes	Glucose	[51]

Aeromonas hydrophila	Acetate	[52]
Alcaligenes faecalis	Glucose	[53]
Enterococcus gallinarum,	Glucose	[53]
Pseudomonas aeruginosa	Glucose	[53]
Clostridium beijerinckii	Glucose,Starch, lactate,molasses	[54]
Clostridium butyricum	Glucose, Starch, lactate, molasses	[55]
Desulfovibrio desulfuricans	Sucrose	[56]
Erwinia dissolven	Glucose	[57]
Escherichia coli	Glucose,Sucrose	[58]
Geobacter metallireducens	Acetate	[59]
Geobacter sulfurreducens	Acetate	[49]
Gluconobacter oxydans	Glucose	[60]
Klebsiella pneumoniae	Glucose	[61]
Lactobacillus plantarum	Glucose	[57]
Proteus mirabilis	Glucose	[62]
Pseudomonas aeruginosa	Glucose	[53]
Rhodoferax ferrireducens	Glucose, xylose, sucrose, maltose	[63]
Shewanella oneidensis	Lactate,pyruvate, acetate, glucose	[64]
Shewanella putrefaciens	Glucose, Lactate	[65]
Streptococcus lactis	Glucose	[57]

2.6.2 **Electron transfer mechanism of MFCs**

Microbial fuel cells oxidize organic and some inorganic substrates using bacteria to generate electric current. Microbes transfer electrons to the anode of the MFC by direct or indirect extracellular electron transfer mechanisms [66, 67, 68].

Direct electron transfer occurs through the cell outer membrane cytochrome c or through electrically conductive bacterial appendages called pilli or nanowires [70]. Electrons are transferred through the physical contact of the cell structures with the anode. Microbes identified as capable of direct electron transfer to an anode include; *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens*, and *Rhodoferax ferrireducens* [59, 71]. These microbes form bio-electrochemically active biofilms which are stable and yield high coulombic efficiencies [71, 72].

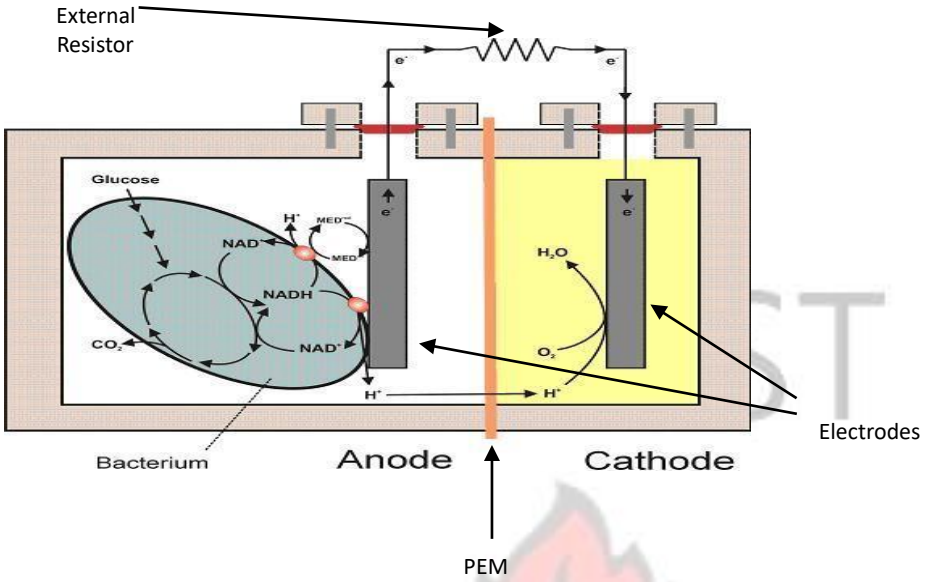


Figure 2-7. Diagram of a typical microbial fuel cell showing transfer mechanisms [69]

Indirect electron transfer involves the presence of exogenous or endogenous soluble molecules called mediators or redox shuttles which carry electrons through the extracellular aqueous matrix from the bacterial cells to the anode [73]. Mediators in MFCs serve as a shuttle between the donor bacteria and the anode for systems in which the bacteria has no cellular mechanism to deposit the electrons. The mediator should favour oxidation at the anode and reduction by the bacteria. Oxidized mediators can easily be reduced by capturing electrons found in the cell membrane. The mediators release the electrons to the anode becoming re-oxidized in the anodic bulk solution.

A very good mediator should possess the capacity to cross cell membranes easily and capture electrons from electron carriers. It should also have a high electrode reaction rate and good solubility in the anode solution [56]. It should be non-biodegradable and non-toxic especially to microbes. Examples of mediators include safranin O, resazurin, methylene blue, humic acids, neutral red, Fe(III)EDTA, thionine and athraquinone [51, 74, 75].

2.6.3 **Substrates used in MFCs**

Substrates in MFCs are considered a vital component in all MFC operations. It is completely indispensable since it is the component which is converted directly to electric current. Its choice therefore is crucial in determining the overall power output of the MFC unit being used. Substrates which have been tested in MFCs are very diverse and are mainly composed of waste materials from either domestic or industrial sources. Bacteria in MFCs have the capacity to degrade an enormous range of substrates be it organic or inorganic. These substrates include; acetate, glucose, starch, cellulose, pyridine, phenols and lipids most of which are present in domestic and industrial wastewater [76, 77, 78]. The capacity of microbes in MFCs to degrade a wide range of contaminants in wastewater makes it a very important potential tool for wastewater treatment with the production of electrical energy as a by-product.

Acetate has been the most extensively used source of substrate in MFC research for electricity generation. It is often used to induce electrogenic bacteria growth due to its inertness towards other microbial conversions such as fermentation and methanogenesis at ambient temperatures [79]. Acetate is also the end product of a number of metabolic pathways for more complex carbon sources. A power density of 506mW/m^2 has been reported for concentrations of 800mg/L COD [79]. Glucose is also a substrate commonly used in MFC research with power densities of 216W/m^3 [80] and 161mW/m^2 [81].

The lists of wastes that can be used as substrates in MFCs are still growing rapidly. Removal efficiencies of wastes currently in use are also being increased through modifications in MFC structure and configuration, operating parameters and conditions and most importantly choice of inoculums and biofilm development. Expansion in choice of substrates to include crude oil processing waste would be an enormous breakthrough in the study due to the environmental hazards it poses.

2.6.4 **Ideal and Actual Performance**

Parameters such as the power density and coulombic efficiency are often used as a means of establishing a common performance measuring tool which is applicable to all types of MFCs under any conditions in any part of the world. Power density refers to the power produced per unit anodic volume (W/m^3) or per unit anode surface area (W/m^2) of the MFC while coulombic efficiency refers to the amount of substrate recovered as electric current.

The ideal performance of an MFC is largely dependent on the electrochemical reactions that occur at the anode between the organic substrate at a low potential and the final electron acceptor at the cathode at a high potential [69]. The ideal cell voltage that can be produced is most often uncertain since electron transfer from substrate to the anode goes through a complex respiratory chain process which varies from one microbial species to another. The ideal potentials therefore are calculated using the Nernst equation from the various electrode reactions.

The actual cell potential is always lower than the ideal cell potential due to internal losses within the unit being operated. These losses include; activation losses caused by an activation energy that must be overcome by reacting species, concentration losses due to slow mass transfer of electrons and protons and Ohmic losses due to resistance to the flow of ions in electrolytes and electron flow between the electrodes [29, 34]. These losses can be minimized by taking measures such as the use of improved electrode materials and configurations, introduction of catalysts, shorter electrode spacing and the use of more conductive electrolytes [25].

The actual performance of BESs with reactor volumes larger than 1L is targeted at

1kW/m^3 which is regarded as the ideal limit for energy recovery from organic wastes [82]. Ideally anaerobic digestion systems are able to convert 1kg of COD to an average of 1kWh with power densities of 400W/m^3 when supplied organic loads of 5 to 25kg COD per m^3 reactor volume [12]. MFCs can theoretically convert 1kg of COD to 4kWh of electrical energy [12]. Development of efficient scale-up models is a major drawback in achieving the full potential from MFCs with the maximum recorded power density at 250 W/m^3 [38]. The theoretical maximum voltage of an MFC is estimated at 1.14V under open circuit conditions while the typical maximum open circuit voltage reported in a study is at 0.8V. Also the typical working voltage under a load is reported at 0.5V [83, 84].

A major hurdle associated with MFCs is the start-up time which varies from 4 to 103 days. Start-up is mainly dependent on factors which include the choice and source of inoculums and electrode materials. It is also influenced by the reactor design, operating conditions and type of substrate [85]. The operating conditions that affect the actual MFC performance include pH, temperature, organic loading rate, solution conductivity, oxygen concentration and internal losses.

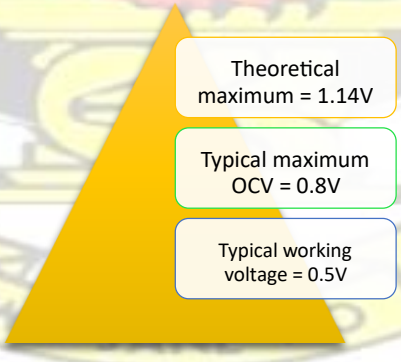


Figure 2-8. Performance pyramid of MFCs

2.7 Electrode materials in MFCs

The electrode is the key component in determining the overall performance of a Microbial fuel cell due to its influence in microbial attachment, biofilm formation and electron transfer [86]. It also influences construction cost by up to 60% [68]. Electrode design has therefore become the biggest challenge in making MFCs a cost-effective and scalable technology [6]. The development of electrode materials is crucial in any MFC and is therefore the primary aim of this study.

Different electrode materials vary in their physical and chemical properties; i.e. surface area, electric conductivity and chemical stability. They also vary in microbial attachment, electron transfer, electrode resistance and the rate of electrode surface reaction.

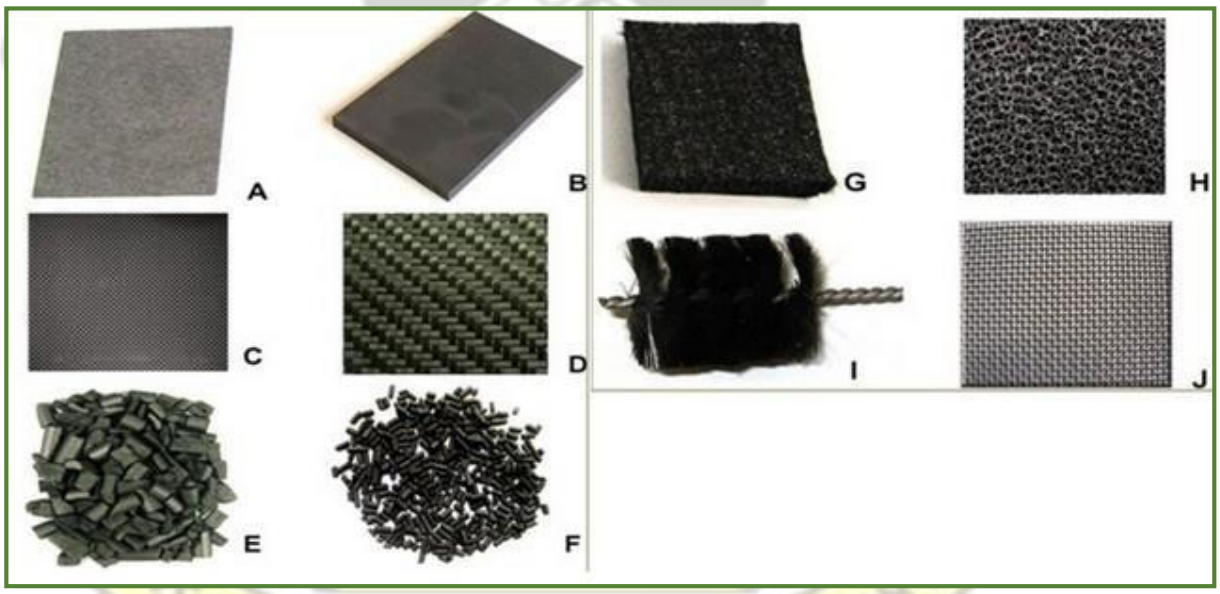


Figure 2-9. Examples of electrode materials commonly used in MFCs [87] (A-carbon paper, B-graphite plate, C-carbon cloth, D-carbon mesh, E-granular graphite, Fgranular activated carbon, G-carbon felt, H-reticulated vitrified carbon, I-carbon brush, J-stainless steel mesh)

2.7.1 **Anode Materials**

A good anode material should have good electrical conductivity, low resistance, strong bio-compatibility, chemical stability and be anti-corrosive. It should also have a large

surface area and appropriate mechanical strength and toughness [88]. Anode materials can be grouped into carbon based and metal based (non-carbon based) materials.

2.7.1.1 Carbon based anode material

Anode electrodes made from carbon materials are the most commonly used due to its excellent electrical conductivity and chemical stability. They include graphite rod, graphite fibre brush, carbon cloth, carbon paper, carbon felt and reticulated vitreous carbon (RVC). The graphite rod has the advantage of good electrical conductivity, chemical stability and low cost but a smaller surface area [89]. In a study, a maximum power output of 26 mW/m^2 was attained using sewage from the primary sedimentation tank of a treatment plant as substrate in the MFC with graphite rod in the anode [71].

The use of carbon paper and carbon cloth helps to reduce electrode spacing and also have a large surface. Carbon cloth is relatively expensive ($1000/$m^2$ in 2010) while carbon paper is fragile [90]. A single chamber MFC using carbon cloth as electrode and brewery wastewater as anode substrate yielded a maximum power density of 483 mW/m^2 [91].

In terms of structural configuration, carbon-based electrodes can be grouped into a plane structure, a packed structure and a brush structure. Materials with a plane structure include carbon paper, graphite plates and carbon cloth. Carbon paper is very thin and slightly brittle. Graphite plates have a higher strength and toughness than carbon paper. Both materials have a relatively smooth surface making it easy to measure biomass per unit surface area.

Electrode materials in packing forms are often used to increase surface area available for bacteria attachment [92]. These materials include graphite granules and granular activated carbon. Tight packing for current collection is usually encouraged to

minimize dead zones which are empty spaces found in packings [93]. Clogging is a potential disadvantage with this structure due to the spaces between granules. Graphite brush has a high surface area, high porosity and is efficient in current collection making it an ideal electrode. The brushes are made of carbon fibres cut to a set length and wound onto a twisted core consisting of two conductive titanium wires [93].

2.7.1.2 Metal based anode material

Metal based materials are used less in MFCs even though they are more conductive than carbon based. This is mainly due to their corrosive nature, higher cost and in some cases toxicity to microbes making them non-biocompatible. Stainless steel and titanium are those in this category that have been tested. The suitability of stainless steel plate was tested in an MFC as both the anode and bio-cathode electrodes. It yielded a power density of 23 mW/m^2 [94].

Highly conductive gold was used as the anode with *Geobacter sulfurreducens* as inoculum producing a steady current of 0.4-0.7mA. Although this is comparable to carbon electrodes, its practical application is very limited by its cost [95]. Titanium is also used in the form of wires in a graphite brush as a current collector but is unsuitable as a standalone anode in comparison with carbon electrodes due to its higher cost [96].

2.7.2 **Cathode Materials**

Cathode materials have a major impact in power generation in MFCs. High redox potential and ease in proton capture are important characteristics of a good cathode material. Common cathode materials used include carbon paper, graphite and carbon cloth. Platinum is often used as a highly active catalyst in modifying cathodes. The aim is to reduce the cathodic reaction activation energy and increase the reaction rate. However its high cost limits its practical application [88].

Cathode configuration also contributes to the choice of electrode material and the performance of the MFC as a whole. It also contributes to the overall internal resistance of the unit. Common configurations include air-cathode, bio-cathode and cathodes aided by chemical oxidizing agents.

2.7.3 **Electrode Modifications**

The modification of electrode materials has proven to be an effective means of improving electrode performance. It changes the physical and chemical properties to provide better microbial attachment and electron transfer. Techniques used include surface treatments, coatings, composite materials and heat treatment. In some cases a combination of treatment techniques are used.

Surface treatments (acid treatment) involves the use of physical or chemical methods to modify surface characteristics which in turn is a deciding factor affecting bacterial attachment. Ammonia gas has been used to increase power and reduce start-up time by up to 50% this increase was as a result of an increase in positive surface charge which favoured biofilm formation [97]. Acid soaking has also been reported to have caused an increase of up to 8%. Further 25% increase was recorded when the acid treatment was combined with heat treatment [98]. The improvement could be caused by an increase in specific area facilitating bacteria adhesion on the electrode. Other surface modifications include chemical vapour deposition, carburization, sintering or soak method.

The addition of surface coating materials such as carbon nanotubes (CNTs), conductive polymers, mediators and metals can also help increase performance. Electrochemical methods have shown that CNTs reduces internal resistance

facilitating electron transfer from bacteria to electrode [99]. Conductive polymers such as polypyrole have been reported to cause an increase in current densities as a result of an increase in surface area and enhanced electron collection at the anode [100].

Heat treatment is reported to aid in the removal of impurities of the electrode surface increasing its active surface area and conductivity. A carbon mesh heated in a muffle furnace at 450°C for 30 minutes resulted in a maximum power density 3% higher than that of carbon mesh cleaned with acetone. This was attributed to the active surface area and charge transfer coefficients [85].

2.7.4 **Local materials considerable as MFC electrodes**

Evidently none of the electrode materials mentioned in the previous sections is available on the Ghanaian market. Therefore constructing an MFC in our localities becomes a challenge. Materials that could be considered should meet the basic MFC electrode properties of good conductivity, bio-compatibility, chemical stability and anti-corrosion. Based on the properties listed, materials such as biochar, charcoal and scrap stainless steel can be considered. The electrodes can also be developed from other forms of domestic and industrial waste materials as examined in this research.

Table 2-2. Some electrode materials and their performance in MFCs [87]

Electrode material	Electrode size	Reactor configuration	Maximum power or current density	References
Carbon paper	22.5cm^2	Two bottle, air cathode	600mW/m^2	[101]
Carbon cloth	7cm^2	Single chamber cube air-cathode	46W/m^3	[77]
Graphite plate	1.92cm^2	Two chamber, air cathode	3290mW/m^2	[102]

Graphite plate	155cm^2	Two chamber, air cathode	1410mW/m^2	[102]
Carbon mesh	7cm^2	Single chamber cube air-cathode	45W/m^3	[85]
Activated carbon cloth	1.5cm^2	Single chamber, air-cathode	0.51mW/cm^2	[103]
Granular graphite	1.5-5mm granular diameter	Tubular MFC	90W/m^3	[43]
Graphite felt	156mL anode chamber	Two chamber MFC	386W/m^3	[104]
Reticulated vitreous carbon	97cm^2	Two chamber cylindrical MFC	170mW/m^2	[105]
Carbon brush	4cm length, 3cm in diameter	Single chamber cube air-cathode	73W/m^3	[101]
Stainless steel plate	0.12cm^2	Artificial marine MFC	23mW/m^2	[94]

CHAPTER 3. METHODOLOGY

3.1 Electrode material development

The selected electrode materials used in the study were from two carbon based sources i.e. carbon butts (CB) and activated carbon (AC). The materials were considered under the basic requirements of an MFC electrode i.e. good conductivity, bio-compatibility, chemical stability and anti-corrosion. Carbon paper (CP) which has been extensively used in MFC research was used as the standard against which the performance of the selected electrode materials would be measured. The two carbon-based materials were used in the granular form.

3.1.1 **Carbon butts (CB)**

These are spent carbon anodes from the electrolytic reduction cells in the aluminium smelting industry. They are primarily made up of calcined petroleum coke and coal tar pitch. The carbon butts used in this study in the granular form were prepared by crushing large samples of 15-20cm diameter collected as waste material from the Volta Aluminium Company (VALCO). The large samples were reduced to granules 2-4mm in diameter to minimize void spaces and improve inter-granular electron transfer.

3.1.2 **Palm Kernel shells-Activated Carbon (AC)**

The activated carbon used in the study was prepared from palm kernel shells. Palm kernel shells are the shell fractions of palm nuts left after the palm fiber and palm kernel nuts have been removed after crushing in a palm oil mill. It is often used as an energy supplement in Palm Oil mills due to its high calorific value of up to 18.8MJ/kg but in some mills it is discarded as waste material. The palm kernel shells used were collected as waste material from oil palm mills in and around the Kumasi metropolis. They were pre-treated by washing, air-drying and screening to remove any impurities. It was then pyrolyzed at 500°C for 4 hours and activated with steam at 130 – 165°C for 3 hours to increase its active surface area. The granules from the activated carbon were obtained by crushing and sieving to sizes of 2 - 4 mm in diameter from an initial size range of 10 -25 mm.

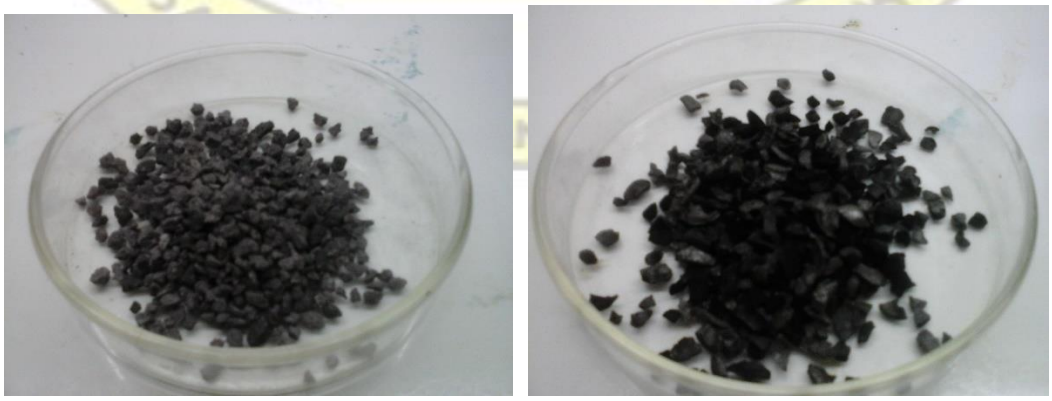


Figure 3-1.Granules of Palm kernel shell activated carbon (right) and Carbon butts (left)

3.2 Microbial Fuel cell construction and operation

3.2.1 MFC Construction

The H-shaped double chamber MFC was constructed with two cylindrical acrylic cylinders with a volume of 300 ml, which were connected with an acrylic tube with an inner diameter of 30 mm. (Centre for Bioprocess Biochemical Engineering; DTU, Denmark). A proton exchange membrane Nafion™ N117 (Fuel Cell Earth LLC, USA) with an area of 9.62 cm^2 was placed between the chambers. The two chambers were tightened by using rubber rings and screw bolts.

As described in the preceding section electrode materials used as anode and cathode materials for the study included;

- Toray Carbon paper (TGPH-120, Permeas USA, Inc., E-TEK division) of size 4cm x 9cm with a thickness of 0.35mm. (Surface area: 0.0144m^2)
- Carbon butt granules (VALCO, Ghana) of size 2mm-4mm, (Bulk density: 1.02g/cm^3)
- Palm kernel shell Activated carbon (AC) granules (Dept. of Chemical Eng. KNUST, Ghana) of size 2mm-4mm in diameter. (Bulk density: 0.57g/cm^3)

Stainless steel rods was used as current collector from the electrode materials in both the anode and cathode electrolytes. They were connected using copper wires to a 1000 Ohm resistor to form an electrical circuit. Reference electrode in the form of Ag/AgCl (BASi, UK) was installed in the anode of the MFC. The anode was operated under anaerobic conditions while the cathode was operated under aerobic conditions.

Figure 3-2. The H-shaped Double chambered MFCs used in the study 3.2.2

MFC Operation

The anolyte used in the study was wastewater with sodium acetate as primary substrate. Sodium acetate was used as primary substrate since it is easily fermentable by most electrogenic bacteria. The wastewater was used as inoculum and a secondary source of substrate. Duplicate MFC reactors were operated to ensure that the results obtained during experiments were repeatable and reproducible.

Wastewater of volume 220mL was filled into the anode chamber. Sodium acetate was added as substrate with a concentration of 33.29mM (2.73g/L). The anode chamber was sealed off tightly with a plastic lid to create an anaerobic environment for bacterial growth. The cathode chamber was filled with 200mL Potassium ferricyanide solution as electron acceptor of concentration 0.10M (32.90g/L). The cathode chamber was partially sealed to allow atmospheric oxygen into the system to support electron reduction. The system was operated in a fed-batch mode and at ambient conditions (i.e. temperatures, 25-32°C). The experiments were operated in several cycles.

3.3 Wastewater sampling

Domestic wastewater in the form of grey water and faecal sludge from the KNUST wastewater treatment plant and the Kumasi Metropolitan Assembly Landfill site were the sources of samples to be used as inoculums. The grey water was composed primarily of wastewater from laundries, washrooms, water closest flushes and kitchens. The faecal sludge was composed primarily of faecal matter, urine and water closest flushes. Wastewater was sampled for use in the MFCs solely for purposes of inoculation and not as sole source of organic substrate. This was due to the limitations posed by sample variability. The organic substrate contribution of each wastewater sampled and used was however determined and noted. The sample technique used was systematic with the collection of composite samples from each source. Samples were collected based on guidelines from Standard Methods for the Examination of Water and Wastewater 1998.

The KNUST wastewater treatment plant receives 35.7m^3/day during lean periods (i.e. when the university is on vacation) and 71.4m^3/day during peak periods (i.e. when the university is in session) [106]. The facility receives approximately 21.21ML in an entire year with the two generation periods alternating twice throughout the year i.e. peak periods are January to May and September to November while the lean periods are June to August and December. Sample variability was not a major concern since the wastewater generation sources were consistent all throughout the year. Sample size and frequency was therefore limited to 6 composite samples (3 samples per generation period) over a period of six months. Samples were collected from the influent of the primary sedimentation tank.

The KMA Landfill site at Dompoase receives 180 to 500m^3/day of faecal sludge from various locations in the Kumasi Metropolis [4]. The faecal sludge is mainly received

from public septic facilities, private septic facilities and pit latrines. Due to the enormous sample source size, sample variability was expected to be very high due to variations in organic load. The purposes of the sampling was purely for inoculation purposes therefore variations in organic load was not a major concern. Sample size and frequency was therefore limited to 6 composite samples over a period of six months. Samples were collected from the influent of the first stabilization pond.

Samples collected received no form of pre-treatment before its use as inoculum in the MFCs. The samples were characterized on the basis of COD (Chemical Oxygen Demand) (Hanna COD HR kit), solution conductivity (VWR CO310 meter), pH (VWR pH110 meter), temperature (VWR pH110 meter), total dissolved solids (VWR CO310 meter), total solids, total nitrogen (Hanna Total Nitrogen HR Test vials), total phosphorus (Hanna Checker Phosphorus HR kit), ammonia content (Hanna Checker Ammonia HR kit) and salinity (VWR CO310 meter). The wastewater characterization was limited to these parameters since these were the most relevant to the study. The inoculation was done under anaerobic conditions which favours the growth of electrogenic bacteria.

3.4 Performance analysis methods

The overall performance of an MFC can be summed up into two namely power generation and substrate removal. The limiting factor such as internal impedance within the system is also measured to assess the extent to which power generation and substrate removal are affected in a particular unit. Performance analysis methods used were focused on three key areas, namely electrical methods, electrochemical methods and biochemical methods.

3.4.1 **Electrical methods**

The cell voltage across a 1000 Ω resistor which was used as load and was measured every 15 minutes using a data logger (PicoLog ADC20 Data logger, PicoTech-UK) connected to a computer. The current and power were calculated as follows;

From Ohm's law: $V = I \times R$, where V is cell voltage, I is the current and R is the external resistance

Therefore: $I = V \div R$; Power (P) was calculated as: $P = I \times V$

The current and power densities were calculated as the current and power per unit electrode surface area and also as the current and power per unit anode active volume respectively.

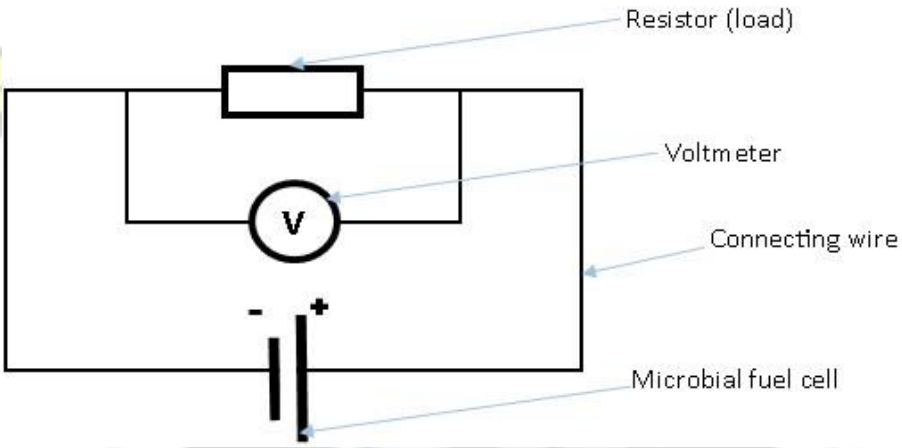


Figure 3-3. Circuit diagram of the experimental set-up used

3.4.2 **Electrochemical method**

The total power output of an MFC is limited by factors such as the high internal resistance. It is therefore important to analyse the various components of the resistance using electrochemical methods so as to adopt measures to minimize them and improve power. The electrochemical method used was electrochemical impedance spectroscopy (EIS). This method was used to evaluate the overall internal resistance.

EIS is the most preferred method for measuring internal resistance since it measures the dynamic response of the system without interrupting the MFC operation. The method requires the use of a potentiostat with EIS software on a computer to obtain impedance data. EIS involves superimposing a sinusoidal signal at varied frequencies over a wide range with small amplitude on the applied potential of a working electrode [93]. It deals with the variation of total impedance in a complex plane as shown in Nyquist and Bode plots.

Nyquist and Bode plots are commonly used to represent the measurement of impedance. In a Nyquist plot, the real part of the impedance is represented in the xaxis and the imaginary part of the impedance is represented on the y-axis while each point in the plot corresponds to the impedance at one frequency. The electrode impedance measurements are carried out at the open circuit potential of the MFC to obtain detailed system information [93]. The overall internal resistance determined via EIS is composed of polarization resistance, ohmic resistance and concentration resistance. The EIS technique was performed as outlined in Appendix A.3.

3.4.3 **Biochemical methods**

These methods are primarily aimed at examining substrate removal efficiencies. These efficiencies include COD removal, coulombic efficiency, total nitrogen removal, total phosphorus removal and ammonia removal. Chemical Oxygen Demand (COD) is a measure of the organic substrate concentration in the sample under analysis. It is obtained as the mass of oxygen consumed per litre of solution by dichromate, a strong oxidizing agent (USEPA Method 8000). *COD removal efficiency* was calculated as the change in COD measured at the beginning (initial) and end (final) of each test cycle expressed as a percentage of the initial COD at the beginning of the test cycle i.e. (Logan, 2007)

$$COD\ removal\ efficiency = \frac{Final\ COD - Initial\ COD}{Initial\ COD} \times 100\%$$

Coulombic efficiency (CE) is a measure of the number of coulombs recovered as electric current compared to the theoretical maximum number of coulombs that can be recovered from the organic substrate initially in the system. The coulombic efficiency was calculated by integrating the measured current relative to the theoretical current based on organic substrate consumed.

The CE for the fed-batch system was calculated as;

$$CE = \frac{M_s \int_0^{t_b} I\ dt}{F b_{es} v_{An} \Delta c}$$

Where M_s is the molecular weight of the substrate, F is Faradays's constant, Δc is the change in substrate concentration over the batch cycle (COD is used as a measure of substrate concentration), t_b is the time over which change in substrate concentration occurs, b_{es} is the moles of electrons produced per mol of oxidized substrate, v_{An} is the volume of anodic solution and I is the average current produced within the operating time [93].

3.5 Microbial Analysis

Two techniques were employed in the analysis of the electrode biofilm species, biofilm morphology and structure, and also the electrode surface structure. Denaturing Gradient Gel Electrophoresis (DGGE) was used in the detection of individual bacterial species present within the biofilm. Scanning Electron Microscopy (SEM) was used to study biofilm structure and morphology and also the electrode surface structure in the absence of a biofilm. Attached biofilms were isolated for analysis by cutting a piece of carbon paper (< 2% of anode material) and also by collecting some granules (< 3% of anode material) for AC and CB.

3.5.1 **Denaturing Gradient Gel Electrophoresis (DGGE)**

16sRNA-DGGE Analysis is a technique that provides characterization of a microbial community diversity and composition. The analysis employs the separation of double stranded DNA (deoxyribonucleic acid) fragments that are identical in length but different in sequence (separation of DNA fragments produced after PCR amplification). The technique takes advantage of the differences in the stability of base pairs (i.e. G-C pairs as against A-T pairs).

The stages of the analysis included;

- DNA Extraction from the anode biofilm on the electrodes
- PCR (Polymerase Chain Reaction) Analysis to amplify the extracted DNA
- DGGE (Denaturing Gradient Gel Electrophoresis) Analysis to detect the individual species DNA

DNA Extraction from the anode samples

The DNA Extraction was carried out using the Power Biofilm Isolation kit (MO-BIO, USA). The procedure was undertaken using the manufacturer's extraction protocol. DNA samples were extracted from all the electrode samples and their concentrations measured using the Synergy HT Multi-detection microplate reader (BioTek, USA). The minimum concentrations required for a proper PCR analysis were > 2ng/μL.

Polymerase Chain Reaction (PCR) Analysis

PCR analysis is a biochemical technique used to amplify a single copy or a few copies of a piece of DNA in several orders in magnitude to generate numerous copies of a particular DNA sequence. The method involves thermal cycling consisting of repeated heating and cooling cycles for DNA melting and enzymatic replication of the DNA. The key components used in the selective replication and amplification include

primers containing sequences complementary to the target region, and a DNA polymerase which enzymatically assembles new DNA strands from nucleotides by using a single stranded DNA as template.

In the PCR analysis of the electrode biofilm DNA extract, Phusion polymerase was used with 16sFw and 16s-Rv as primers in the first PCR tests (for full length 16s rRNA). The second PCR (for specific region for DGGE analysis) test involved the use of Dream Taq polymerase with GC 338f and 518r as primers. Thermal cycling was performed using the Biorad C1000 Thermo-cycler (BIORAD, USA). The PCR products were examined via electrophoresis in agarose gel to assess the presence of well-defined Gel band patterns as an indicator of a successful or failed PCR analysis.

Denaturing Gradient Gel Electrophoresis (DGGE)

The DCODE Universal Mutation Detection System was used in the DGGE analysis using the PCR products. The DCODE Universal Mutation Detection protocol was used for the preparation and electrophoresis of the gel. Each PCR product is added to a lane of a polyacrylamide gel (8% W/V) containing a linear gradient of DNA denaturants ranging from 45% to 60% (urea and formamide). The DGGE was run in a 1x Trisacetate-EDTA (TAE) buffer at 38V for 16h and at 60 degrees. Double-stranded DNA is partially denatured as it migrates through the gel. Separation of differing nucleotide sequences within a sample occurs due to the decreased mobility of partially melted double-stranded DNA molecules migrating through the gel under the influence of an applied electric field.

During the electrophoresis, DNA partially denatures in discrete regions called melting domains. Melting domains are stretches of base-pairs with an identical melting temperature. The melting temperature of each domain is sequence specific i.e. each

unique nucleotide sequence melts differently. Once a domain reaches its melting temperature, the migration of that domain halts. This allows domains with different nucleotide sequences to stop migrating at different positions in the gel. Separation is therefore based on the difference in melting behaviour of individual melting domains as the DNA denatures. Differing sequences of DNA (from different bacteria) will denature at different denaturant concentrations resulting in a pattern of bands. Each band theoretically representing a different bacterial population present in the community.

After the electrophoresis, the gel was stained in TAE buffer (pH 8.0) containing SYBR Green I (1:10000, Bio-Medicine, USA) for 30 minutes before the DNA bands were observed using a UV Illuminator (Biorad Gel Doc 2000, USA). A UV light was passed through the DGGE Gel so as to observe and take images of the Gel bands via the UV illuminator software for further analysis. The Gel Bands produced from the Electrode biofilm samples were analysed against a marker containing bands for 9 known electrogenic species.

3.5.2 **Scanning Electron Microscopy (SEM)**

Scanning Electron Microscopy is a technique that provides characterization of a microbial community diversity and also aids in the examination of microstructure morphology of materials. The SEM is able to achieve magnifications greater than the optical lens since the light source in optical lenses is replaced with high energy electron beam. It utilizes a focused electron beam to scan across the surface of the specimen systematically, producing large numbers of signals which are converted to a visual signal. The images are produced as a result of the interactions between the electron beam and the specimen. The SEM technique was used in the study of anode biofilm

structure and morphology. It was also used in the study of electrode surface structure and its interactions with the anode biofilm.

In order to examine biofilms on the anode electrode surfaces, the anode electrode material (< 3%) was removed without touching its surface. The samples were fixed in 5% v/v glutaraldehyde and 2% paraformaldehyde in 0.1 M Na-acetate in deionized water (pH 7.2). After fixation, the samples were dehydrated in aqueous ethanol using: 20%, 40%, 60%, 80%, 90% and 100% for 20 min in each solution. Subsequent dehydration was performed in 33%, 66% and 100% acetone in ethanol before samples were dried to critical point using Agar E3000 critical point dryer (Agar Scientific, Stansted, UK) with liquid CO_2 as drying agent. Following coating with gold using an Emitech E5000 sputter coater, samples were observed using a Philips XL30 ESEM scanning electron microscope at 50 to 10000 times of magnification [107]. The technique involved the procedures outlined in Appendix A5.

CHAPTER 4. RESULTS AND DISCUSSION

The experimental study involved the selection of an efficient inoculum by operating replicate MFCs with grey water and faecal sludge over 3 unique inoculation cycles with carbon paper as electrode material. The most efficient inoculum was selected and operated with activated carbon and carbon butts to assess their suitability in biofilm development. The electrode materials were run in further post-inoculation cycles to assess their peak performance in electricity generation in an MFC. The influent and effluent wastewater from the MFCs were then analysed for efficiency of selected wastewater constituents' removal. The results from each stage of the study are discussed in detail in the succeeding sections

4.1 Start-up and electricity production for selected inoculum sources The use of bacteria in the generation of electricity in MFCs begins from the start-up phase referred to as inoculation. It is within this stage that electrochemically active biofilm is formed on the anode surface for electron transfer. Microbes capable of electron transfer are very diverse with wastewater being an ideal and rich source of bacteria. Faecal sludge and grey water were the selected sources of wastewater used as inoculum in the study.

The selected sources were both inoculated into the anodes of the MFCs for biofilm formation (bacteria attachment) on the electrode material surface. After 6-11 days of voltage-time profile observation (Fig. 4-1, 4-2), a maximum average voltage and power density of 720mV and 1.98W/m^3 respectively were obtained for faecal sludge from 3 unique inoculation cycles (Table 4-1, Fig. 4-2). Also the maximum average voltage and power density for grey water was 556mV and 1.23W/m^3 respectively from 3 unique inoculation cycles (Table 4-1, Fig. 4-1). The average length of inoculation for faecal sludge was 10 days while grey water was 4 days.

The high voltage produced by faecal sludge relative to the grey water is attributed to two key factors i.e. the high organic load and high solution conductivity of faecal sludge (Appendix B4). The high organic load ensures efficient substrate utilization by electrogenic bacteria in the biofilm. The high solution conductivity favours the movement of ions and the activity of electrogenic mediators in the bulk solution. This reduces internal resistance allowing an efficient flow of electrons and protons. From the voltages and power densities obtained, faecal sludge and grey water were successfully used as inoculums for the generation of electric current after 3 unique cycles of operation.

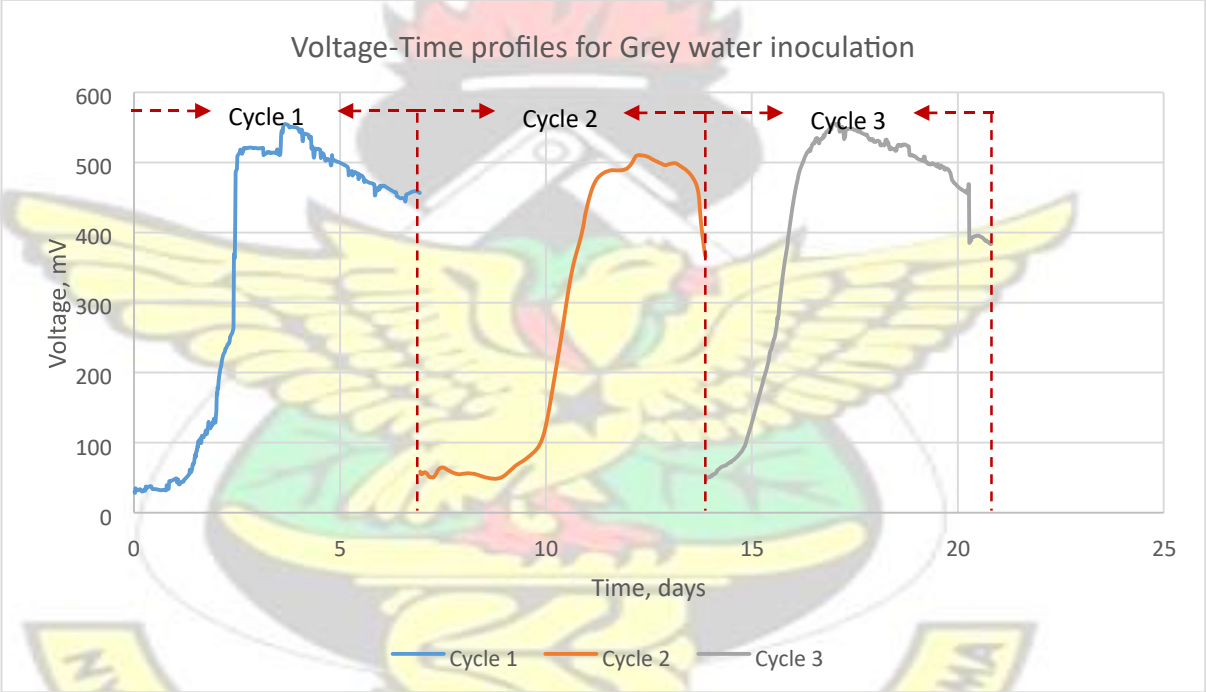


Figure 4-1 Electricity generation during grey water inoculation in three unique cycles

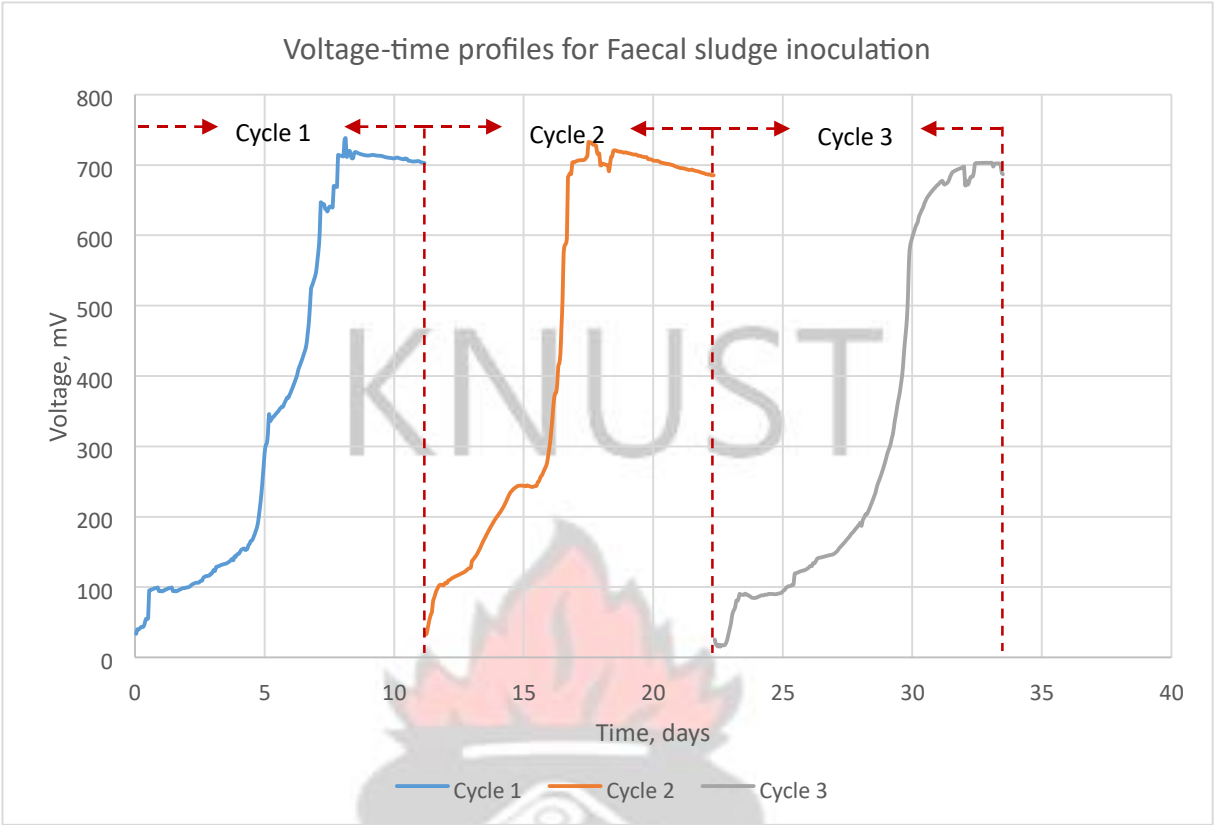


Figure 4-2 Electricity generation during faecal sludge inoculation in three unique cycles

Table 4-1 Summary of results for Inoculation Performance

Inoculum used	Cycle	*Length of startup (Days)	*Maximum voltage under load (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Power density (W/m^3)
Grey water	1	3	555	38.53	21.37	1.23
	2	4	510	35.45	18.10	1.04
	3	3	556	38.59	21.44	1.24
Faecal sludge	1	9	718	49.89	35.85	2.06
	2	8	720	50.04	36.06	2.08
	3	10	703	48.82	34.33	1.98

(*"length of start-up" refers to total time used to achieved maximum voltage during inoculation; "Maximum voltage under load" refers to maximum voltage obtained under the 1000 Ohm resistor.)

4.2 Microbial and electrode surface analysis of wastewater biofilms

The anodic biofilms in the MFCs were sampled at the end of the inoculation cycle for 16sRNA-DGGE (Denaturing gradient gel electrophoresis) analysis in combination with a marker and also for SEM analysis. Bacteria biofilm and electrode surface structure were observed using Scanning electron microscopy (SEM). The DGGE band patterns of the samples analysed are in Figure 4-3 and their gene bank matches in Table 4-2.

4.2.1 16sRNA-DGGE of selected wastewater biofilms

A marker containing 9 bacterial species isolated from a dominant MFC biofilm was used as a standard reference tool to identify electrogenic species present in biofilms from faecal sludge and grey water MFC inoculations. The bacterial species found in faecal sludge and grey water anodic biofilms were identical. They included *Geobacter sulfurreducens, Enterobacter cancerogenus, Thermanaerovibrio acidaminovorans, Desulfuromonas acetexigens.*

Geobacter sulfurreducens identified from the biofilms is the most dominant electrogenic species known in MFCs for direct electron transfer i.e. belonging to a dissimilatory metal reducing microorganisms group. The mixed culture identified in the anode biofilms were involved in a symbiotic breakdown of organic compounds for the enhanced oxidation by other electrogenic species incapable of direct organic substrate breakdown. T*hermanaerovibrio acidaminovorans* is known for the fermenting of amino acids while *Enterobacter cancerogenus* is known for the fermenting of glucose to simpler forms for further oxidation by other electrogenic species.

The identical DGGE band patterns for faecal sludge and grey water can be traced to the initial composition of the samples from their sources. Grey water was composed

of wastewater from laundries, washrooms and some water closest flushes. The faecal sludge was mainly composed of faecal matter from water closest flushes, pit latrines and urinals. The bacteria identified were from the faecal matter present in both sources. The horizontal gel band labels in figure 4-3 represent samples (1-6) analysed and the Marker (M) while the vertical gel band labels indicate the 9 known species of bacteria from the marker used. Carbon paper inoculated with the faecal sludge and grey water is labelled horizontally as 4 and 5 respectively.

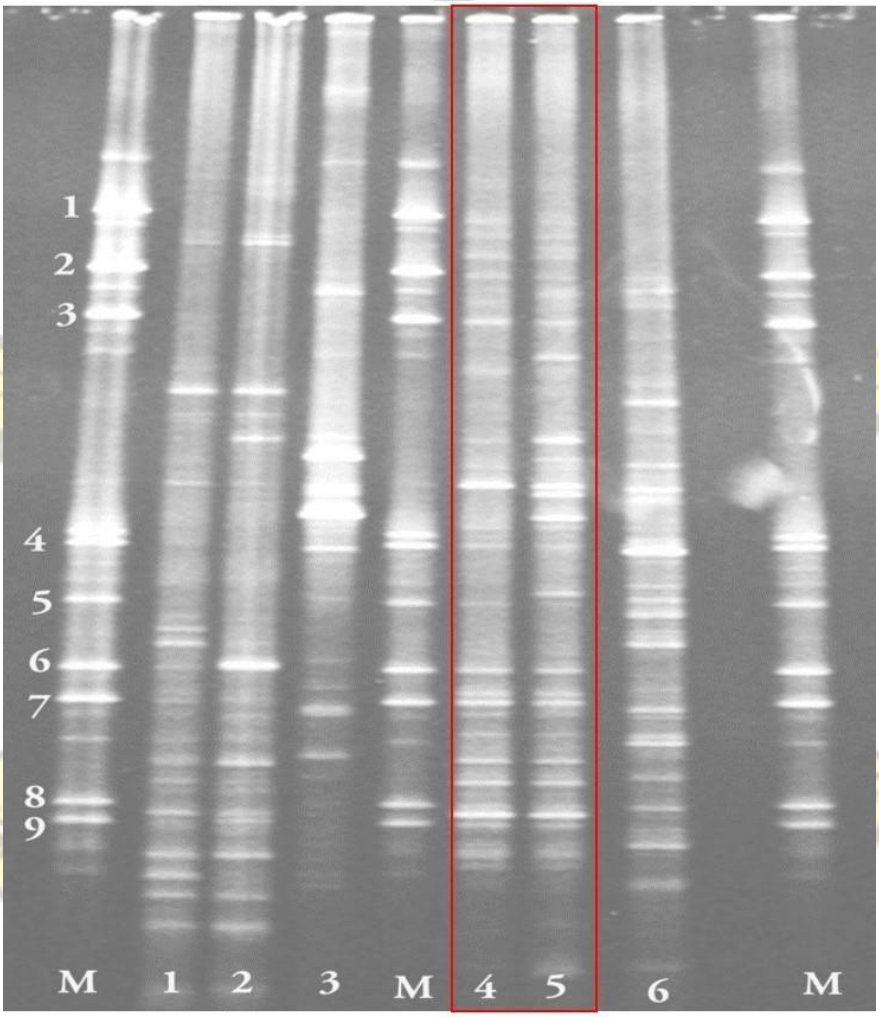


Figure 4-3. An image of the DGGE Gel product from samples analysed

Table 4-2 Gene bank matches for samples tested

SAMPLE	GENE BANK MATCH	SPECIES CHARACTERISTICS

4 (Carbon paper-Grey water)	*Thermanaerovibrio Acidaminovorans (3)*	Thermophilic anaerobe fermenting a variety of amino acids.
	Desulfuromonas Acetexigens (9)	Obligate anaerobic and sulphurreducing eubacterium oxidizing acetate as carbon resource
	Shigella flexneri (4)	Facultative anaerobe failing to ferment lactose or de-carboxylate lysine
	Enterobacter Cancerogenus (6)	Facultative anaerobes fermenting glucose as carbon sources.
	Geobacter Sulfurreducens (7)	Metal-reducing anaerobe oxidizing short chain fatty acids, alcohols, and mono-aromatic compounds with the ability to generate electricity
5 (Carbon paper- Faecal sludge)	*Thermanaerovibrio Acidaminovorans (3)*	Thermophilic anaerobe fermenting a variety of amino acids.
	Desulfuromonas Acetexigens (9)	Obligate anaerobic and sulphurreducing eubacterium oxidizing acetate as carbon resource
	Shigella flexneri (4)	Facultative anaerobe failing to ferment lactose or decarboxylate lysine
	Geobacter Sulfurreducens (7)	Metal-reducing anaerobe oxidizing short chain fatty acids, alcohols, and mono-aromatic compounds with the ability to generate Electricity
	Enterobacter Cancerogenus (6)	Facultative anaerobes fermenting glucose as carbon sources.

4.2.2 **SEM of Carbon Paper anode biofilms**

The surface structure and biofilm adhesion of carbon paper were observed using SEM.

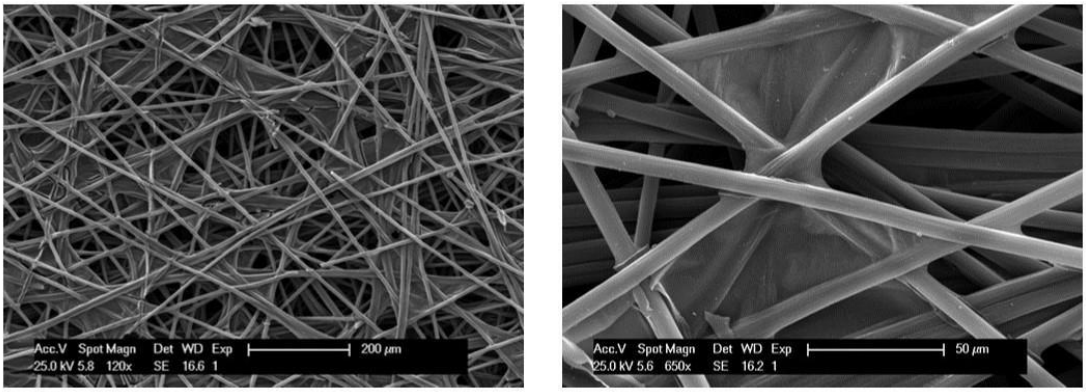
From figure 4-4, (Carbon paper control image) carbon paper is seen to consist of a

structured network of carbon strands. The strand arrangement provides an extensive surface area for the attachment of any microbial material. Figure 4-4 was used as the control images for the study of FS and GW biofilm structure.

The biofilm structure of grey water MFC anodes can be seen in figure 4-5. From figure 4-5, thin organic tissues cover the inter strand networks of the carbon paper. The organic tissues which appeared fibrous in nature were identified as mainly composed of polymeric tissue and microbes. Several forms of bacteria can be seen embedded in the organic tissues in figure 4-5. The bacteria observed in the organic tissues had bacillus (rod-like) and cocci (spherical) forms. It is notable that *Geobacter sp*. belongs to the bacillus bacterial form.

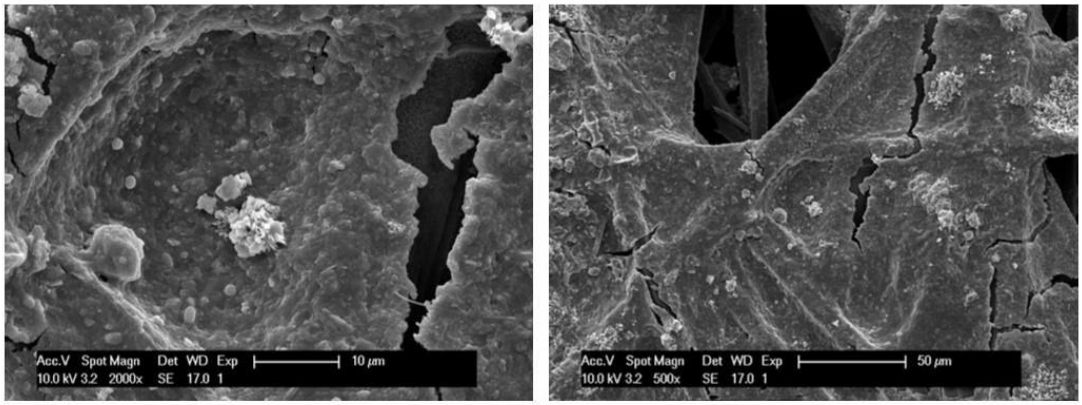
The biofilm structure of faecal sludge MFC anodes is seen in figure 4-6. From figure 4-6, dense organic films cover the vast inter strand networks of the carbon paper. The organic tissue in the FS biofilm also appears fibrous in nature, slimy and mainly composed of polymeric tissue and microbes. Bacterial forms can be seen in figures 4-7. These bacterial forms are dominated by the bacillus (rod-like) forms.

The SEM images for FS and GW anodes indicate the definite presence of microbial biofilm. The images confirm the earlier assertion that a biofilm is formed on the electrode surface during inoculation. These bacteria laden biofilms are known to initiate the organic substrate oxidation for the release of electric current.



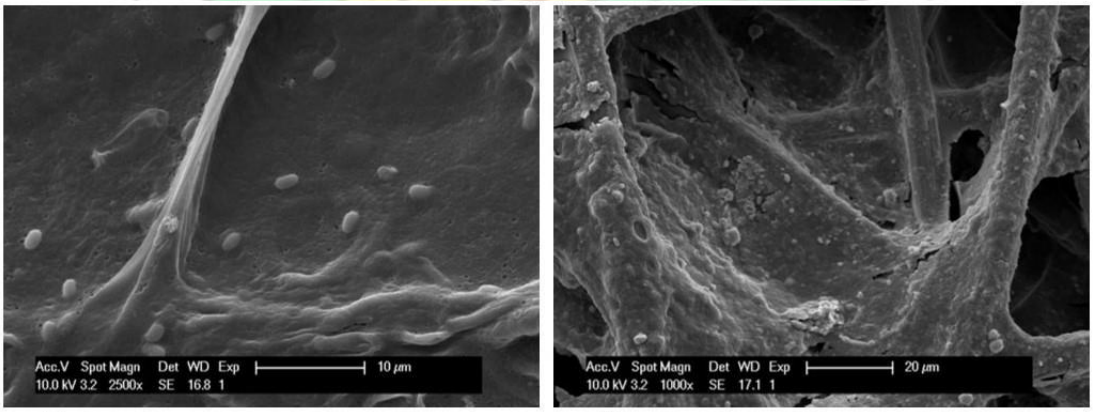
(a) (b)

Figure 4-4 Control Images of Carbon paper



(a) (b)

Figure 4-5 Images for grey water inoculation



(a) (b)

Figure 4-6 Images for faecal sludge inoculation

4.3 Start-up and electricity production of selected electrode materials As part of the development of palm kernel shells activated carbon (AC) and carbon butts (CB) as electrode materials for MFCs a careful observation of its inoculation was critical since without a biofilm there would be little or no electron transfer. The selected electrode materials were inoculated with faecal sludge (FS) which was a higher performing inoculum as against grey water. The materials were used as electrodes in both the anode and cathode of the MFCs supported by a stainless steel rod as electron collector to the external circuit.

The inoculation of Palm kernel activated carbon granules yielded a maximum voltage and power density was 599mV and 1.44W/m^3 respectively (Table 4-3, Fig 4-7). The inoculation of Carbon butts yielded a maximum voltage and power density 19mV and 0.001W/m^3 respectively (Table 4-3, Figure 4-7). The inoculation of Palm kernel activated carbon granules was successful due to the significant power and voltage obtained as seen in Fig 4-8. The inoculation of Carbon butts on the other hand was unsuccessful due to the very low voltage obtained under the load (Fig. 4-7). The significant voltage and power produced by the Palm kernel activated carbon was as a result of the formation of a proper biofilm on the surfaces of the material to initiate the electron transfer indicating a successful inoculation.

Analysis of the post inoculation anolyte and catholyte for MFCs operating with CB indicated a significant rise in pH. This indicated the possible presence of ionic residue deposited on the carbon butts surface from its previous use in an aluminium smelter. The residue is assumed to have dissolved into both the anolyte and catholyte to change its conditions contributing to the negligible power obtained. In order to thoroughly examine the actual cause of the low power further there was the need to examine

carbon butts biofilms using 16sRNA-DGGE and SEM analysis. This is discussed in detail in the succeeding sections.

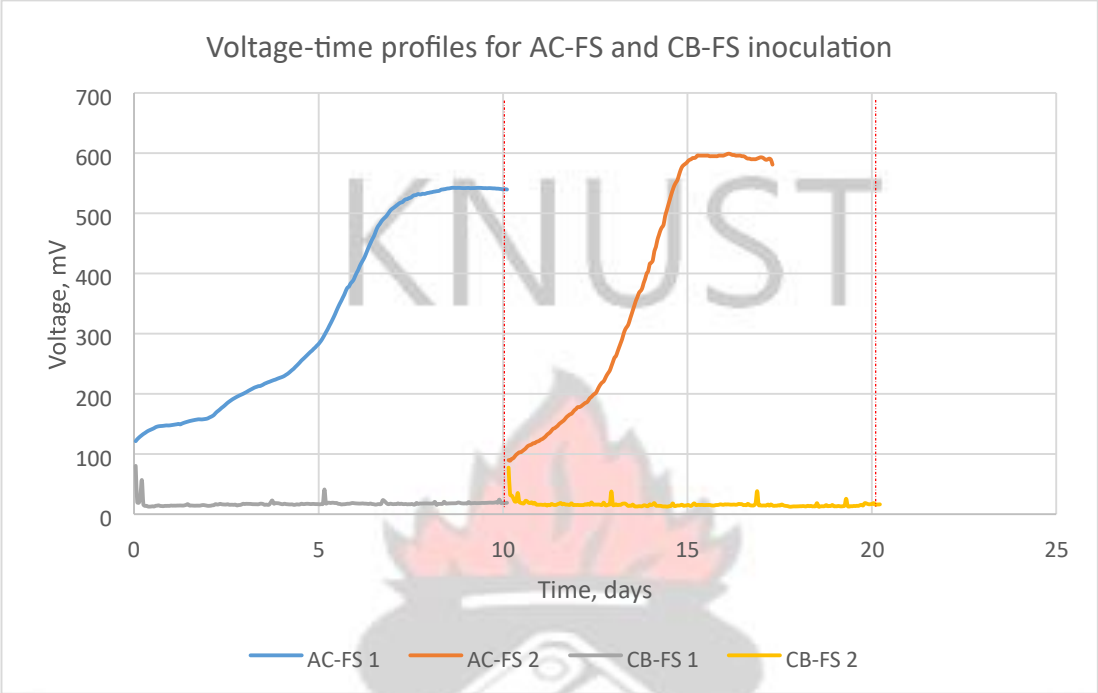


Figure 4-7 Electricity generation profiles for the inoculation of Palm kernel shellactivated carbon and Carbon butts with faecal sludge.

Table 4-3 Summary of results for Inoculation Performance

Electrode material	Inoculum used	Cycle	Startup time (Days)	Maximum voltage under load (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Power density (W/m^3)
Activated carbon	faecal sludge	1	5	542	1.47	0.80	1.18
		2	8	599	1.62	0.97	1.44
Carbon butts	faecal sludge	1	29*	19	0.07	0.001	0.001
		2	29*	16	0.06	0.001	0.001

(* start-up time was undefined since an appreciable voltage was not obtained)

4.4 Microbial and electrode surface analysis of AC and CB

The anodic biofilms in the MFCs operating with AC and CB as electrode materials were sampled at the end of the inoculation cycle for 16sRNA-DGGE analysis in combination with a marker and SEM analysis so as to fully characterize the electrode biofilms. Bacteria biofilm and electrode surface structure were observed using Scanning electron microscopy (SEM). The DGGE profiles of the samples analysed are summarized in Fig. 4-8 and Table 4-4.

4.4.1 16sRNA-DGGE of AC and CB anode biofilms

The bacterial species found in AC inoculation biofilms relative to the marker included *Geobacter sulfurreducens, Enterobacter cancerogenus* and *Desulfuromonas acetexigens.* The bacterial species found in carbon butts biofilms included *Enterobacter cancerogenus* and *Desulfuromonas acetexigens. Geobacter sulfurreducens* identified in the AC biofilm is a known dominant electrogenic species therefore the biofilm from Palm kernel shell activated carbon is very active electrogenically in the production of electric current. The impact of the activity of these microbes can be seen in their organic substrate removal efficiency outlined in closer detail in section 4.7. The presence of bacteria DNA from carbon butts was still not enough to conclude on its poor electrogenic activity therefore SEM analysis was needed for a closer examination of its biofilm. The horizontal gel band labels in figure 4-8 represent samples (1-6) analysed and the Marker used (M) while the vertical gel band labels indicate the 9 known species of bacteria from the marker used. AC and CB inoculated with faecal sludge is labelled horizontally as 2 and 3 respectively

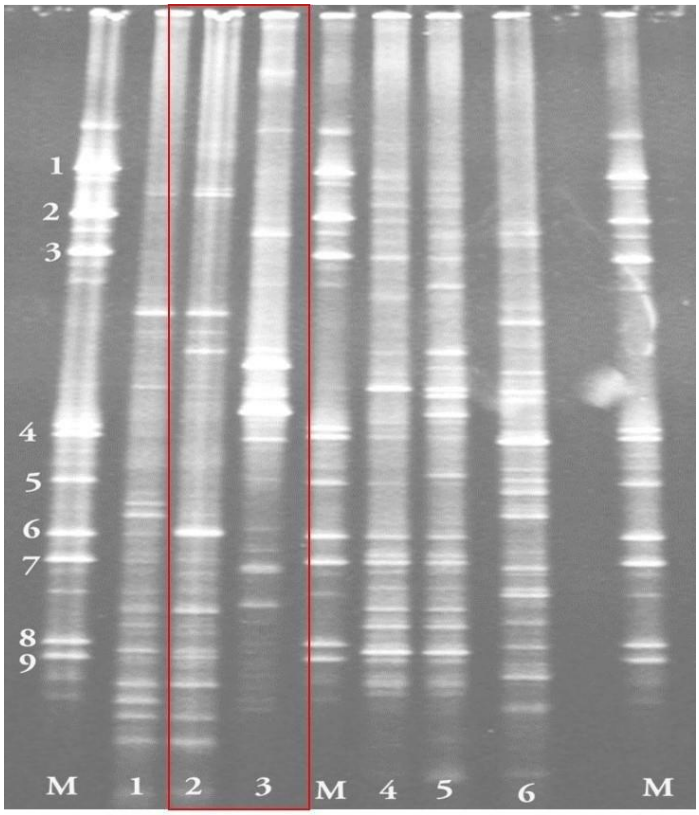


Figure 4-8 An image of the DGGE Gel product from samples analysed

Table 4-4 Gene bank matches for samples tested

SAMPLE	GENE BANK MATCH	SPECIES CHARACTERISTICS
1 (AC-FS)	9 *Desulfuromonas acetexigens*	Obligate anaerobic and sulphur-reducing eubacterium oxidizing acetate as carbon resource
	6 *Enterobacter cancerogenus*	Facultative anaerobes fermenting glucose as carbon sources.
	7 *Geobacter sulfurreducens*	Metal-reducing anaerobe oxidizing shortchain fatty acids, alcohols, and monoaromatic compounds with the ability to generate electricity
2 (CB-FS)	6 *Enterobacter cancerogenus*	Facultative anaerobes fermenting glucose as carbon sources.
	9 *Desulfuromonas acetexigens*	Obligate anaerobic and sulphur-reducing eubacterium oxidizing acetate as carbon resource

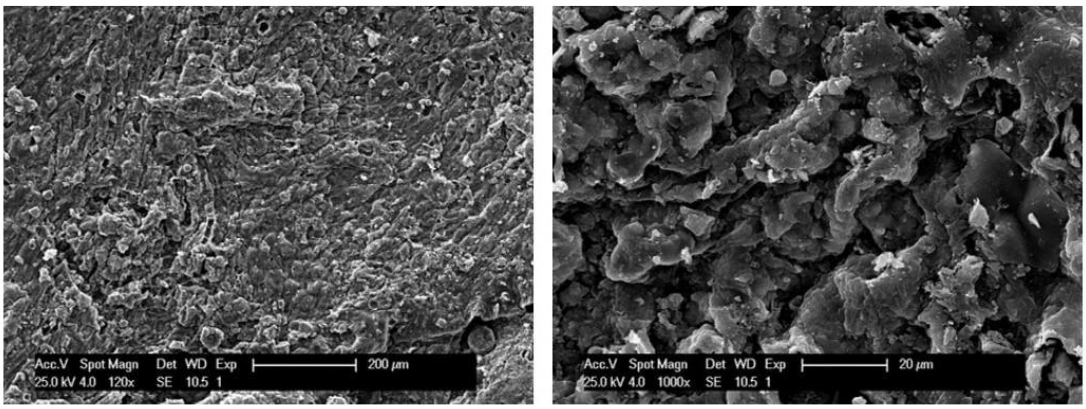
4.4.2 **SEM of AC and CB anode biofilms**

The surface structure and biofilm adhesion of palm kernel shell activated carbon (AC) and carbon butts (CB) were observed using SEM. Figure 4-9 and 4-11 were used as the control images for the study of AC and CB biofilm structure. From figure 4-9, AC is seen to consist of a surface laden with micro-porous networks and layers which were caused by the high efficiency of the steam treatment phase of activated carbon production. A surface of this form is very ideal for bacteria attachment and growth since movements in the bulk solution would not be able to remove any properly adhered biofilm. From figure 4-11, (control image) CB is composed of a solid, uneven surface structure with little or no micro-porous networks for any form of bacterial adhesion.

From figure 4-10, a thin organic tissue can be seen partially covering the micro-porous surface of the AC granule. At a high magnification (Figure 4-10) bacterial forms can be observed embedded in the thin organic tissue and the micro-channels on the granule surface of AC. The bacterial forms observed in the organic tissue had bacillus (rodlike) and cocci (spherical) forms. From the SEM images of AC, a definite biofilm can be seen present to initiate the oxidation of organic substrate for the generation of electric current.

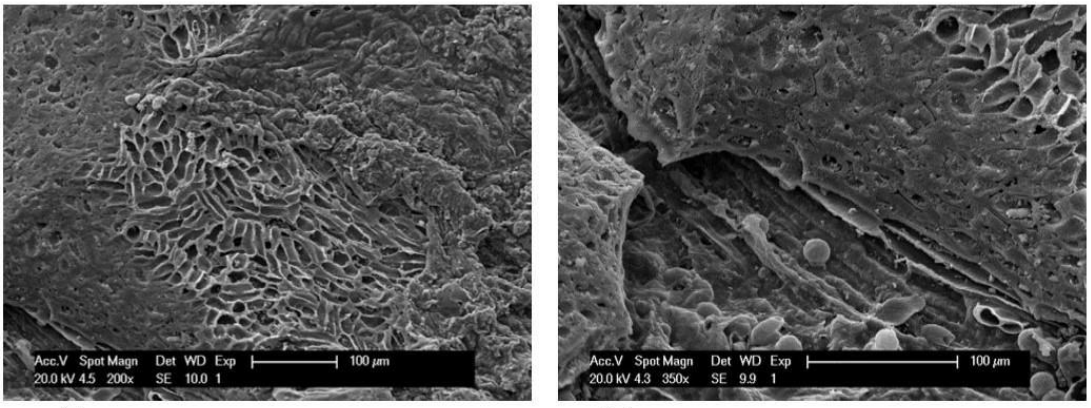
From figure 4-12, small deposits of bacterial forms can be seen on the surface of the CB granule. These bacterial deposits were of the bacillus and cocci forms. This confirms the presence of bacteria as detected in the DGGE analysis. A defined biofilm however could not be found on any SEM image from the CB anode granules which were analysed. This explains the negligible voltage and power obtained. The images of CB (Figures 4-12) showed the deposition of inorganic crystal-like structures on the surfaces. These could be salts formed from the movements of ions within solution or

residue from the electrolytic process from the aluminium smelter. A lack of biocompatibility therefore exists for CB due to its poor surface structure and the large deposition of crystal-like structures. The unsuccessful inoculation of CB indicates that it is not suited for use as an electrode material in an MFC. Further post-inoculation cycle for CB granules could therefore not be carried out.



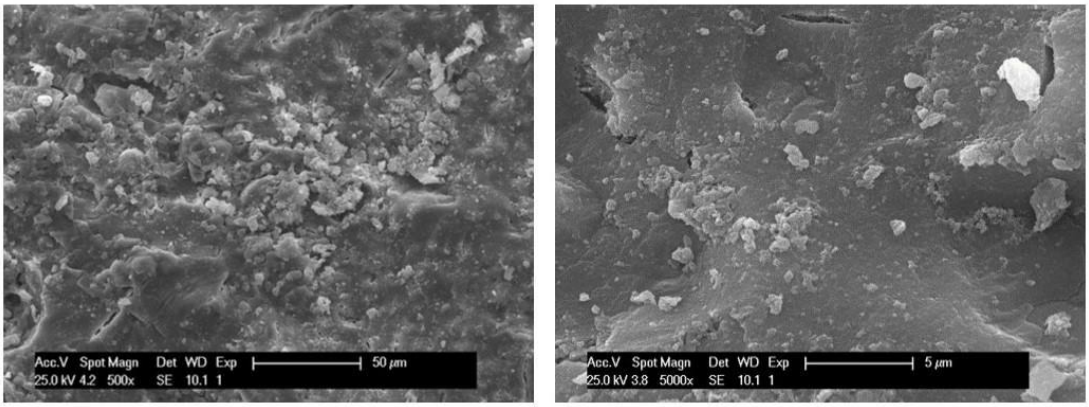
(a) (b)

Figure 4-9 SEM Control images of Palm kernel shell activated carbon



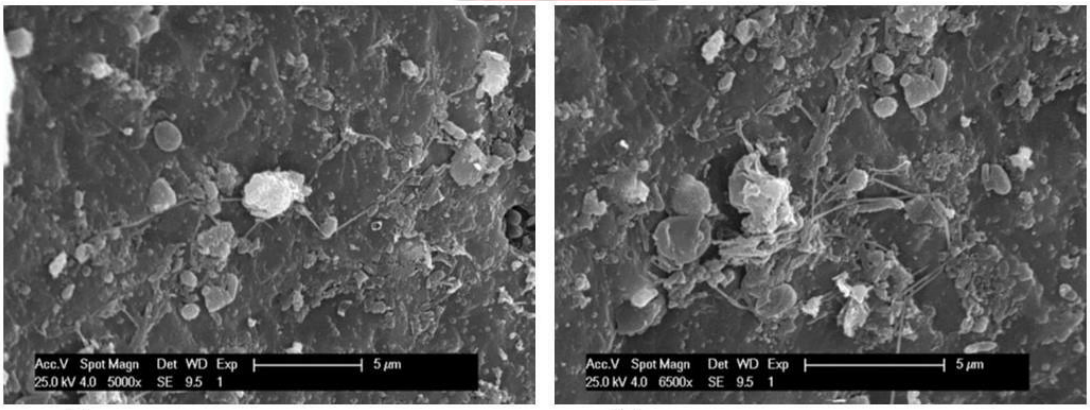
(a) (b)

Figure 4-10 SEM Images of Palm kernel shell activated carbon inoculation



(a) (b)

Figure 4-11 SEM Control Images of carbon butts



(a) (b)

Figure 4-12 SEM Images of carbon butts inoculation

4.5 Electricity generation and impedance for Activated carbon MFC The generation of electrical energy is the primary purpose of MFC research with the electrode materials in the anode and cathode playing a critical role in electron transfer. The selection and development of palm kernel shells activated carbon (AC) was targeted at introducing a cost-effective and efficient electrode material for use in MFCs. The successful inoculation of AC indicated that an improvement in power generation can be achieved through further post-inoculation operating cycles. Numerous cycles of operation are known to improve biofilms and increase the total

power generated. In each post-inoculation cycle fresh substrate was added to the anodes. From figure 4-14, a significant increase in maximum power is achieved (917%) from that initially recorded during inoculation (cycle 1).

From table 4-5, the three post-inoculation cycles yielded an average maximum voltage and power density of 516mV ±7 and 1.07W/m^3 ±0.03 respectively for AC-GW, 657mV ±8 and 1.73W/m^3 ±0.05 respectively for AC-FS. Steady power above 0.6V was available for the entire cycle time (6 days) in all three post inoculation cycles for faecal sludge operated MFCs. Steady power above 0.5V was however available for 540% of post-inoculation cycles for grey water. The longer length of steady high voltage attained for FS than GW was due to its high organic load which enhanced substrate availability and utilization by electrogenic bacteria. A very good inter-granular electron transfer was also achieved as evident in the stable and appreciable overall voltage obtained under the 1000 Ω load (Fig. 4-13, 4-14).

Nyquist plots (Appendix B2) were generated using the electrochemical impedance spectroscopy technique with the aid of a Potentiostat to determine the overall internal resistance (impedance). The overall impedance was determined as the x-intercept on the real impedance axis, Z_{real} of the plot (Appendix B2). An overall impedance of 31Ω ±4 was measured for AC-GW MFCs and 7.5Ω ±1 for AC-FS MFCs (Table 4-5). The overall resistance of AC MFCs were fairly consistent in all cycles of FS and GW operated MFCs. GW operated MFCs recorded a higher impedance than the FS operated MFC due to its low solution conductivity. High solution conductivities of electrolytes in MFCs favours electrogenic activity and proton transfer. The effect of the overall impedance on AC-GW MFCs is visible in its lower power output relative to AC-FS which was caused by several internal losses.

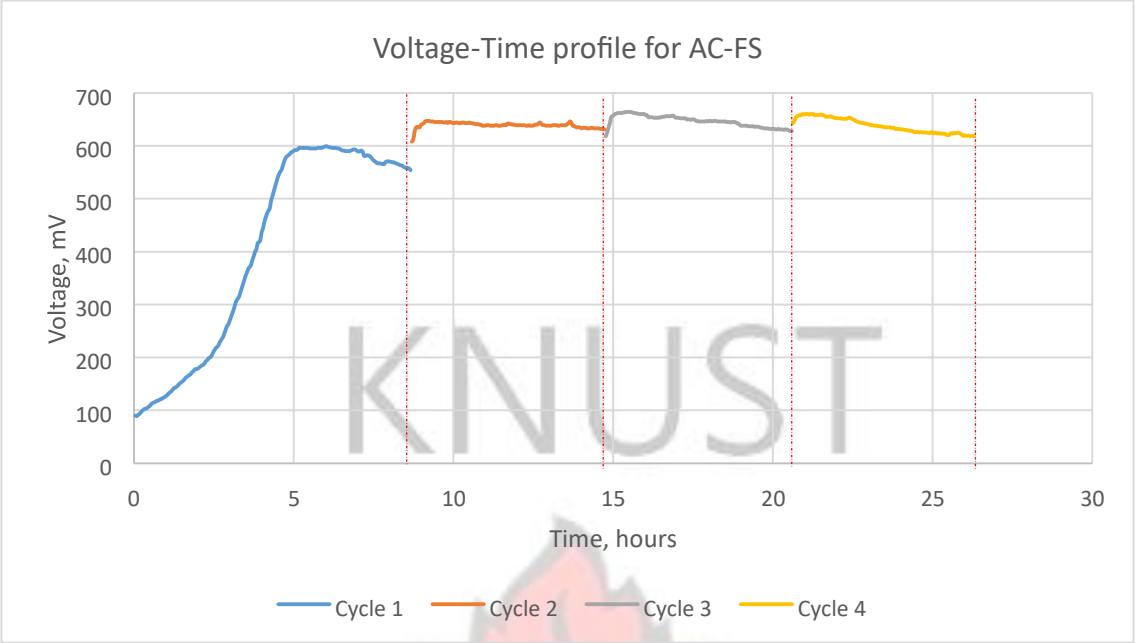


Figure 4-13 Electricity generation profiles for Activated carbon-Faecal sludge MFC (AC-FS)

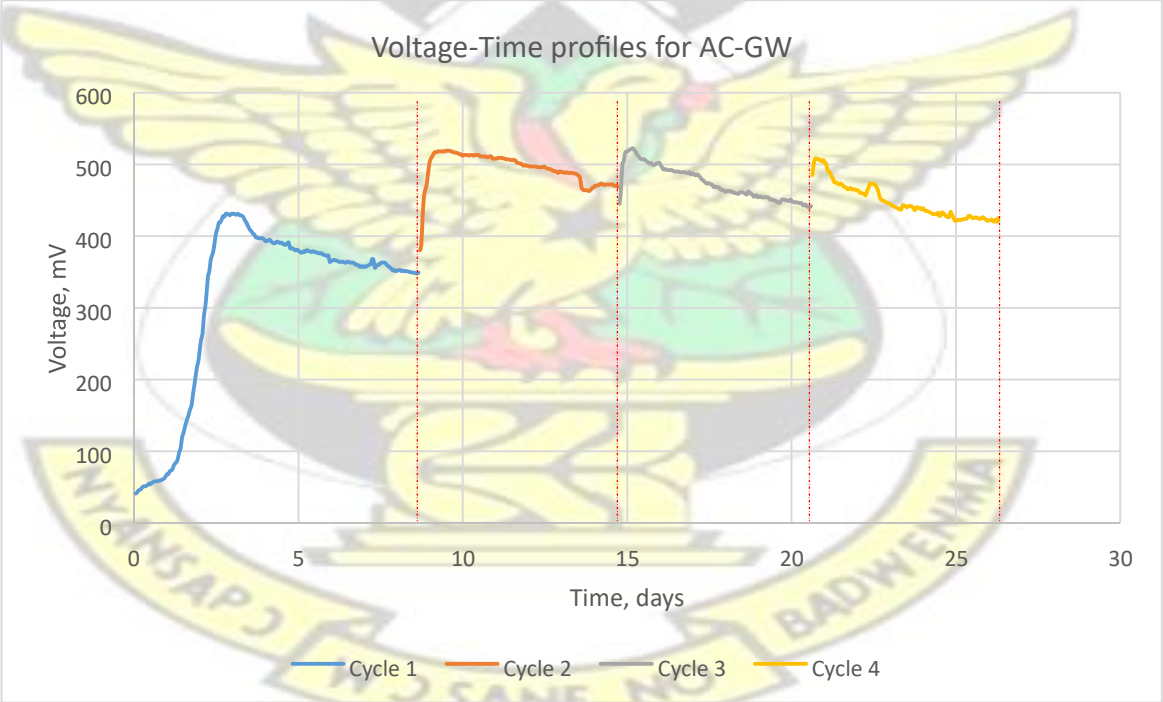


Figure 4-14 Electricity generation profiles for Activated carbon-Grey water MFC (AC-GW)

Table 4-5 Summary of Power performance of Activated carbon MFCs

Electrodeinoculum used	Cycle	Open circuit voltage (mV)	Maximum voltage under load (mV)	Current density (mA/m^2)	Power density (mW/m^3)	Overall Internal resistance (Ω)	Coulombic efficiency (%)
AC-GW	1	-	431	1.17	0.74	32	-
	2	619	519	1.40	1.08	33	5.6
	3	649	522	1.41	1.09	25	2.6
	4	651	508	1.38	1.03	34	3.4
AC-FS	1	-	599	1.62	1.44	9	-
	2	713	647	1.75	1.67	7	1.9
	3	741	664	1.80	1.76	7	3.0
	4	737	660	1.79	1.74	7	2.2

4.6 Performance of Activated carbon relative to Carbon paper

Carbon paper (CP) has been extensively used globally in MFCs as electrode material in both the anode and cathode. This made it a suitable material for the standardization of Palm kernel shell activated carbon (AC). Under identical conditions of operation, MFCs operating with carbon paper yielded 10-15% more power than MFCs operated with Activated carbon (Table 4-6). This difference is appreciable even though AC possesses a much larger surface area than CP. This difference is attributed to the more efficient electron transfer aided by stronger bonding in the carbon strands of carbon paper as against the inter-granular electron transfer in AC. Granules in AC have no binding agent and rely on contact between two or more granules for electron transfer. CP on the hand is composed of micro-carbon strands (Fig. 4-4, SEM control image) bonded together by a binding agent. The overall resistance of AC MFCs (7.5Ω) was very appreciable as compared to CP (118Ω).

Table 4-6 Comparison of the Performance between the new electrode material and standard

Electrode material	Inoculum used	Length of startup (Days)	Maximum voltage under load (mV)	Current density (mA/m^2)	Power density (W/m^3)	Overall internal resistance (Ω)
Carbon Paper (standard)	Faecal sludge	9 ±1	714.0 ±9.6	49.60 ±0.70	2.04 ±0.05	118.00 ±0.06
Activated carbon	Faecal sludge	6 ±2	657.0 ±8.9	1.78 ±0.08	1.72 ±0.05	7.50 ±1.00

4.7 Organic substrate removal by Activated carbon MFC

MFCs are considered very useful in the treatment of wastewater. It is seen by some researchers as the most likely final application of MFCs other than power generation due to the various limitations associated with its use in power generation. Wastewater constituents of concern were measured at the beginning and end of each post inoculation operating cycle for AC-GW and AC-FS to assess the efficiency of its removal. Table 4-7 is a summary of the various efficiencies of constituents' removal in the post-inoculation operating cycles of the MFCs.

The most critical constituent of concern is the organic load which is composed of biodegradable and refractory organics. Biodegradable organics are principally composed of proteins, carbohydrates and fats. If discharged untreated, their biological stabilization leads to the depletion of atmospheric oxygen and development of septic conditions in water resources. The organic load was measured as Chemical oxygen demand (COD). COD removal efficiencies in AC-FS operating cycles were between 36 and 44% (Table 4-7). COD removal efficiency in AC-GW operating cycles were between 69 and 72% (Table 4-7). Wastewater constituents' removal efficiencies were

higher in grey water MFCs (AC-GW) than faecal sludge MFCs (AC-FS) due to the lower initial organic load in grey water i.e. up to 83% less (Appendix B4).

Achieving an average COD removal of 44% and 72% for GW and FS in 6 days is very appreciable for a wastewater treatment system with zero energy input in the entire treatment process (Table 4-7). Higher efficiencies can possibly be achieved in longer operating cycles. COD was removed in the form of electrons, carbon dioxide and water. The electrons were recovered as electric current while the CO_2 formed can be released safely into the environment since it is a replacement of the initial CO_2 removed from the atmosphere during photosynthesis by plants.

Another important parameter measured is coulombic efficiency which is a measure of the total substrate recovered in the form of electric current. AC-FS had coulombic efficiencies between 2 to 3% from its operating cycles. AC-GW had coulombic efficiencies between 2.6 to 5.6% (Table 4-7). The loss of substrate was attributed to the production of the by-products of the MFC operations in the form of carbon dioxide and water. It was also caused by losses to oxygen located in the inter-granular voids (air pockets). Substrate utilization in the generation of electric current was however fairly appreciable.

Other wastewater constituents of concern such as nitrogen, ammonia and phosphorus had removal efficiencies of up to 36 and 88% for AC-GW and AC-FS respectively (Table 4-7). Also, it was observed that the wastewater effluent from the MFCs operated with AC were completely odourless. This was due to the absorption of hydrogen sulphides and the breakdown of volatile organic compounds which are the primary sources of the unpleasant odours in domestic wastewater.

From the wastewater constituents' removal efficiencies obtained, the Ghana EPA standards minimum discharges (Appendix B1.1) can be achieved through the use of

MFCs. An example is seen in the cycle 2 of AC-GW MFC post-inoculation operations as COD was reduced from 2437mg/L to 682mg/L which meets the EPA discharge standard of 1000mg/L (Appendix B4). Nutrients such as nitrogen, phosphorus and ammonia available in MFC effluents can be reduced to safe levels for farm irrigation. The removal efficiencies enhance the development of palm kernel shell activated carbon as a viable and cost effective electrode material for the application of MFCs in wastewater treatment.

Table 4-7 Table of efficiencies in wastewater constituents' removal

Parameter (%)	AC-FS			AC-GW		
	Cycle 2	Cycle 3	Cycle 4	Cycle 2	Cycle 3	Cycle 4
Substrate removal efficiency (COD removal)	44.8	36.2	41.0	72.0	71.9	69.8
Total Dissolved Solids	4.0	7.2	5.9	28.8	14.8	21.4
Salinity	3.8	7.6	6.7	31.2	13.3	21.4
Total nitrogen	27.0	8.4	10.8	88.0	84.0	82.7
Total phosphorus	0.4	2.3	2.1	25.2	84.0	41.1
Ammonia content	36.6	32.3	33.8	82.0	57.8	65.3

CHAPTER 5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The development of simple, low cost and efficient electrode materials is critical in order to possibly recommend the microbial fuel cell technology as a viable solution to decentralized power generation and efficient wastewater treatment in Ghana. This study specifically sought to assess the feasibility of faecal sludge and grey water as inoculum sources for an MFC; test the feasibility of the use of Palm kernel shell activated carbon and Carbon butts as electrodes; analyse the overall power output of an MFC operating with the new electrode materials; and to measure the efficiency of organic substrate removal when the selected electrodes are used.

The study established that faecal sludge and grey water are ideal and efficient sources of inoculum for use in MFCs since they formed a defined biofilm containing electrogenic species such as *Geobacter sp*. Also carbon butts would not be an efficient electrode material for MFCs since inoculations were unsuccessful due to an absence of a defined biofilm to initiate substrate oxidation and electron transfer. On the other hand, Palm kernel shell activated carbon (AC) would be an ideal and efficient electrode material for MFCs since its inoculation was successful. It yielded high power densities from its post-inoculation operating cycles which were comparable to carbon paper (standard) by up to 86%. The study also established that using AC operated MFCs significant efficiencies of organic substrate removal along with other wastewater constituents' of concern can be achieved (organics removal of up to 45% for faecal sludge and 72% for grey water). The effluent from the MFCs can be recommended for use on farms since it is also rich in plant nutrient such as nitrogen and phosphorus.

From these findings, it can be concluded that Palm kernel shell activated carbon can be efficiently used as electrode materials in an MFC to generate electric power and treat wastewater. Carbon butts on the other hand is unsuited for use in an MFC.

5.2 Recommendations

The study has shown that palm kernel shell activated carbon would be an ideal and efficient electrode material for MFCs since it yielded significant power densities. However further improvements in power densities are still required especially for the construction of scale-up models. Modifications in the choice of inoculums is highly recommended as a means of finding a superior biofilm for perhaps higher performance. Modifications could include a mixture of inoculums from various sources or the selection of other sources different from that used in this study.

Also, limitations associated with carbon butts were from its surface structure which hinders biofilm formation and growth. Therefore an extensive study of possible modifications to the surface structure would be needed. Modification methods such as heat and chemical treatment could be considered.

REFERENCES

1. Ghana's Power outlook. Volta River Authority, May 2014 2. Proposal for tariff review. Electricity Company of Ghana June 2013
3. Renewable Energy Act 2011, Act 832 (01).
4. Agodzo, S.K. Huibers, F.P. Chenini, F. van Lier, J.B. Duran, A. (2003) Use of Wastewater in Irrigated Agriculture. Country Studies from Bolivia, Ghana and Tunisia. *Wageningen: WUR*. 2. p.40
5. Wastewater production, treatment and use in Ghana. UNW-AIS. 2012
6. Rabaey, K. Angenent, L. Schroder, U. Keller, J. (2009) Bioelectrochemical Systems: From Extracellular Electron Transfer to Biotechnological Application, 1st ed. IWA Publishing, London.

7. Potter, M.C. (1911) Electrical effects accompanying the decomposition of organic compounds. *Proc. R. Soc. Lond. B Biol. Sci*. 84, p. 160-276.
8. Logan, B.E. Hamelers, B. Rozendal, R. Schröder, U. Keller, J. Freguia, S. (2006) Microbial fuel cells: methodology and technology. *Environ Sci Technol.* 40. p.5181-5192.
9. Lewis, K. (1966) Symposium on bioelectrochemistry of micro-organisms. 4. Biochemical fuel cells. *Bacteriol. Rev.* 30. p.101-113
10. Sundmacher, K. (2010) Fuel cell engineering: toward the design of efficient electrochemical power plants. *Ind. Eng. Chem. Res.* 49. p. 10159-10182.
11. Arends, J.B. Verstraete, W. (2011) 100 years of microbial electricity production; three concepts for the future. *Microbial Biotech*. p. 1-14
12. Pham, T.H. Rabaey, K. Aelterman, P. Clauwert, P. De Schamphelaire L. Boon, N. Verstraete, W. (2006) Microbial fuel cell in relation to conventional anaerobic digestion technology. *Eng. Life Sci.* (6)3 p. 285-292
13. Patil, S.A. Surakasi, V.P. Koul, S. Ijmulwar, S. Vivek, A. Shouche, Y.S. Kapadnis, B.P. (2009) Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. *Bioresour. Technol.* 100. p. 5132-5139.
14. Hatzell, M.C. Kim, Y. Logan, B.E. (2013) Powering microbial electrolysis cells by capacitor circuits charged using microbial fuel cell; *J. of Power sources* 229, p.198 – 202
15. Liu, H. Cheng, S.A. Logan, B.E. (2005) Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. *Environ. Sci. Technol*. 39(14) p.5488-5493
16. Virdis, B. Rabaey, K. Yuan, Z. Keller, J. (2008) Microbial fuel cells for simultaneous carbon and nitrogen. *Water research* 42. p. 3013 – 3024
17. Gerardi, M.H. (2006) *Wastewater Bacteria*, John Wiley & Sons, Inc.: Hoboken.
18. Gerardi, M.H. Zimmerman M.C. (2005) *Wastewater Pathogens*, John Wiley & Sons, Inc.: Hoboken.
19. Gates E. (2013) *Introduction to Basic Electricity and Electronics Technology*. 1st Ed. Cengage Learning

20. Sekar, N. Ramasamy, R.P. (2013) Electrochemical Impedance Spectroscopy for Microbial Fuel Cell Characterization. *J. Microbial Biochem. Technol.* S6004
21. Du, Z. Li, H. Gu, T. (2007) A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnology Advances* (25) p.464-482
22. Daniel, D.K. Mankidy, B.D. Ambarish, K. Manogari, R. (2009) Construction and operation of a microbial fuel cell for electricity generation from wastewater. *Int. Journal of Hydrogen energy* 34. p. 7555-7560
23. Thygesen, A. Poulsen, F.P. Min, B. Angelidaki, I. Thomsen, A.B. (2009) The effect of different substrates and humic acid on power generation in microbial fuel cell operation. *Bioresource Tech.*100. p. 1186–1191
24. Fan, Y. Hu, H.Q. Liu, H. (2007) Sustainable power generation in microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. *Environ. Sci. Technol.* (41) p.8154–8158.
25. Gil, G. C. Chang, I. S. Kim, B. H. Kim, M. Jang, J. K. Park, H. S. Kim, H. J. (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. *Biosens. Bioelectron*. 18(4). p.327-334
26. Pham, T.H. Jang, J. K. Chang, I.S. Kim, B. H. (2004) Improvement of cathode reaction of a mediator-less microbial fuel cell. *J. Microbiol. Biotechnol*. 14. p.324-329
27. Wei, L. Yuan, Z. Cui, M. Han, H. Shen, J. (2012) Study on electricity generation characteristic of two-chambered microbial fuel cell in continuous flow mode. *Int. J. of Hydrogen Energy* (37) p. 1067-1073
28. You, S.J. Zhao, Q.L. Zhang, J.N. Jiang, J.Q. Zhao, S.Q. (2006) A microbial fuel cell using permanganate as the cathodic electron acceptor. *J Power Sources* 162. p. 1409-1415
29. Oh, S. Min, B. Logan, B.E. (2004) Cathode performance as a factor in electricity generation in microbial fuel cells. *Environ. Sci. Technol*. 38. p. 4900-4904
30. Clauwaert, P. Van der Ha, D. Boon, N. Verbeken, K. Verhaege, M. Rabaey, K. Verstraete, W. (2007) Open air biocathode enables effective electricity generation with microbial fuel cells. *Environ. Sci. Technol.* 41(21). p. 75647569.

31. He, Z. Angenent, L.T. (2006) Application of bacterial biocathodes in microbial fuel cells. *Electroanalysis* 18. p.2009-2015
32. Mauritz, K.A. Moore, R.B. 2004. State of understanding of Nafion. *Chem. Rev.* 104 p.4535-4585.
33. Pant, D. Van Bogaert, G. De Smet, M. Diels, L. Vanbroekhoven, K. (2010) Use of novel permeable membrane and air cathodes in acetate microbial fuel cells. Electrochim. Acta. 55. p.7710–7716.
34. Cheng, S.A. Liu, H. Logan, B.E. (2006) Increased performance of singlechamber microbial fuel cells using an improved cathode structure. *Electrochem. Commun.* 8, p.489-494.
35. Kim, I.S. Chae, K.J. Choi, M.J. Verstraete, W. (2008) Microbial Fuel Cells: Recent advances, bacterial communities and application beyond electricity generation. *Environ. Eng. Res*. 13. p.51 – 65
36. Moon, H. Chang, I.S. Jang, J.K. Kim, B. H. (2005) Residence time distribution in microbial fuel cell and its influence on COD removal with electricity generation. *Biochem. Eng. J.* 27(1). p. 59-65.
37. Jana, S.P. Behera, M. Ghangrekar, M.M. (2010) Performance comparison of up-flow microbial fuel cells fabricated using proton exchange membrane and earthen cylinder. *Int. J. of Hydrogen Energy* (35) p.5681-5686
38. Aelterman, P. Rabaey, K. Pham, H.T. Boon, N. Verstraete, W. (2006) Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. *Environ Sci Technol* 40. p. 3388-3394.
39. Watanabe, K. (2008) Recent developments in microbial fuel cell technologies for sustainable bioenergy. J. Biosci Bioeng. 106(6) p. 528-536
40. Franks, A.E. Nevin, K.P. (2010) Microbial Fuel cells, a current review; *Energies* 3 p.899 - 919
41. Lovley, D.R. (2006) Bug juice: harvesting electricity with microorganisms. *Nature Rev. Microbiol*. 4. p.497-508.
42. Kim, B.H., Chang, I.S., Gadd, G.M., (2007a) Challenges in microbial fuel cell development and operation. *Appl. Microbiol. Biotechnol*. 76. p.485494
43. Rabaey, K. Ossieur, W. Verhaege, M. Verstraete, W. (2005) Continuous microbial fuel cells convert carbohydrates to electricity, Water Sci. Technol. 52. p. 515-523

44. Toutain, C.M. Caiazza, N.C. O'Toole, G.A. (2004) Molecular basis of biofilm development by pseudomonas. Washington: ASM Press 2004
45. Hall-Stoodley, L. Costerton, J.W. Stoodley, P. (2004) Bacterial biofilms: from the Natural environment to infectious diseases. *Nat Rev Microbiol* 2. p. 95108
46. Reguera, G. Nevin, K.P. Nicoll, J.S. Covalla, S.F. Woodard T.L. Lovley, D.R. (2006) Biofilm and nanowire production leads to increased current in Geobacter sulfurreducens fuel cells. *Appl. Environ. Microbiol.* 72(11). p. 73457348
47. Choo, Y.F. Lee, J. Chang, I.S. Kim, B.H. (2006) Bacterial communities in microbial fuel cells enriched with high concentrations of glucose and glutamate. *J. Microbiol. Biotechnol.* 16. p.1481-1484
48. Jong, B.C. Kim, B.H. Chang, I.S. Liew, P.W.Y. Choo, Y.F. Kang, G.S. (2006) Enrichment, performance, and microbial diversity of a thermophilic mediatorless microbial fuel cell. *Environ Sci Technol* 40 p.6449-6454
49. Bond, D. R. Holmes, D. E. Tender, L. M. and Lovley, D.R. (2002) Electrodereducing microorganisms that harvest energy from marine sediments. *Science* 295(5554). p. 483-485
50. Read, S.T. Dutta, P. Bond, P.L Keller, J. Rabaey, K. (2010) Initial development and structure of biofilms on microbial fuel cell anodes. *BMC Microbiology* 10(98). 1-10
51. Park, D.H. Zeikus, J.G. (2000) Electricity generation in microbial fuel cells using neutral red as an electronophore. *Appl. Environ. Microb.* 66. p. 12921297.
52. Pham, C.A. Jung, S.J. Phung, N.T. Lee, J. Chang, I.S. Kim, B.H. (2003) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell. *FEMS Microbiol. Lett.* 223. p. 129-34.
53. Rabaey, K. Boon, N. Siciliano, S.D. Verhaege, M. Verstraete, W. (2004) Biofuel cells select for microbial consortia that self-mediate electron transfer. *Appl. Environ. Microbiol*. 70. p. 5373-5382.
54. Niessen, J. Schroder, U. Scholz, F. (2004) Exploiting complex carbohydrates for microbial electricity generation, a bacterial fuel cell operating on starch. *Electrochem. Commun.* 6. p. 955-958.

55. Park, H.S. Kim, B.H. Kim, H.S. Kim, H.J. Kim, G.T. Kim, M. Chang, I.S. Park, Y.K. Chang, H.I. (2001) A novel electrochemically active and Fe(III)reducing bacterium phylogenetically related to Clostridium butyricum isolated from a microbial fuel cell. *Anaerobe* 7. p. 297-306.
56. Ieropoulos, I.A. Greenman, J. Melhuish, C. Hart, J. (2005) Comparative study of three types of microbial fuel cell. Enzyme Microb Tech 37. p. 238-245.
57. Vega, C.A. Fernandez, I. (1987) Mediating effect of ferric chelate compounds in microbial fuel cells with Lactobacillus plantarum, Streptococcus lactis, and Erwinia dissolvens. *Bioelectrochem Bioenerg* 17. p. 217-222.
58. Schroder, U. Niessen, J. Scholz, F. (2003) A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. *Angew. Chem., Int. Ed*. 42 (25). p. 2880-2883.
59. Min, B. Cheng, S. Logan, B.E. (2005) Electricity generation using membrane and salt bridge microbial fuel cells. *Water Res* 39. p.1675-86
60. Lee, S.A. Choi, Y. Jung, S. Kim, S. (2002) Effect of initial carbon sources on the electrochemical detection of glucose by Gluconobacter oxydans. *Bioelectrochemistry* 57. p.173-178
61. Rhoads, A. Beyenal, H. Lewandowski, Z. (2005) Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant. *Environ. Sci. Technol*. 39. p. 4666-4671.
62. Choi, Y. Jung, E. Kim, S. Jung, S. (2003) Membrane fluidity sensoring microbial fuel cell. *Bioelectrochemistry* 59 p.121-127.
63. Liu, Z.D. Lian, J. Du, Z.W. Li, H.R. (2006) Construction of sugar-based microbial fuel cells by dissimilatory metal reduction bacteria. *Chin J Biotech* 21. p131-137
64. Ringeisen, B.R. Henderson, E. Wu, P.K. Pietron, J. Ray, R. Little, B. (2006) High power density from a miniature microbial fuel cell using Shewanella oneidensis DSP10. *Environ. Sci. Technol.* 40. p. 2629-2634.
65. Park, D.H. Zeikus, J.G. (2002) Impact of electrode composition on electricity generation in a single-compartment fuel cell using Shewanella putrefaciens. *Appl. Microbiol. Biotechnol.* 59. p. 58-61.
66. Liu, H. Logan, B.E. (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environ. Sci. Technol.* 38, p.4040-4046.

67. Rabaey, K. Rodríguez, J. Blackall, L.L. Keller, J. Gross, P. Batstone, D. Verstraete, W. Nealson, K.H. (2007) Microbial ecology meets electrochemistry: electricity-driven and driving communities. ISME J. 1, 9-18.
68. Rozendal, R.A. Hamelers, H.V.M. Rabaey, K. Keller, J. Buisman, C.J.N. (2008) Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol*. 26 (8). p. 450-459.
69. Rabaey, K. Verstraete, W. (2005) Microbial fuel cells: novel biotechnology for energy generation. *Trends Biotechnol*. 23. p.291-298.
70. Reguera, G. McCarthy, K.D. Mehta, T. Nicoll, J.S. Tuominen, M.T. Lovley, D.R. (2005) Extracellular electron transfer via microbial nanowires. *Nature* 435. p. 1098-1101.
71. Chaudhuri, S.K. Lovley, D.R. (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat. Biotechnol.* 21. p.1229-1232.
72. Feng, Y. Yang, Q, Wang, X. Liu, Y. Lee, H. Ren, N. (2011) Treatment of biodiesel production wastes with simultaneous electricity generation using a single-chamber microbial fuel cell. *Bioresource Tech.* 102. p. 411–415
73. Rabaey, K. Clauwaert, P. Aelteman, P. Verstraete, W. (2005) Tubular microbial fuel cells for efficient electricity generation. *Environ. Sci. Technol.* 3. p. 8077-8082.
74. Tokuji, I. Kenji, K. (2003) Vioelectrocatalyses-based application of quinoproteins and quinprotein-containing bacterial cells in biosensors and biofuel cells. *Biochim Biophys Acta* 1647. p.121-126.
75. Sund, C.J. McMasters, S. Crittenden, R.S. Harrell, L.E. Sumner, J.J. (2007) Effect of electron mediators on current generation and fermentation in a microbial fuel cell. *Appl. Microbiol. Biotechnol*. (76) p. 561-568
76. Ren, Z. Steinberg, L.M. Regan, J.M. (2008) Electricity production and microbial biofilm characterization in cellulose-fed microbial fuel cells. *Water Sci. Technol*. 58. p. 617-622.
77. Zhang, C. Li, M. Liu, G. Luo, H. Zhang, R. (2009) Pyridine degradation in the microbial fuel cells. *J. Hazard. Mat*. 172. p. 465-471.
78. Freguia, S. Teh, E.H. Boon, N. Leung, K.M. Keller, J. Rabaey, K. (2009) Microbial fuel cells operating on mixed fatty acids. *Bioresour. Technol.* 101. p.1233-1238.

79. Aelterman, P. (2009). Microbial fuel cells for the treatment of waste streams with energy recovery. Ph.D. Thesis, Gent University, Belgium.
80. Rabaey, K. Lissens, G. Siciliano, S.D. Verstraete, W. (2003) A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.* 25(18). p. 1531-1535
81. Hu, Z. (2008) Electricity generation by a baffle-chamber membraneless microbial fuel cell. *J. Power Sources* 179. p. 27-33.
82. Pham, T.H., Aelterman, P. Verstraete, W. (2009) Bio-anode performance in bioelectrochemical systems: recent improvements and prospects. *Trends Biotechnol.* 27 (3). p. 168-178.
83. Ieropoulos, I. Greenman, J. Melhuish, C. (2008) Microbial fuel cells based on carbon veils electrodes: stack configuration and scalability. *Int. J. Energy Res.* (32) p. 1228-1240
84. Kim, Y. Hatzell, M.C. Hutchinson, A.J. Logan, B.E. (2011) Capturing power at higher voltages from arrays of microbial fuel cells without voltage reversal. *Energy Environ. Sci.* (4) p. 4662-4667
85. Wang, X. Cheng, S.A. Feng, Y.J. Merrill, M.D. Saito, T. Logan, B.E. (2009) Use of carbon mesh anodes and the effect of different pretreatment methods on power production in microbial fuel cells. *Environ. Sci. Technol.* 43. p. 6870-6874.
86. Rabaey, K. Rozendal, R.A. (2010) Microbial electrosynthesis-revisiting the electrical route for microbial production. *Nat. Rev. Microbiol.* 8. p. 706716.
87. Wei, J. Liang, P. Huang, X. (2011) Recent progress in electrodes for microbial fuel cells. *Bioresource Tech*. (102) p. 9335-9344
88. Zhou, M. Chi, M. Luo, J. He, H. Jin, T. (2011) An overview of electrode materials in microbial fuel cells. *Journal of Power sources* (196) p. 4427-4435
89. Logan, B.E. Murano, C. Scott, K. Gray, N.D. Head, I.M. (2005) Electricity generation from cysteine in a microbial fuel cell. *Water Res* 39. p. 942-952.
90. Zhang, F. Saito, T. Cheng, S.A. Hickner, M.A. Logan, B.E. (2010) Microbial fuel cell cathodes with poly (dimethylsiloxane) diffusion layers constructed around stainless steel mesh current collectors. *Environ. Sci. Technol.* 44. p. 1490-1495.

91. Wang, X. Feng, Y.J. Qu, Y.P. Li, D.M. Li, H. Ren, N.Q. (2008) Effect of temperature on performance of microbial fuel cell using beer wastewater, *Huanjing Kexue/Environ. Sci.* 29. p. 3128–3132.
92. Di Lorenzo, M. Scott, K. Curtis, T. Head, I.M. (2010) Effect of increasing anode surface area on the performance of a single chamber microbial fuel cell. *Chem. Eng. J.* (156) p. 40-48
93. Logan, B.E. (2007) Microbial Fuel Cell, 1st ed. John Wiley & Sons, Inc., Hoboken.
94. Dumas, C. Mollica, A. Feron, D. Basseguy, R. Etcheverry, L. Bergel, A. (2007) Marine microbial fuel cell: use of stainless steel electrodes as anode and cathode materials. *Electrochim. Acta* (53) p.468-473.
95. Richter, H. McCarthy, K. Nevin, K.P. Johnson, J.P. Rotello, V.M. Lovley, D.R. (2008) Electricity generation by Geobacter sulfurreducens attached to gold electrodes. *Langmuir* 24. p. 4376-4379.
96. Ter Heijne, A. Hamelers, H.V.M. Saakes, M. Buisman, C.J.N. (2008) Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. *Electrochim. Acta* 53. p. 5697-5703.
97. Cheng, S. Logan, B. E. (2007) Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. *Electrochem. Commun.* 9. p.492–496.
98. Feng, Y. Yang, Q. Wang, X. Logan, B.E. (2010) Treatment of carbon fibre brush anodes for improving power generation in air-cathode microbial fuel cells. *J. Power Sources* 195 p.1841-1844.
99. Peng, L. You, S.J. Wang, J.Y. (2010) Carbon nanotubes as electrode modifier promoting direct electron transfer from Shewanella oneidensis. *Biosens. Bioelectron.* 25. p. 1248-1251.
100. Zhao, Y.K. Watanabe, K. Nakamura, R. Mori, S. Liu, H. Ishii, K. (2010) Three-dimensional conductive nanowire networks for maximizing anode performance in microbial fuel cells. *Chem. Eur. J.* 16. p. 4982-4985.
101. Logan, B.E. Cheng, S.A. Watson, V. Estadt, G. (2007) Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ. Sci. Technol*. (41) p. 3341-3346.
102. Dewan, A. Beyenal, H. Lewandowski, Z. (2008) Scaling up microbial fuel cells. *Environ. Sci. Technol.* (42) p. 7643-7648.

103. Zhao, F. Rahunen, N. Varcoe, J.R. Chandra, A. Avignone-Rossa, C. Thumser, A.E. Slade, R.C.T. (2008) Activated carbon cloth as anode for sulfate removal in a microbial fuel cell. *Environ. Sci. Technol.* (42) p. 4971-4976.

104. Aelterman, P. Versichele, M. Marzorati, M. Boon, N. Verstraete, W. (2008) Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. *Bioresour. Technol.* (99) p. 8895-8902.

105. He, Z. Minteer, S.D. Angenent, L.T. (2005) Electricity generation from artificial wastewater using an upflow microbial fuel cell. *Environ. Sci. Technol*. (39) p. 5262-5267.

106. Arthur, R. Brew-Hammond, A. (2010) Potential biogas production from sewage sludge: A case study of the sewage treatment plant at Kwame Nkrumah University of science and technology, Ghana. *Int. J. of Energy and Env.* 1(6) p.1009-1016

107. Sun, G. Thygesen, A. Meyer, A.S. (2015) Acetate is a superior substrate for microbial fuel cell initiation preceding bioethanol effluent utilization. *Appl. Microbiol. Biotechnol*. 99. p.4905–4915.

APPENDIX APPENDIX A: Experimental Procedures A1: Measurement of voltage with time

Equipment: V-T data logger (PicoLog ADC-20 data logger), Duplicate H-shaped Microbial Fuel Cells, Personal Computer.

Procedure: Copper wires were connected between the input channels of the V-T data logger and the MFCs whose voltages were to be measured. The V-T data logger was connected via its USB cable to the Personal Computer with the PicoLog reader software installed. The software was started with the following parameters; recording method: real-time continuous, sampling interval: 1800 seconds, stop time: 1000000 seconds, voltage range: ±2.5V. The voltage of the MFCs were recorded and saved by the V-T data logger at 30 minutes interval.

A2: Determination of Chemical Oxygen Demand (COD)

(Dichromate, Mercuric sulphate method; Adaptation of USEPA 410.4)

Equipment: COD Reactor (Hanna Reactor), Bench Photometer (Hanna C214 Multiparameter Photometer), Test tube cooling rack.

Reagents: reagent vials (Hanna MR COD vials), deionized water

Procedure: A homogeneous sampled was chosen to be measured. The COD reactor was pre-heated to 150°C. The cap of two reagent vials was removed. 2.0mL of sample was added to one vial (sample vial) and 2.0mL of deionized water was added to the other vial (blank vial). The caps of the vials were replaced tightly and mixed by inverting each vial a few times. The vials were inserted into the COD reactor and heated at 150°C for 2 hours. At the end of the digestion period the vials were allowed to cool to about 120°C. The vials were inverted several times while warm. The vials were placed in the test tube rack to cool to room temperature.

The blank vial was placed into the holder of the photometer and was zeroed for measurement. The blank was removed and the sample vial was placed in the holder of the photometer. The measurement was allowed to stabilize before the reading was taken.

A3: Determination of Internal resistance using Electrochemical Impedance Spectroscopy (As described in Thygesen et al. 2009)

Equipment: Potentiostat (Gamry 750), Microbial Fuel Cells, Personal Computer.

Procedure

A Gamry series G750 potentiostat and the Gamry Framework software was used in performing the EIS tests. The potentiostat was equipped with leads (wires) for working (WE), working sense (WES), reference (RE), counter (CE) and counter sense (CES) electrodes. The overall impedance was measured between the anode and cathode in a two terminal mode by connecting WE and WES to the anode, and CE, CES and RE to cathode. The experiment was carried out in EIS galvanostatic mode with AC-current amplitude of 0.3mA. The frequencies were varied from 20 kHz to 0.1Hz, with 6 steps per decade. The measurements were conducted when the MFCs were operating at steady state. Nyquist plots generated from the experiment were analysed for the overall internal resistance.

A4: Extraction of Biofilm DNA using the Power Biofilm Isolation kit

Equipment: PowerBiofilm Bead Tubes, 2mL Collection tubes, Incubator, Microcentrifuge, Vortex Adapter, Spin filters, Pipettors (100-1000uL)

Reagents: PowerBiofilm Solutions BF1, BF2, BF3, BF4, BF5, BF6 and BF7

Procedure

1. 0.05 to 2g of anode biofilm material was weighed and added to the PowerBiofilm Bead tube. 350uL of solution BF1 was added directly to the Bead tube. 100uL of Solution BF2 was also added and vortex briefly. The PowerBiofilm Bead tube was incubated at 65C for 5 minutes. Solution BF1 and BF4 were warmed prior to use at 55C for 5-10 minutes.
2. The Bead Tube was secured horizontally to a Vortex Adapter and vortex at maximum speed for 10 minutes. The Bead tube was then centrifuged at 13000x for 1 minute at room temperature. The supernatant was transferred to a clean 2 mL Collection Tube.
3. 100uL of Solution BF3 was added to the Collection tube and vortex briefly to mix. It was then incubated at 4C for 5 minutes. The tube was centrigued at 13000x for 1 minute at room temperature.
4. Avoiding the pellet, the entire volume of supernatant was transferred to a clean 2mL Collection tube. 900uL of Solution BF4 was added and vortex briefly to mix.
5. 650uL of supernatant was loaded onto a Spin Filter and centrifuge at 13000x for 1 minute. The flow was discarded and the step repeated until all the supernatant had been loaded onto the Spin filter.
6. The Spin filter basket was loaded into a clean 2mL Collection tube. 650uL of Solution BF5 was added to the tube and centrifuge at 13000x for 1 minute at room temperature. The flow through was discarded and 650uL of Solution BF6 was added and centrifuge at 13000x for 1 minute. The flow was discarded and centrifuged again at 13000x for 2 minutes to remove residual wash. The Spin filter basket was placed into a clean 2mL Collection tube.

7. 100uL of Solution BF7 was added to the centre of the white filter membrane. The tube was centrifuged at 13000x for 1 minute. The Spin filter basket was then discarded. The DNA extract remains in the 2mL Collection tube and is now ready for further application. The DNA extract was stored frozen at -20C.

A5: Scanning Electron Microscopy Analysis

Equipment: Scanning Electron Microscope (PHILIPS XL 30), Gold sputter (EMITECH K550X), stainless steel studs, oven

Reagents: Fixation solution (4% formaldehyde and 2.5% glutaraldehyde), ethanol, acetone, distilled water

Procedure

The anode material was sampled for analysis by cutting a piece of carbon paper (< 2% of anode material) and also by collecting some granules (< 3% of anode material) for AC and CB. The anode samples were stored in the fixation solution before their analysis. The samples were dehydrated with ethanol-water mixtures of 20%, 40%, 60%, 80%, 90% and 100% ethanol for 20 minutes each. The samples were further dehydrated with acetone-ethanol mixtures of ratios 1:2, 2:1 and pure acetone for 20 minutes each. Control samples (unused electrode materials) and the anode samples were critical point dried using Agar E3000 critical point dryer (Agar Scientific, Stansted, UK) with liquid CO_2 as drying agent. The samples were mounted on studs after drying. The samples were sputtered with gold in the EMITECH K550X sputter. The samples were viewed and microscopic images collected using the PHILIPS XL 30 SEM at different resolutions and magnifications.

APPENDIX B: Tables and Figures B1: Wastewater and Electrode materials

Table B1.1 Summary of wastewater characterisation for grey water and faecal sludge.

Parameter	Grey water (KNUST wastewater treatment plant)	Faecal sludge (KMA Landfill site – Dompoase)	EPA Wastewater quality guidelines for minimum discharge
pH	7.6 ±0.4	8.2 ±0.4	6 – 9
Temperature, °C	30.3 ±0.8	28.3 ±0.2	< 3°
Conductivity, mS/cm	1.35 ±0.15	17.19 ±2.38	1.5
Chemical Oxygen Demand (COD), mg/L	1482 ±150	7248 ±1604	1000
Total Dissolved solids, g/L	0.80 ±0.08	10.50 ±1.44	1.0
Total solids, g/L	1.05 ±0.04	12.73 ±1.6	-
Salinity, ppt	0.6 ±0.1	9.4 ±1.4	-
Total nitrogen, g/L	0.15 ±0.02	1.85 ±0.12	0.1
Total phosphorus, g/L	0.017 ±0.007	0.23 ±0.10	0.010
Ammonia content, g/L	0.17 ±0.05	2.01 ±0.73	0.010

Table B1.2 Electrode materials used for the MFC operations.

Parameter	Quantity	
	Activated carbon	Carbon butts
Mass of granules	57g	101g
Volume occupied by granules	40% (per chamber)	40% (per chamber)
Size of granules (diameter)	2 - 4mm	2 - 4mm
Projected surface area	0.37m^2	0.26m^2
Bulk density	0.57g/cm^3	1.02g/cm^3
Granular bed height	3.5cm	3.5cm

B2: Nyquist plots from Electrochemical Impedance Spectroscopy *Experimental Parameters for EIS:* AC current: 0.0003A rms, Initial frequency:

20000Hz, Final frequency: 0.1Hz, Points/Decade: 6

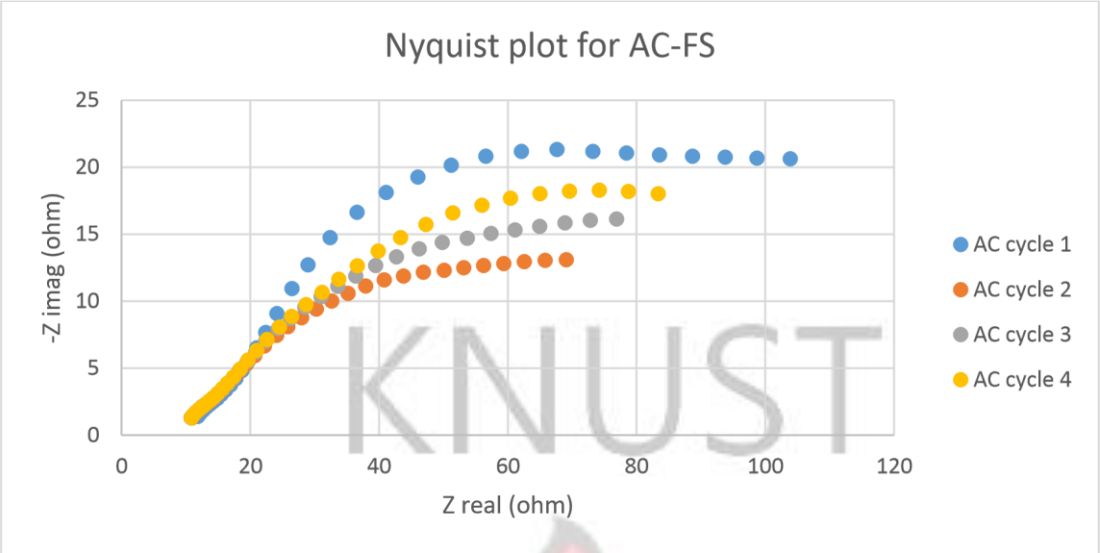


Fig.B2.1 Nyquist plot for overall internal resistance determination in AC-FS cycles

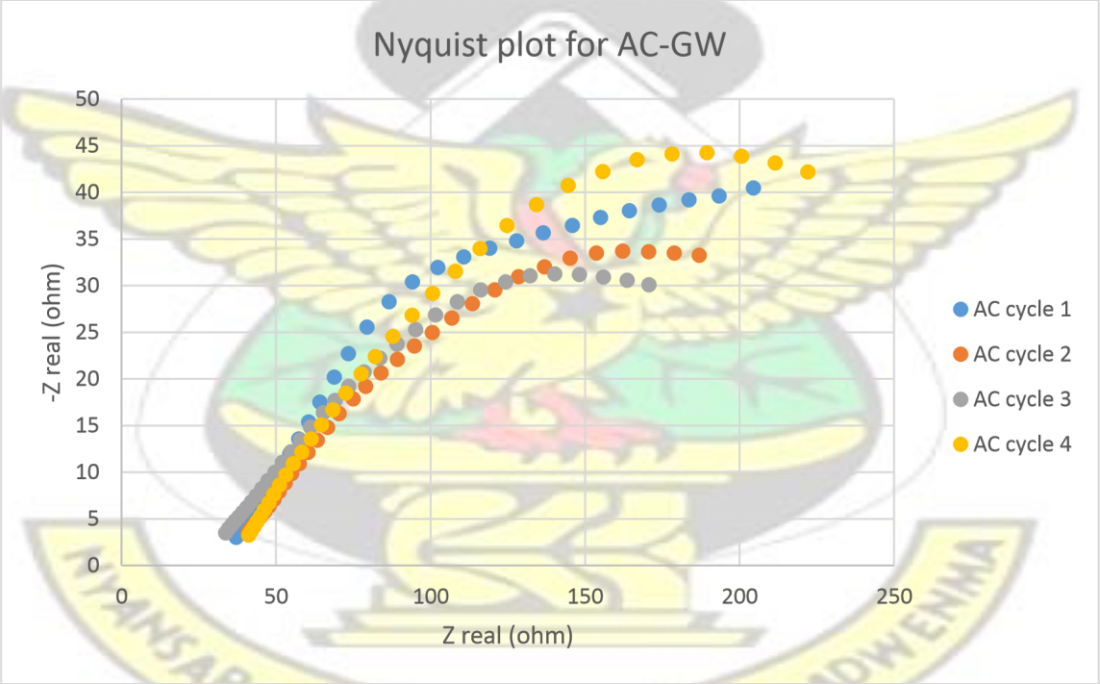


Fig.B2.2 Nyquist plot for overall internal resistance determination in AC-GW cycles

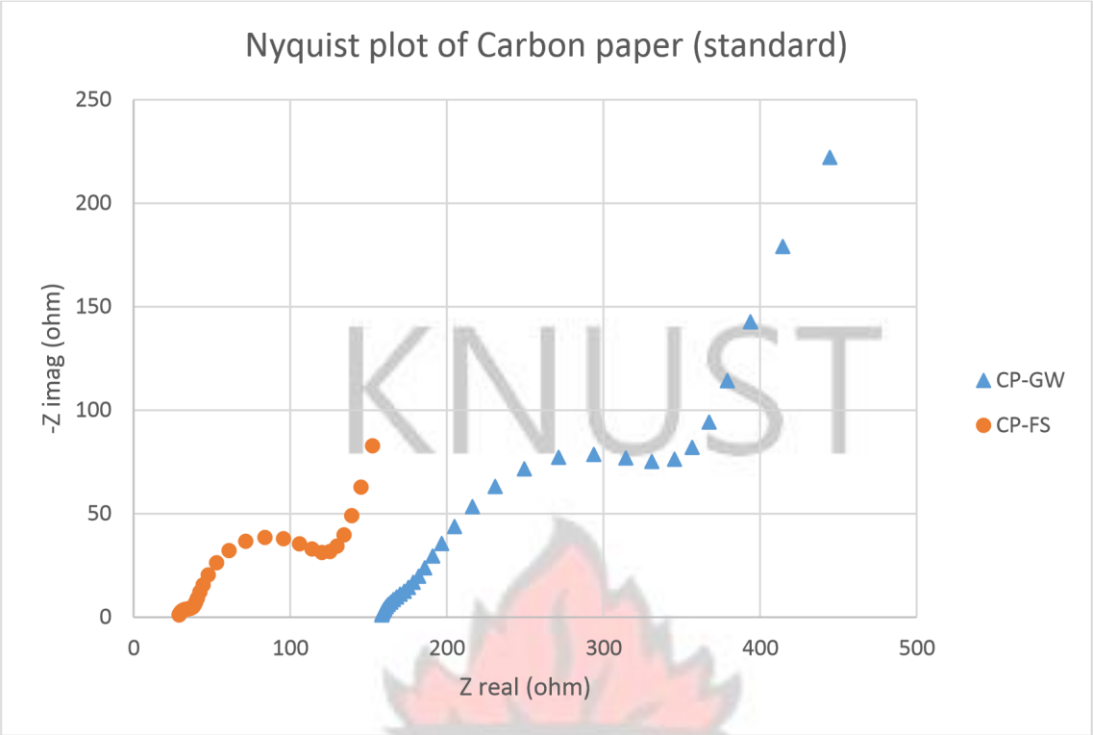


Fig.B2.3 Nyquist plot for overall internal resistance determination in Carbon paper with faecal sludge (CP-FS) and Carbon paper with grey water (CP-GW)

B3: Table of DNA Extraction concentration *Concentrations of extracted DNA.*

Sample	Duplicate 1 (ng/µL)	Duplicate 2(ng/µL)
CP-GW (4)	0.848	0.85
CP-FS (5)	2.884	1.865
AC-FS (1)	5.899	4.89
CB-FS (2)	1.857	1.86
Blank	-2.191	-1.181

B4: Tables of operating conditions at the anode

Parameter		Activated Carbon-Faecal sludge				Activated Carbon-Grey water			
		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4
pH	Initial	8.27	7.89	7.70	8.16	7.22	7.34	7.17	7.65
	Final	7.22	6.84	7.11	6.96	6.29	6.32	6.32	7.01
Conductivity, mS/cm	Initial	22.06	17.99	19.18	19.17	4.84	3.17	3.17	2.97
	Final	15.87	17.60	17.90	17.98	2.25	2.32	2.78	2.33
Total dissolved solids, g/L	Initial	13.28	11.14	11.63	11.63	2.92	1.98	1.96	1.82
	Final	9.80	10.70	10.79	10.94	1.39	1.41	1.67	1.43
Total phosphorus mg/L	Initial	–	134.3	134.3	132.7	–	20.6	41.2	26.3
	Final	–	133.7	131.2	129.9	–	15.4	6.6	15.5
Total nitrogen, mg/L	Initial	–	1468.7	752.3	1533.7	–	219.7	311.3	166.7
	Final	–	1071.5	688.8	1368.4	–	26.4	49.9	28.8
Ammonia content, mg/L	Initial	–	1978.2	1166.7	2078.2	–	207.4	113.4	228.8
	Final	–	1254.0	789.6	1376.6	–	37.4	47.8	79.4
Salinity, ppt	Initial	12.1	9.98	10.5	10.5	2.4	1.6	1.5	1.4
	Final	8.7	9.60	9.7	9.8	1.1	1.1	1.3	1.1
Chemical Oxygen Demand, mg/L	Initial	–	14776.6	11507.9	13142.0	–	2437.6	4852.4	3477.0
	Final	–	8159	7339	7749.0	–	682	1360	1050.1
Temperature, °C	28.5 ±0.6								