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Design, construction and operation of a membrane- and mediator-less Microbial Fuel Cell to generate electrical energy from artificial wastewater with a concomitant bio-remediation of the wastewater.

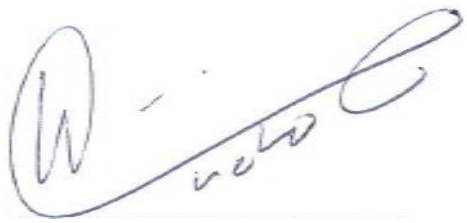
Winnie Mpumelelo Mahlangu

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science

April, 2015

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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Winnie Mpumelelo Mahlangu

April 2015

Abstract

Microbial fuel cell (MFC) technology presents great potential for use as a dual system for industrial waste water remediation and electricity generation. The hurdle in up-scaling this technology has been identified as MFC-bioreactor architecture, both with regards to bioremediation and carbon source to electricity conversion rates. In addition to the latter's limitations, the use of expensive mediators and membrane to enhance MFC performance renders the technology uneconomic to employ industrially. A 60mm high double chamber membrane and mediator-less MFC-bioreactor was designed, and constructed. The novel MFC-bioreactor made of transparent polyacrylic plastic had a total working volume of 8 litres with the anode chamber situated at the bottom and the cathode chamber at the top separated by a 10cm deep artificial membrane made up of glass wool, glass beads and marble balls. The MFC was operated under various operating parameters including; feeding modes (batch and continuous), with different substrate concentration at a range of external resistance (100-9000Ω) .The voltage produced during MFC operation was monitored and used to estimate the power density output of the MFC. The pseudo membrane was able to sufficiently separate the anode and cathode chambers allowing the development of potential difference and hence generation of current. The MFC demonstrated the potential for sustainable operation by producing and maintaining a stable power density of 2000mW/m^2 when operated with an external resistance of 1000Ω. This power density was accompanied by a 73% remediation efficiency of the synthetic wastewater. It was concluded that the results of this research show proof of concept for a membrane-less MFC that can produce electrical energy in the absence of an electron shuffling mediator.

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%	percent
^{o}C	degree Celsius
I	current
P_d	power density
i	current density
W/m^2	watt per square meter
mW/m	milli watt per square meter
Δ	change
Ω	Ohm
m^2	square metre
mL	millilitre
ml/min	millilitre per minute
g/L	grams per litre

Nomenclature

CFC	chemical fuel cell
MFC	microbial fuel cell
R	resistance
tCOD	total chemical oxygen demand
V	voltage

List of research outputs

Poster Presentations

Mahlangu, W.M. and Gray, V. (2012). Assessing the potential of a novel single chamber, membrane- and mediator-less microbial fuel cell to generate electrical energy synthetic wastewater with a concomitant bio-remediation of the wastewater. University of the Witwatersrand Postgraduate research symposium 2012

Mahlangu, W.M. and Gray, V. (2012). Design, construction and operation of a membrane- and mediator-less Microbial Fuel Cell to generate electrical energy from artificial wastewater with a concomitant bio-remediation of the wastewater. University of the Witwatersrand Postgraduate research symposium 2013

Mahlangu, W.M. and Gray, V. (2012) Design, construction and operation of a membrane- and mediator-less Microbial Fuel Cell to generate electrical energy from artificial wastewater with a concomitant bio-remediation of the wastewater.. University of the Witwatersrand Molecular Biosciences Research day 2013

Chapter 1: Literature Review

1.1. Introduction

In light of the global energy and water crisis caused by the adverse effects of industrialization on the environment, extensive research is been carried out in a quest to find alternative energy sources which have a minimum impact on the environment. To this end, current research is focused on generating knowledge in the field of renewable such as; solar panels, wind mills, hydro power, ethanol, biodiesel, methane and hydrogen gas as alternatives to burning fossil fuels such as oil, coal and natural gas for generating electricity (Sung *et al*., 2010).

Further, to address the water crisis, wastewater needs to be remediated to acceptable contaminants concentrations to be recycled, thus, more economic water treatment processes that are sustainable and their production have a minimum net carbon emission into the ecosystem such as anaerobic based treatment are currently investigated (Sung *et al*., 2010). Anaerobic based bioremediation of wastewater produces energy rich by-products in the form of reduced protons or hydrogen, which is usually a substrate for methane production. However, the reducing potential generated in anaerobic digestion can be used to directly generate electricity in Microbial Fuel Cells (MFC) reactors (Sung *et al*., 2010).

Anaerobic wastewater treatment employs the catalytic action of anaerobic bacteria to break down complex reduced compounds found in various industrial effluents into monomers which are less or non-toxic and easier to degrade with traditional anaerobic water treatment processes (Ghangrekar and Shinde, 2006). This degradation of reduced compounds by bacteria is accompanied by a release of energy. This informs the current efforts to attempt to couple wastewater bioremediation and biofuel production (Ghangrekar and Shinde, 2006).

Literature well documents the potential use of such biofuels (bio-hydrogen and methane) as alternative energy sources to use industrially (Rabaey and Verstraete, 2005). The potential use of biofuels is currently suggested based on laboratory scale experiments however, scaling up of

such technologies is still in its infancy (Das and Mangwani, 2010). The hurdle in moving from laboratory to industrial scale research or implementation in industry is mainly due to the relatively low energy outputs attained from bio-fuel reactor systems reported to date compared to fossil.

Consequently, more energy efficient and economically viable bio-fuel technologies are imperative to achieve sustained industrial use of bioenergy production (Du *et al*., 2010). Until recently, the main focus in bio-fuel research has been on the producing of bio-diesel and bio-ethanol from carbon rich plant materials such as sugar cane, maize meal and a variety of agricultural wastes. To this end, a number of anaerobic treatment reactors have also been optimised to produce hydrogen and methane gas.

The main challenge with the wide spread production of bio-diesel and ethanol is the threat the production of plant based fuels place on food security and are thus viewed as not being sustainable. On the other hand, the production of bio-gases currently yields low energy and is thus rendered uneconomic to up-scale. The focus over the last decade has therefore moved to Microbial Fuel Cell research as a single step process for electricity generation from organic substances, rather than attempting to generate electricity from anaerobic generated bio-gases such as methane or bio-hydrogen.

Microbial Fuel Cells have operational and functional advantages over other technologies that are currently used for bio-fuel production with regards to the following: 1) In MFC, electrical energy is produced when the energy available for the bio-conversion of substrate is converted directly into (usable) electricity. This is in contrast to other technologies where the bio-fuel produced cannot be used directly (like in bio-gas production), i.e. MFC have superior conversion rates. 2) MFC operate efficiently at ambient temperatures, distinguishing them from all current bio-fuel producing process which require energy input to heat up the system for efficient conversion rates i.e. those that rely on thermophilic bacterial culture for. 3) They have a relatively simple configuration which gives them a widespread application potential in locations lacking electrical generation energy infrastructure and where MFC may be incorporated to expand the diversity of fuels than can be used to satisfy the energy requirements. 4) MFC can generate electricity while accomplishing biodegradation of organic matter and hence remediation of wastes (Kaekannetra *et al*., 2011; Rabaey and Verstraete, 2005).

MFC thus provides the potential use of a technology with a dual function of wastewater bioremediation accompanied by the production of electrical energy which can be used to power a wide variety of wastewater remediation plants (Kassongo and Togo, 2010; Kaekannetra *et al*., 2011). Successful implementation of such technologies industrially would respond to two key environmental problems the global community is currently faced with; 1) Waste management and 2) green energy production (Kaekannetra, 2011; Pant *et al*., 2010).

1.2. History of MFC

The earliest concept of a MFC was demonstrated by Potter in 1910 (Du *et al*., 2007). Potter, 1910 illustrated that current can be generated from live cultures of *Escherichia coli* and *Saccharomyces* using a platinum electrode (Pant *et al*., 2010). But surprisingly, MFC technology is currently still in its infancy, with regards to the scientific community's understanding of its operation and microorganisms interactions that drive electricity generation.

This idea of generating electrical energy from microorganisms did not get much attention until the 1980 when it was discovered that current density and power output could be substantially enhanced with the addition of chemical electron transport mediators in MFC reactors (Du *et al*., 2007). Nonetheless, post this discovery in the 1980s, MFC technology still received little attention, with a lot of interest in bio-fuels production being focused on the development of bio-gases generating and combusting technologies such as those that produce bio-H_2 and CH_4 (Du *et al*., 2007).

An interest in MFC technology has only resumed in the past decade (Pant *et al*., 2010). The growing interest and body of research in MFC technology is indicated by the tremendous global increase in the number of publications focused on MFC technology (Pant *et al*, 2010; Du *et al*., 2007). Figure 1.1 illustrates an increase in the number of published papers on MFC between the years 1991 to 2009. The graph shows an increasing trend of publications with only 1 paper published in 1991 to 295 and 225 research papers published in 2008 and 2009 respectively, this indeed is indicative of a growing interest MFC technology (Pant *et al*., 2010).

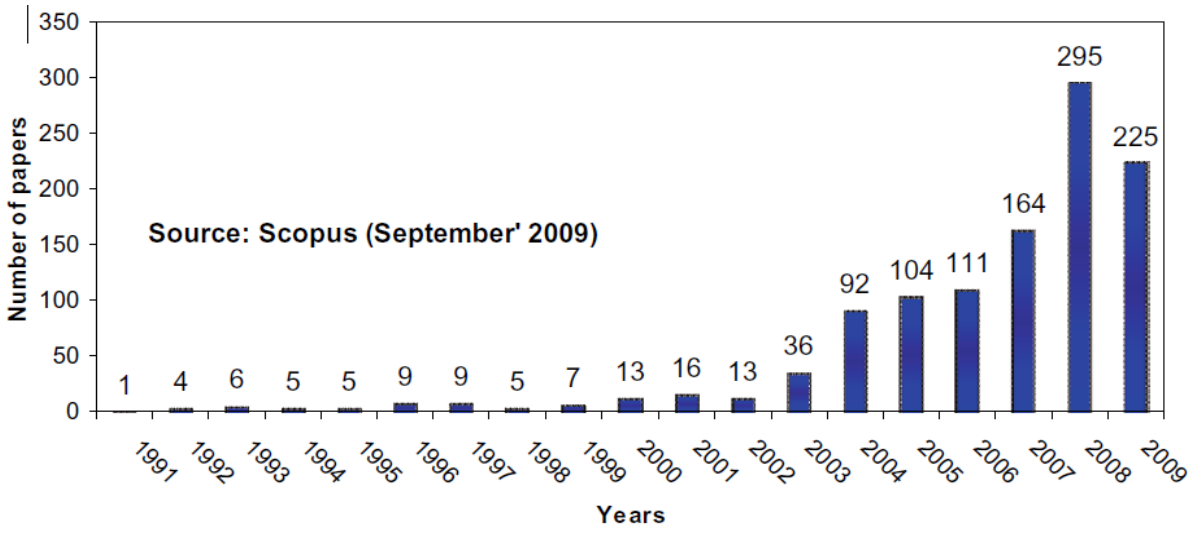

Figure 1.1: An illustration of the number of microbial fuel cell based research papers published from the year 1991 to 2009 according to Scopus (Pant *et al*., 2010).

1.3. Microbial Fuel Cell working principle

The overall reaction in MFC reactors is the oxidation of an organic electron donor substrate to carbon dioxide and water with a concomitant production of protons and electrons as a by-product (Kim *et al*., 2007; Lovely, 2006). MFC function the same as chemical fuel cells, except that instead of using chemical redox energy as a source of electrons, biological redox energy is used as fuel. The biological redox energy used in MFC is harnessed from the redox reactions of an organic substrate catalysed by anaerobic bacteria (Lovely, 2006).

1.3.1. Oxidation reduction reactions in MFC

The redox reactions in MFC are catalysed by anaerobic bacteria (Debabov, 2006; Lovely, 2006). Equation 1 and 2 below illustrates typical redox reaction pairs that occur in MFC resulting in the generation of electricity, for example when acetic acid and acetate are used respectively:

Anode: $\mathbf{C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-}$

Cathode: $2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$ **(1)**

Anode: $\mathbf{CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-}$

Cathode: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ **(2)**

Traditionally, MFC consists of anodic and cathode chambers separated by a proton exchange membrane (PEM) (Figure 1.2) (Sung *et al*., 2010). The anode chamber houses the fuel (organic substrate) and catalyst (anaerobic bacteria) while the cathode chamber contains the terminal electron acceptor- O_2 (Debabov, 2007). The anode is connected to the cathode electrode through an external circuit, usually connected to an external resistor or some form of load on which electrical work can be carried out (Lovely, 2006).

As indicated in equation 1 and 2, the bacteria in the anode chamber catalyse the anaerobic oxidation of the substrate liberating CO_2, electrons and protons (Rabaey and Verstraete, 2005). Depending on the reducing efficiency of the cathode chamber, it seems possible that complete anaerobic oxidation of organic substrate to CO_2, protons and electrons may be an achievable goal (Mohan *et al*., 2008). In contrast to the direct combustion process used as a second step to produce energy in bio-gases producing systems, in MFC, the electrons are absorbed directly by the anode electrode in the anode chamber and transported through the external circuit to the cathode electrode in the cathode chamber (Logan *et al*., 2006). The corresponding protons then migrate from the anode chamber to the cathode chamber through the PEM where they combine with a terminal electron acceptor such as oxygen to complete the redox reaction and thus the electric circuit (Schwartz, 2007).

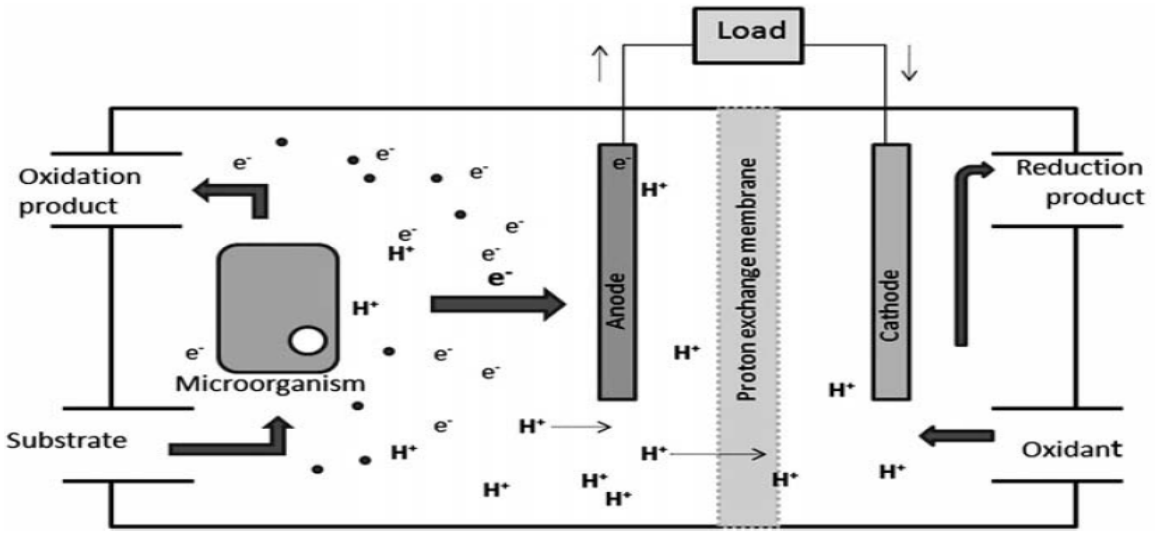

Figure 1.2: A schematic representation of a classic two chamber, H-type microbial fuel cell showing the main components of the reactor (Das and Mangwani, 2010; Logan and Regan, 2006).

Based on the anode and cathode reaction pair illustrated in equation 1 and 2 above, a MFC reactor can generate usable electrical power from the flow of electrons from the anode electrode to the cathode electrode via the external circuit (Rabaey and Verstraete, 2005). Current generation is made possible by keeping the anaerobic anode and aerobic cathode separate because efficient electricity generation in MFC requires the anode chamber to remain strictly anaerobic and the cathode electrode to be the sole terminal electron acceptor, to avoid electrical short circuiting by availing an electron acceptor in the anode chamber (Liu and Li, 2007; Logan *et al*., 2006).

1.3.2. Respiratory chain and electron transport

Electrical energy generation in MFC is made possible by anaerobic and facultative anaerobic bacterial cells performing catabolic metabolic activities to gain energy from the anaerobic oxidation of organic substrates (Rabaey and Verstraete, 2005). Bacterial cells mainly gain their metabolic energy via the transport of electrons in transport chain during respiration which results in the phosphorylation of a chain of electron transporting molecules ADP. ADP's phosphorylation is coupled to energy releasing redox reactions, either by substrate level phosphorylation or by oxidative phosphorylation via the electron the transport chain (Rabaey and Verstraete, 2005).

Energy in MFC is thus generated when a surplus of electrons are produced during the anaerobic metabolism of organic compounds in the anode and transferred to the terminal electron acceptor in the cathode chamber, rather than to protons or CO_2 in which case H_2 and CH_4 would be generated (Liu *et al*., 2004). Since the terminal electron acceptor is confined to the aerobic cathode chamber, the only way the electrons can reach the terminal electron acceptor is via the attachment and transfer to the anode electrode and transport to the cathode electrode through the external circuit (Liu *et al*., 2004).

Further, prior to the external electron transfer to the terminal acceptor, excess electrons produced in the cell cytoplasm must be transported to cell surface and then to the anode electrode (Liu and Li, 2007). This is a rate limiting and critical step in MFC technology (Mohan *et al*., 2008). Until recently, addition of chemicals to mediate transport and transfer of electrons (called mediators) was thought to be essential for MFC operation (Logan and Regan, 2006).

One of the ground breaking discoveries in MFC research to date has been the invalidating of the above theory on the dependency of chemical mediators for electron transfer by the discovery of electrogenic bacteria (Kim *et al*, 1999; Debabov, 2008). Electrogenic bacteria such has *Desulfuromonas acetoxidans, Rhodoferax ferrireducens, Shewella* and *Geobacter metallireducens* are capable of catalysing and coupling the complete oxidation of organic compounds such as glucose and starch with electron transfer directly to the electrode surface (Debabov, 2008).

These findings illustrated that MFC that use anaerobic microbial consortia can efficiently generate electrical energy without the addition of exogenous chemical mediators (Debabov, 2008). Although the process of direct electron transfer to the anode electrode by these bacteria is not fully understood, we know that this can either occur indirectly via production of exogenous and exogenous mediators or by direct contact of the bacterial cell membrane and the electrode surface (Sharma and Kundu, 2010; Kim *et al*, 1999).

The terminal electron acceptor should be a non-toxic sustainable compound and should not interfere in any way with the bacteria in the cathode chamber (Park and Zeikus, 2003). Naturally occurring oxygen is thus an ideal electron acceptor to use in MFC (Das and Mangwani, 2010). Oxygen is non-toxic (not always) and does not interact adversely to bacteria (except obligate anaerobic bacteria in the anode chamber) (Liu and Li, 2007). The only disadvantage with using oxygen is that it can diffuse into the anode chamber and act as a terminal acceptor, thus preventing electron transfer to the electrode (Logan and Regan, 2006). This is another rate limiting and critical factor to consider in MFC design, an efficient barrier is thus required to physically separate the anode and cathode chamber and prevent oxygen diffusion into the anode chamber (Das and Mangwani, 2010).

1.4. Ideal vs actual performance in MFC

Selecting a terminal electron acceptor with a higher electron affinity or oxidizing potential compared to the organic substrate used in the anode chamber is critical because the ideal performance of a MFC ultimately depends on the thermodynamic efficiency of electrochemical reactions that occur between the oxidised substrate and the reduction of the electron acceptor (Du *et al*., 2007; Das and Mangwani, 2010).

1.4.1. Rate limiting reactions

In addition to ensuring that a significant potential difference exists between the organic substrate and the terminal electron acceptor, it is also important to lower the activation energy required for both the anode and cathode reactions to take place (Du *et al*., 2007). Usually, this energy barrier must be overcome for electrons to flow in the electrode connecting wires and thus produce current (Liu and Li, 2007). This activation energy is a major limiting step in MFC when the rate of the electrochemical reactions at the anode and cathode surface is governed by slow reaction kinetics (Du *et al*., 2007).

Consequently MFC design, choice of anode and cathode solution, and inoculum must aim to increase reaction kinetics of the following rate limiting steps with high activation energies: 1) attachment of electrogenic bacteria to the anode electrode surface, and 2) transfer of electrons across the cells double membrane (Liu and Li, 2007). Mediators are added to lower this energy barrier and thus make the electron transfer to anode more efficient hence higher power densities are observed when mediators are used compared to when they aren't such as in mediator-less MFC (Du *et al*., 2007).

Mediator-less MFC rely on the movement of electrogenic bacteria and attachment to the anode surface (Jaing *et al*., 2010). However, in the absence of a mediator, the presence of conducting pili on the bacterial cell surface is critical to lower the activation energy of the electron transfer to the anode surface (Logan *et al*., 2006). In mixed culture inoculated MFCs, formation of biofilms on the electrode surface is an effective way of improving the interaction of bacteria and the electrode surface to lower the activation energy of electron transfer (Du *et al*., 2007). Jaing *et al*., 2010 also supports this by suggesting that higher extents of bacterial adhesion enable bulk transfer of electrons from electrogens to electrode surface (Jaing *et al*., 2010).

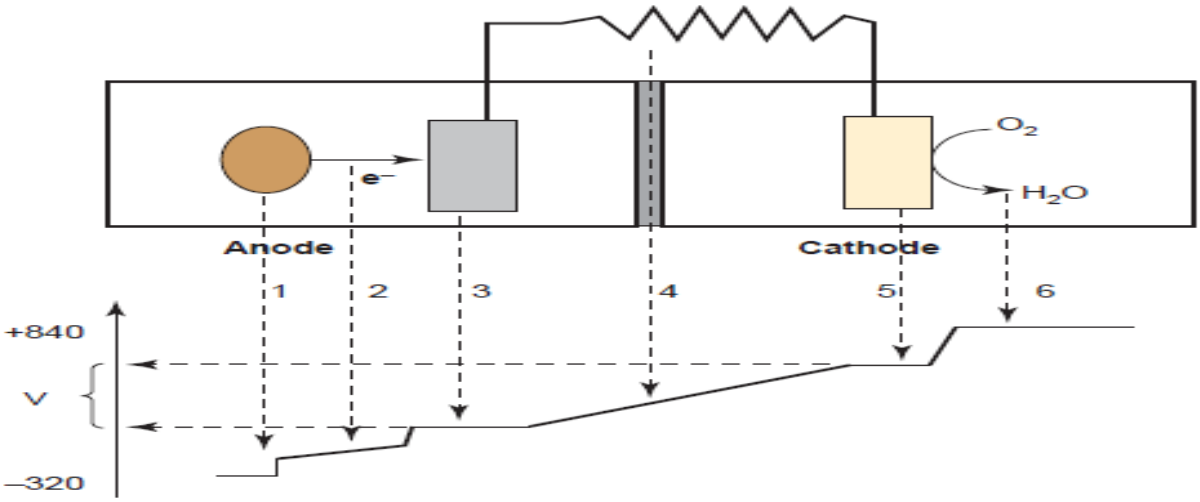

Figure 1.3: An illustration of where potential losses occur in MFC during electron transfer; 1) Loss owing to bacterial electron transfer, 2) Loss owing to electrolyte resistance, 3) loss at the anode, 4) loss at the MFC resistance (useful potential difference), 5) losses at the cathode and 5) losses owing to terminal electron acceptor reduction (Rabaey and Verstraete, 2005).

1.4.2. Ohmic losses

Supplementary to the above stated rate limiting reactions that occur due to the transfer of electrons from bacterial cell membrane to the anode surface (Figure 1.3), the efficacy of MFC to produce electric energy is lowered by Ohmic losses (Logan and Regan, 2006). Ohmic losses are caused by the resistance of the electrolytes (anode and cathode solutions), electrodes and connecting wires to the flow of electrons (Aldrovandi *et al*., 2009).

Since power in MFCs is generated by the flow of electrons through the connecting wires in the external circuit and their reaction to the terminal electron acceptor in the cathode chamber, Ohmic losses greatly decreases the ideal performance of MFC (Jiang *et al*., 2010). Ohmic losses in MFC can be reduced by increasing the ionic conductivity of the anode electrolyte and reducing the distance the electrons travel in the external circuit to reach the terminal electron acceptor (Schwartz, 2007). This can be achieved by shortening the distance between the electrodes and thus decreasing the length of the connecting wire (Du *et al*., 2007).

1.4.3. Efficiency of converting matter to current

As mentioned in section 1.3, the electrons that power MFC are produced from the degradation of organic substrates such as acetate, glucose and various industrial wastewaters with high concentration of organic compounds (Kassongo and Togo, 2010; Wen *et al*.,2010). The efficacy of MFC to convert matter into current is measured by its ability to recover the electrons liberated from the oxidative degradation of organic material and harness them for current generation (Sharma and Kundu, 2010). This is termed columbic efficiency (CE) and is defined as; the percentage of electrons recovered for current generation vs the theoretical maximum that is produced by the complete oxidation of a certain organic substrate (Du *et al*., 2007).

For example, the complete oxidation of one mole of glucose yields 24 moles of electrons theoretically (Du *et al*., 2007). However, the actual cell potential is always lower than its theoretical equilibrium potential due to a number of irreversible losses inherent to MFC operation and bacterial cell activities (Du *et al*., 2007; Jiang *et al*., 2010).

In addition to energy losses due to the energy barrier used to overcome the rate limiting steps, bacterial cells use a portion of the electrons for cell division and some is lost in the anode electrolyte (Liu and Li, 2007). This electron loss is mandatory for the survival of bacteria and hence the continual operation of MFC. It is thus worth acknowledging that unlike in chemical fuel cells (CFC), CE in MFC cannot be 100% (Jiang *et al*., 2010; Du *et al*., 2007).

The additional losses however can be decreased by improving MFC design (Jiang *et al*., 2010). These are predominantly due to; 1) diffusion of electrons into the cathode chamber, 2) diffusion of terminal electron acceptor into the anode (if in the anode chamber, acceptors such as oxygen can be used aerobic respiration by facultative bacteria), and 3) when a consortium of bacteria is used, some electrons can be lost to other electron acceptors that involve bacteria such as methanogenesic and denitrifying bacteria (Jiang *et al*., 2010).

Reported CE varies for different MFC (to date CE of ranges between 0-90% have been reported) but in general it has been found that CE is proportional to power density produced by the MFC (Du *et al*., 2007). It is understood that, when a MFC produces a high power density, the system works to obtain a electron equilibrium and so doing more electrons are preferentially directed for transfer to the anode and less are lost through competing biological and physical processes (Park

and Zeikus, 2003; Du *et al*., 2007). The choice of inoculum and electrolyte thus need to be considered and improved to reduce electron losses and increase CE (Wen *et al*., 2010).

1.5. MFC design elements that affect decrease in voltage

In addition to the intrinsic system losses due to Ohmic and activation energy losses that decrease the efficiency of MFC, a number of design elements also affect the potential to reach near ideal performance current output (Logan *et al*., 2006). These include the choice of 1) electrode material and surface area (Kim *et al*., 2007); 2) type and size of membrane or separator; 3) use of electron transport and catalyst mediators; 4) pH of anode and cathode electrolyte (Kim et al., 2007); 5) external resistance; 6) substrate used (Pant *et al*., 2010) and 7) MFC reactor architecture (Logn *et al*., 2006). A brief description of how each of the latter stated elements affects MFC operation is detailed in the following sections.

1.5.1. Electrode material and surface area

Since electrode material directly impact on the magnitude of the activation energy losses and because using different anode and cathode electrode material results in different activation losses, the choice of material is critical in MFC optimisation with regards to current and power output (Elakkiya and Matheswaran, 2013). An electrode material that yields superior current outputs is biocompatible, conductive, and chemically stable over long periods in the electrolyte (Logan *et al*., 2006).

The most adaptable and widely used electrode material for both anode and cathode is carbon derived graphite plates, rods, cloth and paper (Logan *et al*., 2006). Carbon derived electrodes are easy to handle, relatively inexpensive (compared to better performing electrodes such as Pt based electrodes) and have an easily defined surface area (Du *et al*., 2007).

Electrocatalytic materials such as Pt increase current generation by assisting the direct oxidation of microbial metabolism and thus lowering the activation energy of electron transfer at the electrode (Logan *et al*., 2006). Pt electro-catalysis by Pt is similar to the action of chemical electron transfer mediators such as Mn (IV) and Fe (III) that are used to improve power output in MFC (Logan *et al*., 2006).

Pt and Pt black derived electrode are available and have been used in MFCs and were found to be superior to carbon derived electrodes (Du *et al*., 2008). Pt electrodes thus have a higher catalytic activity to lower activation energy than carbon material (Logan *et al*., 2006). The downside of using Pt electrodes is that their activity declines over time due to the formation of a Pt-O layer at the electrode surface which limits bacteria interaction with the electrode (Logan *et al*., 2006). Another drawback in addition to its unsustainable use in up scaling is the much higher cost of Pt compared to carbon electrode (Liu and Li, 2007).

1.5.2. Membranes

Majority of MFC designs include the addition of a proton exchange membrane (PEM) to separate the anolyte from the catholyte. A PEM is added to mediate proton transfer from the anode to the cathode and prevent diffusion of oxygen from the aerobic cathode chamber to te anaerobic anode chamber (Das *et al*., 2010). Exceptions to these are the recently widely explored single compartment MFC and naturally occurring systems such as sediments (Logan *et al*., 2006). A good quality PEM is highly selective to the permeability of protons such as Nafion and Utex CM- 1700 are used for upper limit power outputs reported to date (Liu and Logan, 2004).

However a big challenge with PEM efficacy is that the movement of other cations through the membrane even with the best performing PEM such as Nafion is unavoidable. This is due to the concentration of competing electrolyte components such as Na^+, K^+,NH_4^+,Ca^{2+} and Mg^{2+} being much higher than that of protons generated from the degradation, these ions occupy most of the PEM surface area and thus limit proton transfer (Du *et al*., 2008). The diffusion of these affects the internal resistance and concentration polarisation losses is the electrolyte and thus limits power output (Logan *et al*., 2006). The efficiency of proton transfer to the cathode can be facilitated by increasing the surface area of the PEM with the size of the MFC (Du *et al*., 2008). It has been shown that MFC internal resistance is inversely related to the increase in PEM surface area (Du *et al*., 2008; Logan *et al*., 2006).

1.5.3. Electrolyte properties

Anode

Fuel type, concentration, feeding rate, pH, mediators and microorganism used in the anode chamber are all important factors that impact performance of MFC (Mardnopour *et al*., 2012). A number of studies have illustrated that electricity generation is dependent on the type of fuel and concentration used in MFC (Kassongo and Togo, 2010). Thus increasing fuel concentration increases the power output in MFC. This relationship however has a growth phase type of shape graph, so power output will increase over a range of concentration until it reaches saturation and then start to decline (Jang *et al*., 2003).

MFC feeding rate has the same correlation to power outputs in MFC as fuel concentration, in both parameters, it is thought that a high concentration of fuel in the anolyte promote growth of other bacteria such as fermentative and methanogenesic bacteria faster than that of electrochemically active bacteria in a mixed culture inoculated anode (Du *et al*., 2008). These degrade the substrate but do not transfer electron to the anode electrode and electrolyte, the MFC would thus consume fuel but yield low current generation (Liu and Logan, 2004).

In addition to promoting growth of non electrogenic and electron consuming bacteria, a high fuel concentration and feeding rate causes, an increase in the concentration of various ions in the electrolyte which are added to promote bacterial growth (Na^+, K^+,NH_4^+,Ca^{2+} and Mg^{2+}). This is undesirable and limits power output (this has been detailed in the section above) (Logan *et al*., 2006).

Cathode

Ferricynanide ($K_3(CN)_6$) is a good example of a good chemical terminal electron acceptor, it has a low resistance which overcomes the activation energy limitation for oxygen reduction at the cathode. Ferricynanide is widely used in MFC for optimal operation in lab scale MFC. Additional ideal characteristic of terminal electron acceptors are 1) It should not interfere with bacterial activities in any way, 2) It must be relatively easy to access and affordable and 3) Its use must be sustainable (remain active in solution for long periods of time) and 4) have a low dissolved oxygen saturation concentration (Das *et al*., 2010).

While ferricyanide is a good electron acceptor, its greatest disadvantage for use in industrial scale MFC is that it has a number of the undesirable characteristics mentioned above. Oxygen does not sufficiently re-oxidise ($K_3(CN)_6$), its activity declines over time and thus cathothyte requires constant changing (Das *et al*., 2010). ($K_3(CN)_6$) can also diffuse into the anode chamber and interfere with bacterial cells activity in the anolyte (Logan *et al*., 2006).

Oxygen has been reported to have poor reduction kinetics that leads to high activation energy requirements for the cathode reaction and thus yield low power output compared to ferricyanide (Kim *et al*., 2007) .Oxygen is still the preferred terminal electron acceptor because of its low cost (it occurs naturally and is thus free), it does not interfere adversely with bacterial activity and only water is formed as the only end product in the cathode (Logan *et al*., 2006). The disadvantage of using oxygen is that, if it diffuses to the anode it can cause a consumption of electrons by facultative and aerobic bacteria and reduce current generation when a mixed culture is used to inoculate the anolyte (Das *et al*., 2010).

1.5.4. Electrolyte buffering

It follows then from the section above, that a buffer solution is needed in the electrolyte because if it is not used, it is clear that there will be a significant difference in pH between the anode and the cathode chamber (Schwartz, 2007). Although theoretically there should be no difference in pH when the consumption rate of protons, electrons and oxygen in the cathode matches the production rate of protons from degradation at the anode, the limitations of PEM decrease the proton transport rate to the cathode and therefore cause acidification of the anode and decreasing cathode pH to basic conditions (Kaewkannetra *et al*, 2011). A buffering solution such as usually PBS is used to reduce the impacts of the pH shifts in the two chambers, decrease internal resistance and thus increase current output (Du *et al*., 2008). It has been reported that an appropriate buffer to use is one with a pK_a similar to the pH of the anolyte (Pant *et al*., 2010).

Substrate

Organic substrates are important in MFC as it provides a carbon/ fuel energy source for the bacteria to degrade and metabolise it to produce electrons which in turn produce the current harnessed in MFC operation (Aelterman *et al*., 2006; Kassongo and Togo, 2010). The efficacy and economic viability of MFC to produce electrical energy from converting organic matter depends on the characteristics of the matter which ultimately determine how the catalysing microbes will interact with and degrade it completely (Pant *et al*., 2010).

Substrate characteristic are crucial in MFC operation because their chemical composition determines the following 1) The bacterial communities that will survive and thrive in the anolyte, 2) The kind of communities and metabolic relationships between biofilm forming bacteria on the anode and cathode electrodes and ultimately 3) The coulombic efficiency and power output in MFC (Das *et al*., 2010).

There is a large and extensive range of substrates that can be used as fuel in MFC (Logan *et al*., 2007). The types of substrate used in MFC to date range from pure compounds such as acetate (Logan *et al*., 2007) and glucose (Catal *et al*., 2008) to complex mixtures of organic wastes present in a variety of industrial wastewater such as that produced from breweries, paper mill and diary (Feng *et al*., 2008; Wang *et al*., 2009 and Aldrovandi, *et al*., 2009).

1.5.5. External resistance

A large range of external resistors with magnitudes ranging from 0-10000Ω have been used in most MFC research studies, and in most results reported to date, it was found that the amount of power generated is proportional to increases external resistance (Feng *et al*., 2008). It is also reported that changing the external resistance alters microbial interactions of bacterial communities attached to electrodes and thus increasing the activation energy of the redox reaction at the anode (Jang *et al*., 2004). It is recommended for power output optimisation studies that an external resistance close to the internal resistance be used in MFC operation (Kasango and Togo, 2010).

1.6. Improving MFC reactor architecture

In addition to the above-mentioned system parameters that affect power output in MFC, the MFC design has also being identified as a limiting factor in MFC power output (Park *et al*., 2002). On-going studies in laboratories around the world is focused on improving MFC reactor architecture to optimise power output, at the same time efforts are made to design less expensive, more practical and economically viable to upscale designs (Qian and Morse, 2011).

One of the fundamental changes that changed the course of MFC research in recent years is the move from two-compartment MFC to single chambered MFC designs (Feng *et al*., 2008); Rabaey *et al*., 2003). The two compartment design has been widely used in lab-scale experiments and has probably yielded the bulk of literature on MFC to date however, such designs would be difficult to upscale for continuous operation as an industrial size system (Lui *et al*., 2004).

Although the move to single compartment was initially made from an architectural point of view, its extensive use was promoted by the observation that MFC can be effectively operated in the absence of a PEM (Liu and Logan 2004). This was one of the most significant breakthroughs in MFC research and has led to the elimination of PEM in the MFC system making MFC more viable for sustainable operation industrially (Lui *et al*., 2004). It has also been noted that the more efficient MFC reactor designs for continuous operation as wastewater remediation and electrical energy generation systems, a MFC must have a large surface area for biofilm formation and a high internal volume (Lui *et al*., 2004).

Biofilm formation on electrode and reactor surfaces is critical because it allows inter-specie electron transfer in the EPS of the biofilm; this lowers the activation energy of electron transfer to the anode surface and thus lower internal resistance (Mohan *et al*., 2008). Further, an ideal biofilm design is one that has a short distance between electrodes and hence low Ohmic losses due to internal resistance of electron flow to the cathode (Logan *et al*., 2006).

Another significant breakthrough in MFC research was the observation that chemical mediators in both the cathode and the anode compartment can be eliminated and replaced by biological catalysts (Feng *et al*., 2008). Chemical electron transport chain mediators in the anode can be eliminated by using a consortium of anaerobic, electrogenic bacteria. Similarly, cathode

mediators such as the traditionally used ferricyanide can be replaced by using aerobic bacteria and their oxidases which have a high affinity for oxygen to catalyse the cathode reaction (Kim *et al*., 2007). Thus to improve and produce sustainable power, formation of anaerobic and aerobic biofilm is thus important in both anode and cathode compartment respectively to facilitate the mediation of electron transport by electrogenic bacteria.

1.7. Problem statement

In order for a MFC system to be viable for both wastewater bioremediation and electricity cogeneration industrially, its design and operation should have the necessary properties which enable this goal to be achieved at the lowest possible cost and to be amenable to scaling up. With MFC, simple designs are superior to complicated designs especially for cost reduction, ease of operation, and ease of scaling up. The elimination of membranes and mediators in MFC reactor design is informed by the cost reduction that accompanies their exclusion in MFC designs and further, the dismissal of toxic by-products that are produced with the use of chemical mediators in the electrolyte.

1.8. Hypothesis

It hypothesised that a more diffuse barrier (relative to the conventional PEM) splitting the anaerobic anode and aerobic cathode chambers will still allow sufficient separation of the anode and cathode chamber while permitting diffusion of positive ions to the cathode chamber thus permitting generation of potential difference between the two chambers in the absence of a PEM and consequently promote generation of current in the external circuit.

Further, that anaerobic sludge collected from an anaerobic wastewater treatment plant contains a consortium of bio-electrochemical bacteria that can facilitate degradation of synthetic wastewater (sucrose based) and transport electrons to the anode electrode allowing the generation of electron flow in the external circuit in the absence of any exogenous chemical mediators. Support for this hypothesis will provide proof of concept that membrane- and mediator-less MFC can be operated for both bioremediation and bio-energy production.

1.9. Objective

The niche of MFC research this work seeks to addresses is the designing of less expensive, easy to operate and economic to upscale MFC reactor design (Qian and Morse, 2011). The objective of this work was thus; to design, construct and operate a double chamber MFC reactor and operate it under various operational parameters to determine its optimum operational parameters (feeding mode, substrate concentration and external resistance) for both synthetic wastewater bioremediation (COD removal) and electricity generation (measured in voltage, current and power density output) using anaerobic sludge as inoculum.

This objective was achieved through investigating the following specific aims:

Aims

1. To design and construct a double chamber, membrane-less and mediator-less MFC bioreactor.

2. To assess the potential and optimal operation parameters of the membrane-less MFC for electrical power generation using Endo-sucrose media based synthetic wastewater as fuel.

3. To evaluate the potential and optimal operation parameters of the membrane-less MFC for bioremediation using Endo-sucrose media based synthetic wastewater as fuel.

Chapter 2: MFC assembly and development (Pilot study)

2.1. Introduction

To date, no industrial size MFC reactor has been studied or used in any application, however the past decade has observed great advances in the development of laboratory scale MFC reactor technology and this has been achieved by making rational changes in MFC design (Du *et al*., 2007). The current direction is to move away from the traditional H-type double chamber MFC reactors that employ proton exchange membranes, single culture inoculation and electron transfer mediators to developing less complex, single chamber reactors without membranes and mediators that are rather catalyzed by a consortia of bacterial species (Park and Zeikus, 2002).

It is thus timely that currently the major focus of MFC research knowledge generation is on designing and exploration with various configurations to improve MFC output (Qian and Morse, 2011). This is the gap in knowledge that this work aims to fit in and contribute towards. Lab scale MFC reactors serve as great devices for exploring the relationship between reactor architecture and substrate degrading microbes in the MFC as well as the interactions involved in electron transfer at the anode and cathode electrodes (Qian and Morse, 2011).

Other on-going research in MFC technology is that focused on designing micro-MFC in which chamber volumes are reduced to sub-microliter scales (Qian and Morse, 2011). Micro-MFC are great devices to use in generating in-depth understanding of the molecular level interactions that occur during the extracellular electron transfer process at the electrode surface (Qian and Morse, 2011). In contrast to lab scale MFC reactors, micro-MFCs offer a unique opportunity to analyse the biochemistry of MFC interactions at small micro-milliliter scales in a controlled microenvironment (Qian and Morse, 2011).

Numerous different configurations are possible to implement in designing MFC reactors (Logan *et al*., 2006). Two-compartment MFC are currently only used in laboratory scale studies and typically operated in batch mode. A key element of the traditional “H-type” MFC is the separation of the two chambers using a PEM. The need for a PEM makes the design expensive to upscale and its batch feeding mode makes it impractical for continuous operation (Du *et al*., 2007).

Contrary to traditional PEM double compartment MFCs, membrane MFC reactors offer simpler designs that can be operated in up-flow mode both in batch and continuous feeding mode (Logan *et al*., 2006; Du *et al*., 2007). Also since there is no need for separation using a PEM, the design is more suitable for sustainable operation and relatively easy to up-scale because they offer cost saving opportunities (Du *et al*., 2007).

In this study, an 8L double chamber MFC reactor made from plastic material that enhance bacteria attachment for biofilm formation was designed. The electrode material chosen was carbon derived graphite felt rods. The graphite rods as both anode and cathode electrode was chosen due to its relatively inexpensive cost, ease of handling characteristics, durable and non-toxic nature. These characteristic make it suitable for sustainable long term use and are desirable for industrial scale MFC systems (Liu *et al*., 2007).

A mixture of anaerobic bacteria enriched from a wastewater treatment plant anaerobic sludge was used to catalyse the anaerobic degradation of fuel (sucrose) in the anode chamber. Oxygen was used as the terminal electron acceptor in the cathode chamber and, to promote growth and development of an aerobic biofilm in the second cathode chamber, the cathode chamber was not sterilized. The use of bacteria as bio-catalysts in the design eliminates the use of chemical mediators which are expensive and unsustainable to use for continuous operation due to their toxicity to bacteria (Lui *et al*., 2007).

Objectives

The objectives of the development study were to:

a) design, construct and assemble a membrane-less MFC.
b) inoculate and develop a microorganism culture in the anode chamber.
c) assess functionality and structural limitations of the MFC.

2.2. Materials and Methods

2.2.1. Microbial Fuel Cell-reactor design

The MFC-bioreactor designed for construction was an up-flow, double chamber, cylindrical vessel constructed from transparent polyacrylic plastic. The MFC had a height of 60cm and external diameter of 18cm with a total working volume of 8L. The vessel was divided into two chambers with 4L for the anode and cathode chamber respectively. The anode chamber was positioned at the bottom and the cathode chamber at the top as illustrated in Figure 2.1. The two chambers were separated by a 5cm perforated polyacrylic plastic plate that acted as a membrane to allow liquid and ion exchange from the anode to the cathode compartment.

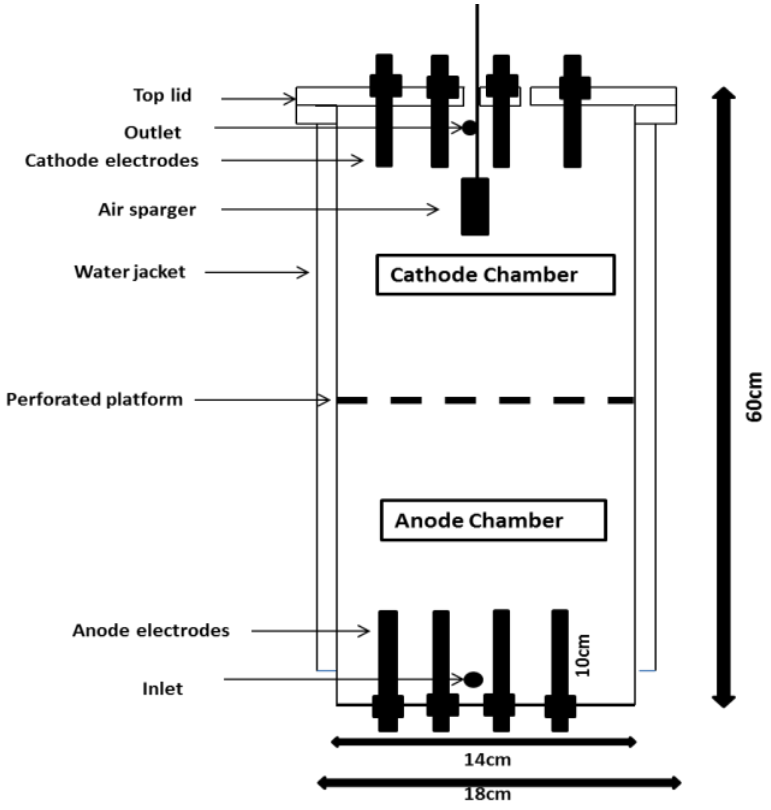

Figure 2.1: A schematic diagram of the MFC bioreactor design

Since the electrodes are at the top and bottom extreme of a 60cm high reactor, the distance between the electrodes in thus 60cm (Figure 2.1.). The internal diameter of the MFC was 14cm and in addition to this, a 2cm wide water jacket covered the entire circumference of the MFC. An

inlet was positioned at the bottom of the node chamber to allow influent feeding into the anode. The outlet was placed at the top of the cathode chamber to allow effluent out of the reactor. The outlet was also used as a sampling plot for effluent analysis from the cathode chamber. In addition to the inlet at the bottom center of the anode chamber, two more were positioned on either side of the chamber to allow stirring of the anode compartment (Figure 2.1).

Eight 10cm long graphite carbon rods were used as electrodes in both the anode and cathode chamber. The electrodes in the anode chamber were fitted onto the lid and protruded into the bottom of the compartment. Similarly, the electrodes in the cathode chamber were fitted on to the top lid and protruded into the top exterior of the compartment. This positioning of the electrodes resulted in a 60cm distance between the electrodes of each chamber (Figure 2.1).

2.2.2. MFC assembly

The 8 electrodes fitted on the lid of each chamber were each connected to a rubber insulated copper wire and all 8 wires were joined at a single point. A single wire from this focal point connected the anode and cathode electrode in series to a digital voltmeter from which voltage (potential difference) and current was monitored.

A pseudo-membrane to separate the anode and the cathode chamber was created by placing two layers of glass wool sheets on to the perforated plate. 450g glass beads (5cm thick) were added on top of the glass wool to complete the membrane. The resulting height of the membrane was 15cm and protruded into the cathode chamber, thus decreasing the working volume of the chamber (Figure 2.2).

A peristaltic pump was connected to the feeding tank which was used to store influent and the inlet at the bottom of the anode chamber by thick rubber pump pipes to allow feeding. The MFC was operated in an up-flow mode with the influent entering at the inlet in the anode chamber and exiting through the outlet at the top of the cathode chamber. The outlet was connected to the waste tank by a rubber pipe.

An air-pump was connected to the air sparger on the top lid (Figure 2.2). The sparger bubbled air to the top of the cathode chamber. Figure 2.2 below illustrates the overall assembly of the MFC bioreactor and all its functional supporting and monitoring equipment.

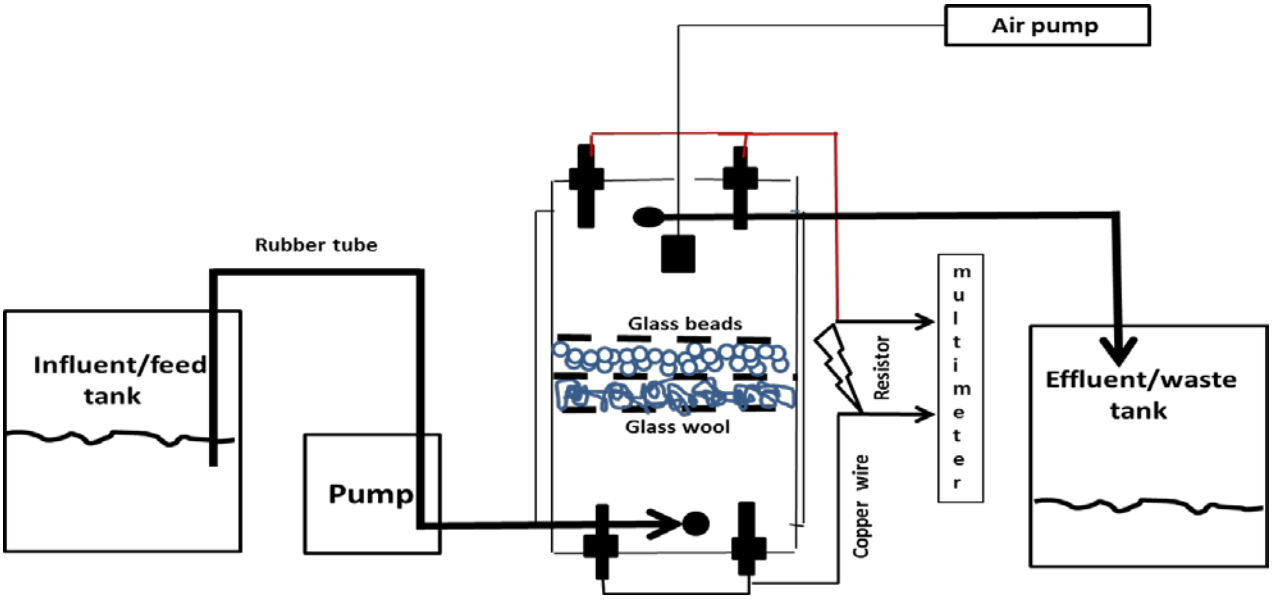

Figure 2.2: A schematic diagram of the MFC-reactor assembly

2.2.3. MFC development (Pilot Study)

Study controls

Control 1: The MFC was filled with sterile Endo-sucrose media in the anode chamber and water in the cathode chamber and run for 5 days. Control 2: inoculated Endo-media (0g/L sucrose) was added to the anode chamber. Voltage was monitored daily using a digital multimeter to assess if un-inoculated Endo-sucrose and inoculated Endo (only) media can produce potential difference in the MFC.

2.2.3 MFC reactor inoculation and anodic culture development

Activated anaerobic sludge collected from a full scale municipal domestic wastewater treatment plant was used to inoculate the anode chamber in the study. The sludge was pre-treated by heat cooking the anaerobic sludge at 100°C for 15 minutes to eliminate pathogenic and methanogenic bacteria prior to inoculating the anode chamber.

A modified buffering and anaerobic culture enriching media i.e. Endo-media (Endo *et al.*, 1982), containing glucose was used as organic substrate and model wastewater throughout the study. The Endo-sucrose media contained (g/L); 6.72 NH_4HCO_3; 0.20 $CaCl_2$; 0.699 K_2HPO_4; 3.36 $NaHCO_3$; 0.015 $MgCl_2.6H_2O$; 0.0225 $FeSO_4.7H_2O$; 0.005 $CuSO_4.5H_2O$; 1.24 x 10^{-4} $CoCl_2.H_2O$ and sucrose(17.63g/L sucrose).

100ml of the heat treated sludge was inoculated in 400ml of Endo media with 17g/L sucrose and incubated at 37^oC overnight to acclimatize the bacterial culture to the media condition. The overnight incubated culture was used to inoculate 3.5L of wastewater (17.63g/L sucrose) in the anode chamber. The inoculated anolyte was left to stand without further feeding for 2days to settle the culture and create an anaerobic bacterial culture gradient at the bottom anode chamber.

To acclimatize the bacterial culture to the reactor conditions and build a stable anaerobic culture, the MFC was fed wastewater (17.63g/L sucrose) continuously at 1ml/min for 35days. Potential (voltage) of the MFC without external resistance was monitored daily. This development phase also tested the capability of the MFC reactor to properly carry out its faction as a continuous system and whether all components are accurately fitted to endure long term operation.

2.3. Results and Discussion

2.3.1. Microbial Fuel Cell reactor design

The MFC reactor was accurately constructed according to the schematic design. The MFC was made up of four separate components i.e., the main reactor hollow cylinder, the perforated plate and two lids as illustrated in Figure 2.3 below. This design was based on a membrane-less MFC prototypes used by Lui and Logan, (2006) and Hu (2008). The use of glass beads and glass wool to create a pseudo membrane by Lui and Logan was modified by adding bigger marble balls and incorporated Hu (2006) design by making placing a complete by perforated disc instead of a baffled one (Hu, 2006).

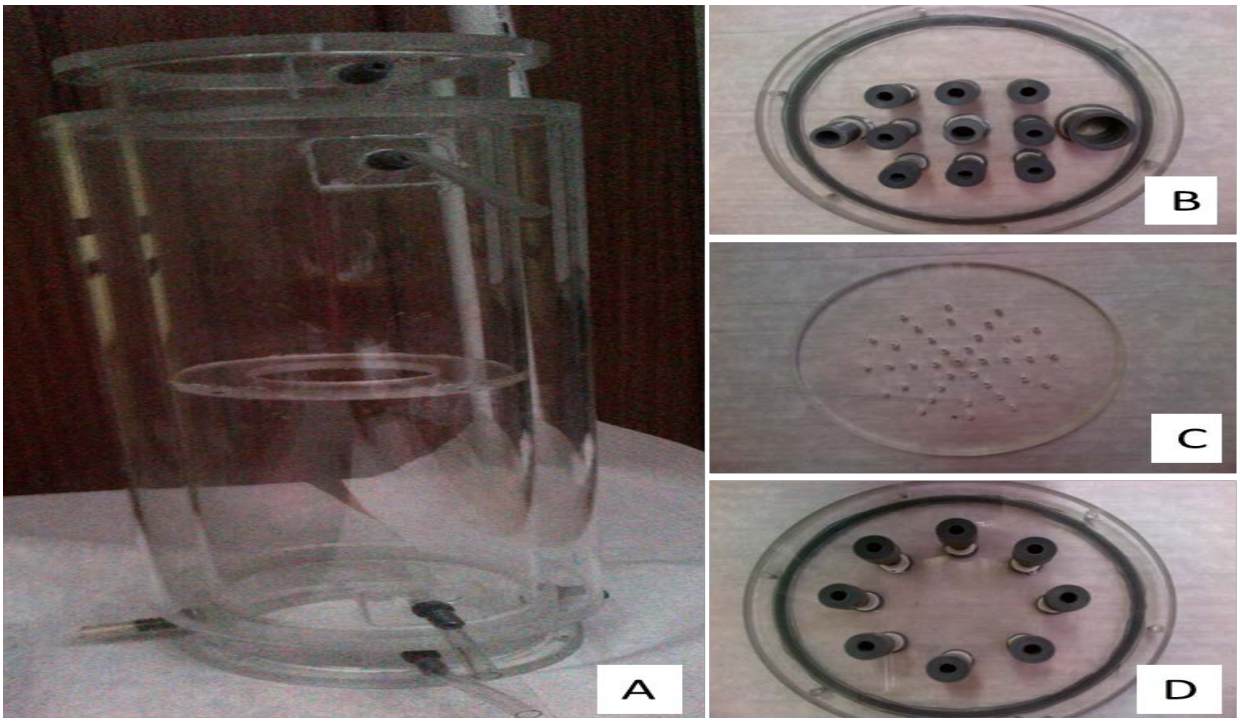

Figure 2.3: A picture illustrating the four main components of the MFC reactor (A). The main cylinder (B) the top lid, (C) perforated plate and (D) the bottom lid.

As illustrated in Figure 2.3 (A), both the anode influent (bottom) and cathode effluent (top) pots are on the main cylinder and the reactor is a single hollow cylinder. This design mimics that of a traditional wastewater treatment plant with an internal settling bed (Hu, 2008). The MFC designed in this study can thus be easily incorporated into existing system at relatively cheaper cost since only upgrades will need to be made to change the systems into MFC reactor.

The MFC had 8 electrode inlets on both lids, in contrast to the bottom lid, the top lid had two additional inlets for an oxygen sparger and air outlet funnel pipe on top of the cathode chamber as illustrated in Figure 2.3 (B). Figure 2.4 below demonstrates how all four components of the MFC are fitted together to develop the completed MFC reactor system. It is critical that all the inlets on the MFC body and lids are sealed to avoid any leakage of the electrolyte out of the reactor and air into the anode compartment. It is particularly important for the anode chamber inlets to be insulated to ensure that anaerobic conditions are maintained in the anode chamber.

It is common practice for the anode to be purged with nitrogen gas to remove dissolved oxygen and inhibit aerobic growth that has been reported to compete with anode reactions for electrons and thus decrease current generation (Du *et al*., 2010). Since the MFC used here was not

sparged, diffusion of oxygen to the anode may promote presence of aerobic growth (Ellakiyu *et al*., 2013).

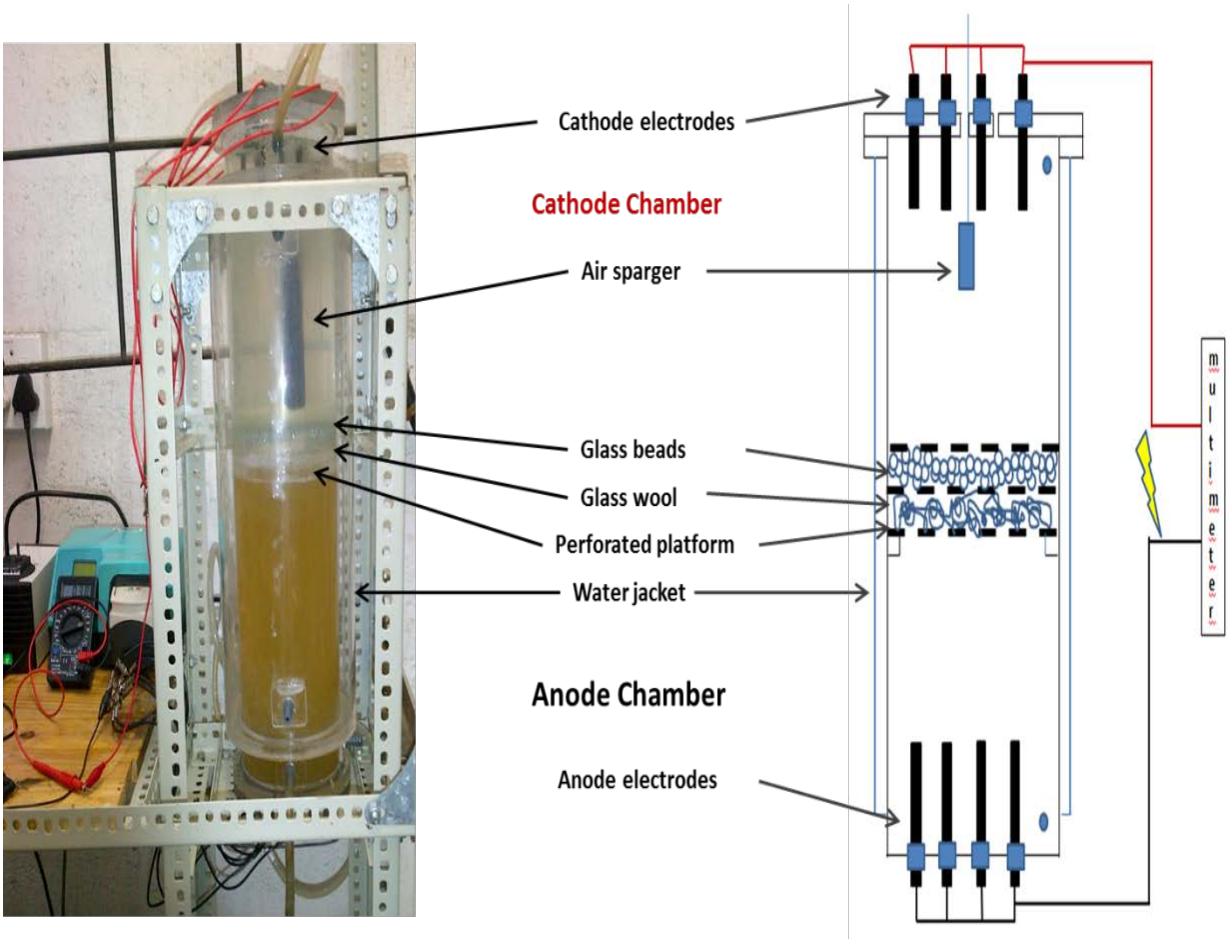

Figure 2.4: An illustration of the completed MFC reactor

2.3.2. MFC reactor assembly

The influent inlet was connected to a tube via a peristaltic pump to the wastewater feeding tank while the effluent outlet was connected to an effluent transporting tube leading to the effluent collection tank. Feeding the anode chamber with synthetic wastewater was done by pumping the synthetic wastewater into the inlet, up the anode chamber, through the artificial membrane and exit as effluent at the top of the cathode chamber. This upwards flow operation of the MFC renders it an up-flow system (Jang *et al*., 2004). Although up flow systems are reported to have higher energy outputs, as elucidated in Chapter one, the energy required to pump water up the system increases the cost of operating this type of MFC system (Figure 2.5).

This energy requirement is a limitation of the MFC design and assembly and may be eliminated by operating it in a horizontal flow with the main body of the reactor lying parallel to the ground such as that used by Hu, (2008). Investigating how the horizontal operation affects the output and assessing the difference in energy requirement for the MFC compared to it is operated in when up-flow designs would be a beneficial to fining more economic ways of operating MFCs.

Initially, each electrode rod was connected to rubber insulated copper wires and all 8 wires from each chamber were joined at a single point which was then connected to the multimeter (Figure 2.5). While it is important that the connecting wires are sealed to avoid electricity leakages in the external circuit, results here indicate that having too many wire contact points also lead to loss due to leakage in the external circuit.



Figure 2.5: A picture of the fully assembled MFC-reactor connected to the pumps (green), O_2 sparger (black and white) and the digital multimeter (orange and grey). The feeding and waste-tank are not shown.

The above mentioned is supported by the observed increase in voltage output when wire contact points were reduced using one unsealed copper wire to join all 8 electrodes in each chamber and connecting one wires between the now combined electrodes to the voltage (results not shown). The use of an unsealed copper during electricity production is however not safe. Thus, safer ways of consolidating multiple electrodes from a single source are needed to reduce electricity losses (Logan, 2006).

Table 1: Different MFC designs and materials reported in literature

MFC type	Membrane	Anode material	Anode area (cm^2)	Working volume (L)	Reference
double chamber	perforated plate, glass marbles, wool	graphite carbon rods	128.3	8	this study
double chamber	salt bridge	carbon	13	6	Al-Sheri *et al*,, 2011
double chamber	baffle disc	carbon paper	4.3	0.2	Hu, 2008
single chamber	none	carbon cloth			Han *et al*., 2011
double chamber	glass beds, wool	graphite felt roll	465		Jang *et al*., 2004

The MFC design used in the study is in line with the current research, the use of PEM is gradually becoming limited in MFC research. This is due to the move towards designing systems that are more economical to up-scale (Jang *et al*., 2004). This observation is also supported by the wide use of carbon based electrodes in various forms (Table 1). Although carbon based electrodes are reported to be less efficient than Pt-based electrodes, they are cheap, easy to handle and suitable for long term continuous operation and hence the preferred choice of electrode material in most recently published work (Al-Sheri *et al*., 2011; Elakkiyu *et al*., 2013).

It is also notable that the working volume of 8L chosen is sensible to address the gradual move to increase the size (in working volume) of the lab scale MFC from milliliters to liter scales (Aldrovandi *et al*., 2009). Aldrovandi *et al*., (2009) used a 22L triple chamber MFC reactor to successfully produce electricity. This indicates a shift in MFC research from focusing on the bio-reaction mechanisms of that affect performance to larger MFC. This will give more insight on the real potential MFC technology has for scaling up. It is with due cause then to highlight that this work is focusing on building new and timely knowledge in MFC research.

Experiments control

Both the un-inoculated anolyte and the no organic substrate (sucrose) controls operated at 0Ω external resistance did not produce any voltage during the experiments (Figure 2.6). This observation is supported by numerous studies that both substrate and electrochemically active bacteria are needed for electricity generation in MFC operation (Jang *et al*., 2004; Ghangreka and Shinde, 2006).

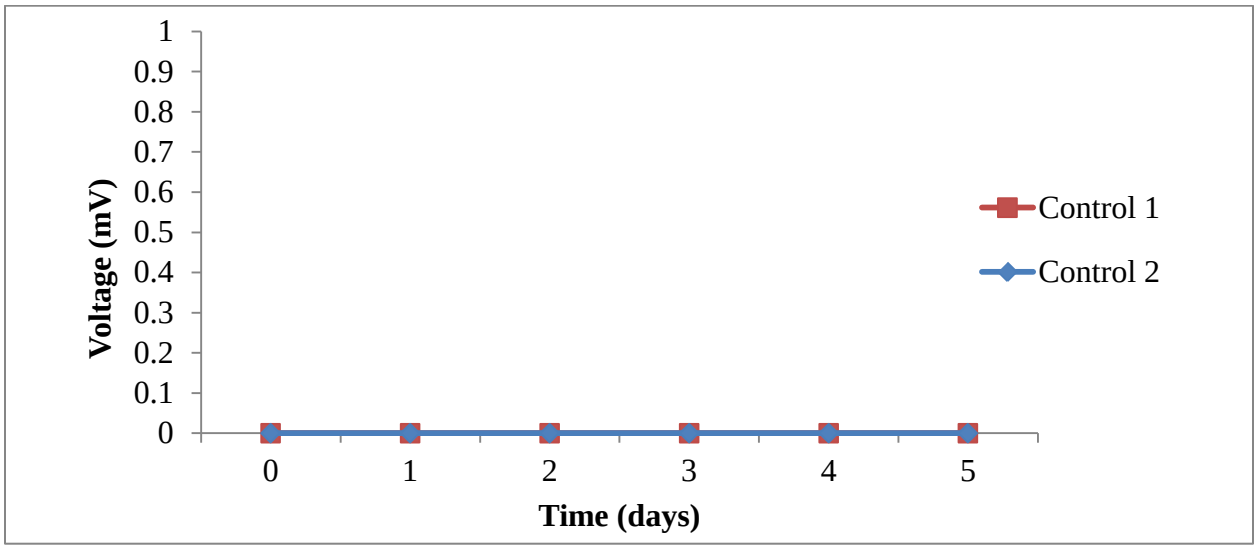

Figure 2.6: Amount of voltage produced by the experimental controls.

Pilot study

Very low voltage was produced during the first 15days of the development phase. This was followed by 10 days of exponential increase after which stable power was produced and sustained until the cycle was terminated (Figure 2.7). This bacteria growth curve like voltage output profile is consistent with what Jang *et al*., 2004 and Al-Sheri *et al*., 2011 has reported. The duration of the lag phase before voltage and current generation differs for various studies (Jang *et al* 2004; Al-Sheri *et al*., 2011). The length of the lag phase is said to depend of substrate type, concentration and the source of inoculate.

Since the mixed culture used in this study was collected from a wastewater treatment plant and incubated in a new media (synthetic wastewater), it is expected that the bacteria will take some time to acclimate to their new conditions and hence the requirement of a lag phase (Logan and

Regan, 2006). Jang *et al*., 2004 also reported a two week long waiting period in a MFC treating wastewater, this is consistent with the results presented here (Figure 2.7).

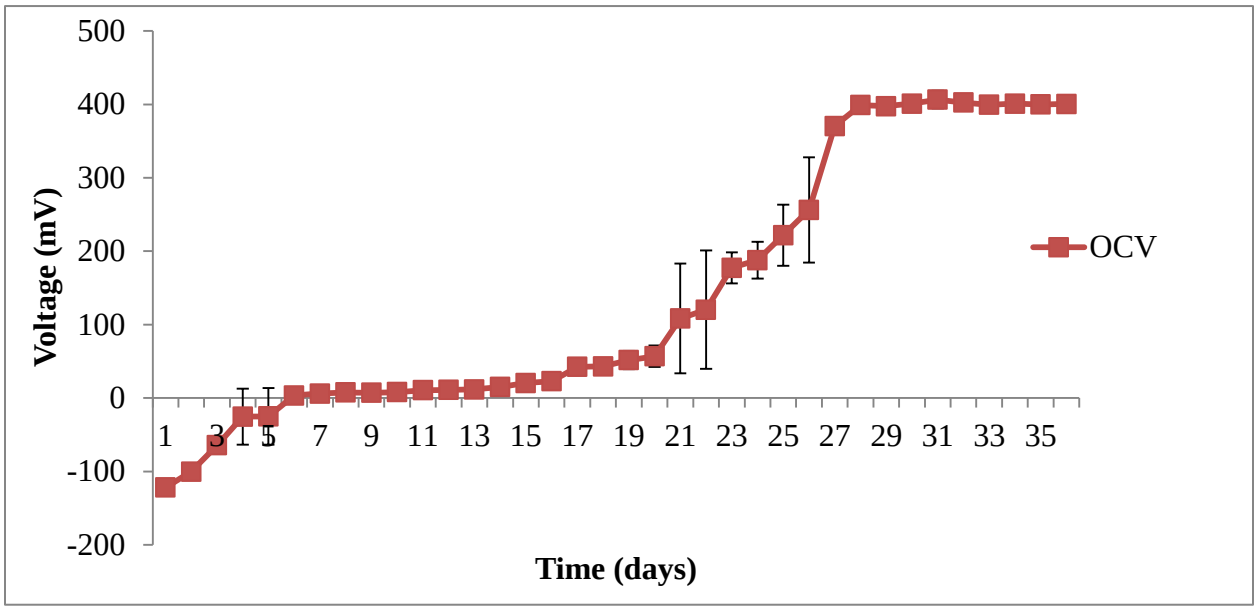

Figure 2.7: Amount of voltage produced during the development of the MFC-reactor. The bars on each data point represent the mean values ± standard deviation.

Aldrovandi *et al*., (2009), reported a 58days long lag phase using a 22 L MFC reactor, while a smaller MFC like that used by Han *et al*, (2011) generally report a shorter waiting period. This contrast in lag phase duration suggests that there might also be a correlation between MFC size and the period of bacteria to adapt to anode conditions.

Potential use of MFC as a capacitor

When the voltage reading was taken from the multimeter, the voltage value on the multimeter fluctuated for 5±2minutes before a stable reading could be taken. This meant that a 5minutes waiting time had to be allowed for the voltage to stabilise before a reading could be recorded. This observation is widely reported in literature and the commonality in the studies that report it in their methodology is the use of a hand held digital multimeter for recording voltage (Elakkiyu *et al*., 2013; Kassango and Togo, 2010).

Although the "stabilisation period" is fairly reported in literature, to date there has been no studies that attempted to really investigate the electrochemical reactions that accompany it. Many researchers are rather moving towards using computer linked data capturing systems such as the one used by Han (2011), the DAS 5020 (Jiehan Technology Corporation) (Han *et al*., 2011). The acquisition systems allow more frequent and accurate data collection. Hand held multimeters such as the one used in this study may thus limit the collection of accurate, high quality data that would allow in-depth analysis of electricity generation profile of MFC which can be achieved by computer linked systems.

On the other hand, this move to collect data via a computer might divert researchers from the need to explore the voltage stabilization period. A closer observation and monitoring of this period revealed that the contrary to what most studies report, the voltage does not fluctuate but rather, that it decreases with time from a high voltage until a stable, lower voltage is reached. This could be indicative of the MFC charge discharging stored charge in the system and thus acting like a capacitor. Investigating the voltage stabilisation period will be a good study to assess MFCs potential to be used to store charge in non-continuous systems. Proof of this concept would open a new niche for MFC application in rural places where a slow influx of domestic or agricultural waste can be used to supplement electricity usage.

MFC architecture development

Subsequent to 6days of continuous feeding, the entire barrier was displaced from the resting position on the supporting disc and was pushed into the cathode compartment (Figure 2.8 B). This displacement increased the anolyte volume and caused the undesirable mixing of the anode with the cathode solution (Figure 2.8 A). Since the barrier did not have sufficient weight to remain intact, an additional 250g of marble balls was added to the membrane. The resultant membrane with 500g marble balls was sufficient to hold the barrier in position and it was not displaced following the addition of extra marble balls.

The displacement of the disc raises a concern with how suitable the design is for industrial use. The movement of a structure like that would be disastrous and cost a lot of money to manage if it was an industrial scale size MFC. The design would thus need to be modified to suit industrial

scale parameters. On the positive side, the displacement of the membrane was caused by a build - up of air pressure from the anode chamber. When sufficient gas failed to pass through the perforation on the membrane, the entire structure was lifted into the cathode chamber (Figure 2.8)

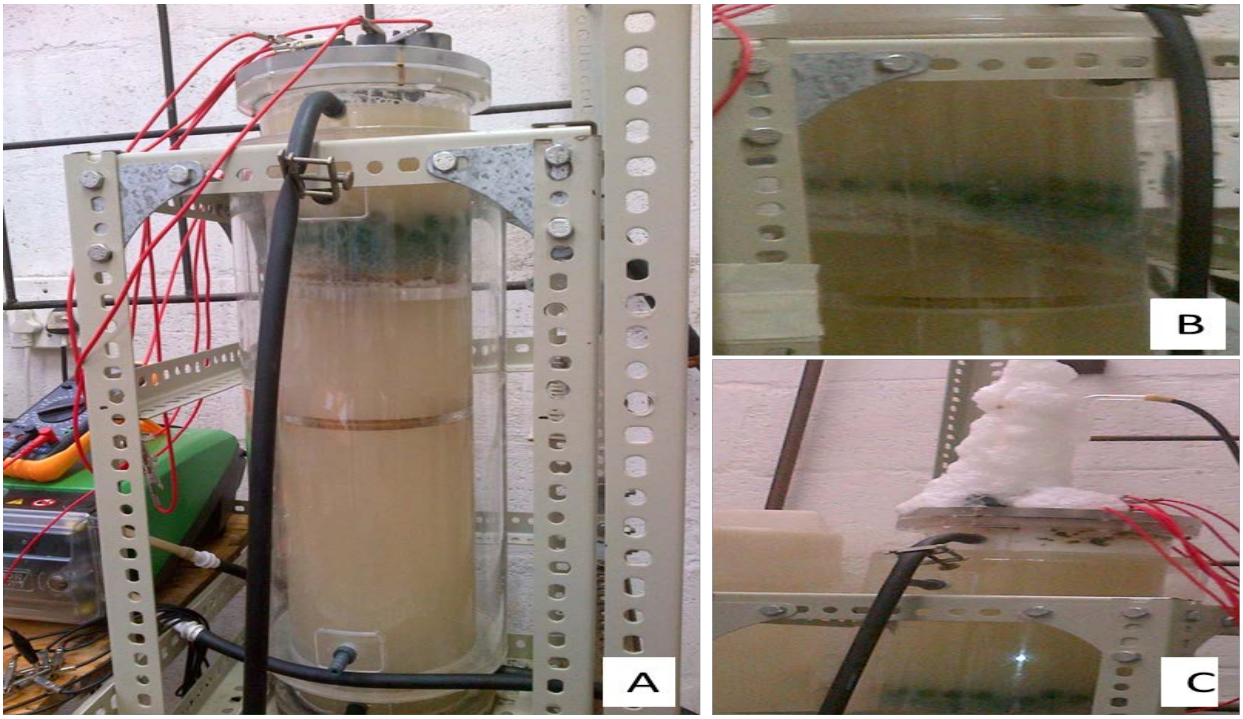

Figure 2.8: Photograph of the displaced membrane (A-B) and overflowing bubbles from the funnel inlet (C).

In addition to the membrane displacement, there was excess bubbling in the cathode compartment that resulted in the formation and overflowing of form through the air sparger inlet. This was rectified by redesigning the top lid to include a funnel inlet (Figure 2.3 B) and fitting a 2meter plastic funnel to relieve the air pressure from the cathode chamber. The funnel was successful in eliminating the excess foaming and overflowing. Both the air pressure and presence of bubbles indicate that bacteria are successfully degrading the synthetic wastewater in the anode chamber (Schwartz, 2007). The air pressure indicates the degradation of sucrose into H_2 and CO_2.

Conclusions

Based on the results presented in this chapter, the following conclusions can be drawn:

a) The MFC-reactor can be used as a continuously feed system to produce electrical energy.

b) Both bacteria and organic substrate (sucrose) are required for electricity generation.

c) The drop in initial voltage during reading suggests that MFC-reactor can act as a capacitor.

Chapter 3: Selecting optimal parameters for electrical power generation

3.1. Introduction

The performance of MFC with regards to the amount of energy it produces has been evaluated and reported in various ways. To date, energy generated in MFC research has been reported as voltage, current, power, power and current density, and as coulombic efficiency (Logan *et al.*, 2006; Aldrovandi *et al.*, 2009). However, to permit unambiguous comparison between different studies taking into account the large variety of MFC configurations used, the reported power should be normalised to the anode surface area and reported as power density (W/m^2) (Logan *et al.*, 2006).

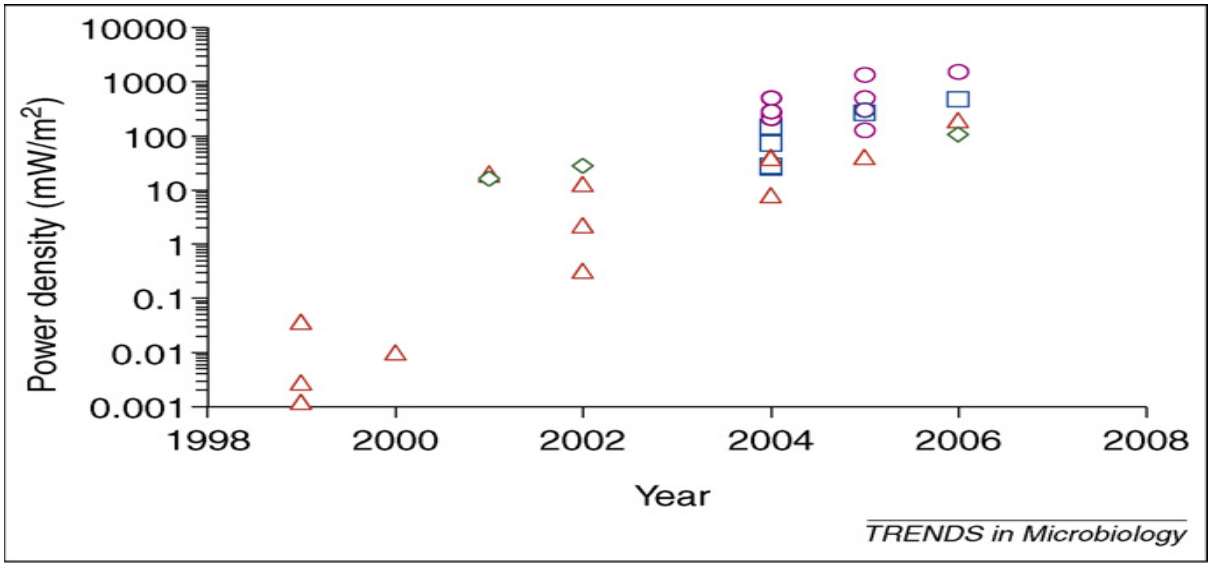

Figure 3: Illustration of MFC power output reported in literature from 1998-2006

Early systems produced relatively low power outputs ($\leq$ 0.1mW/m^2) compared to current systems (Logan and Regan, 2006). Improvements in MFC architecture and operational parameters have increased power output over the past two decades (Figure 3) with outputs reaching 1000 mW/m^2 by the end of the last decade (Logan and Regan, 2006). However, power output is still very low and it is still reported in the milli-watts range.

To put energy production capabilities of MFC into perspective, one AA size chemical cell battery can produce around 3000Wh of energy while a MFC using glucose as a substrate,

operating at 100% CE can in theory produce only 3kWh of energy for every kilogram of organic matter degraded (Aelterman *et al*., 2006; Rabaey *et al*., 2003). Even though a 100% CE is not possible in MFC as mentioned in Chapter 1, it would be favorable to work on increasing the efficiency of MFC systems to produce a steady power (density) output of $1kW/m^2$ if they are to be economically viable to operate industrially and compete with more mature renewable energy technologies such as solar and wind power (Aldrovandi et al., 2009).

It remains a predicament to many researchers that the most significant hurdle in achieving high power output lies in MFC architecture design and operation rather than in the composition of substrate and the bacterial community used for inoculation (Logan and Regan, 2006). It has been demonstrated in numerous studies that power output can be improved by MFC configuration designs and operating parameters that reduce internal resistance and promote MFC biofilm development (Logan and Regan 2006).

In Chapter 2 the MFC reactor design modifications made to increase output in this research was examined. Further, the operational parameters that affect MFC performance were detailed in Chapter 1, these include; substrate type and concentration, feeding rate and magnitude of external resistance (Logan et al., 2006). This chapter addresses the second aim of the research (Chapter 1).

Objectives

The objective of this chapter was to assess the efficiency of electrical energy production and investigating the effect the following parameters have on the MFC performance:

a) batch and continuous fed mode.
b) anode compartment stirring.
c) organic substrate (sucrose) concentration
d) external resistance.

3.2. Materials and Methods

The MFC-reactor was operated under various operation conditions to elucidate its best how parameters can be optimized to increase efficiency. The following parameters were compared; batch and continuous fed mode, effect of organic substrate concentration, effect of anode chamber stirring, effect of external resistance and the effect of biofilm formation on electrical energy generation.

Each experiment investigating the above parameter is herein referred to as a “Cycle”. Each cycle was done in duplicate and each replicate thereof is referred to as a “Run”. The voltage was monitored and recorded daily. The reading on the multimeter was allowed a 5±2minutes waiting period to stabilize before a reading was recorded.

Sections 3.2.1 and 3.2.2 below will detail the cycle while section 3.2.3 explains the methods used to calculate current, power, power density and current density corresponding to the monitored voltage.

3.2.1. Batch-fed mode

In batch feed mode, the anode chamber was fed 3.5L wastewater and 4L unsterilized tap water was added to the cathode compartment. Air (O2) was sparged into the cathode compartment and the voltage was monitored for 10days. The following parameters were altered and investigated with each cycle:

Effect of stirring (cycle 1)

The two inlets in addition to the influent feeding inlet at the bottom of the anode compartment were connected to a peristaltic pump which was used to cycle the anolyte in and out of the chamber and thus mixing the anode compartment. 3.5L wastewater (17.63g/L) was used to feed the anode compartment and as mentioned above, the voltage produced was measured daily for 10 days.

Effect of sucrose concentration (cycle 2)

In this cycle the MFC-reactor was fed wastewater with sucrose concentration of 4.4g/L and 17.63g/L respectively.

Effect of resistance

To investigate the effect of external resistance on the MFC-reactor performance, each cycle (1 and 2) was run at various external resistances. The external resistance used for each run in a cycle ranged from 0; 10; 100; 500; 1000; 5000 to 9000Ω.

3.2.2. Continuous-feed operation (cycle 3)

Effect of feeding rate

The MFC-reactor was fed wastewater (17.63g/L sucrose) continuously at 1.2ml/min and 0.6ml/min to investigate its performance under varying feeding rates. The MFC was operated with an external resistance of 1000Ω in this cycle and the voltage was monitored daily for 60 days.

3.2.3. Analysis

Since voltage was the only parameter monitored directly from the MFC reactor, the voltage (mV) was used to calculate other parameters important in evaluating MFC performance. These are; current (mV), power (mW), power normalised to anode surface area (power density mW/m^2), current normalised to anode surface area (mA/m^2). The calculated power and current will be expressed normalised to the anode surface area to allow accurate comparison to other studies as power density.

Current (mA)

The Ohm's law was used to calculate current from the monitored voltage

I = (V)/(R) where; (I) is the current (Amperes), (V) the Voltage (voltage), and (R) the external resistance (Ω)

Power (mW)

$P = (I)(V)$ where; (P) is power (Watts) and (I), (V) are defined in above.

Power density (mW/m^2)

$P_d = (V)(I)/A$ where P_d is power density and A is the anode surface area in meters^2.

3.3. Results and Discussion

3.3.1. Batch-fed operation

The MFC-reactor was able to produce electrical energy in batch mode. The output profile was characterised by an initial 2day lag phase, followed by a 5day stable voltage production phase and a drop after 7day of incubation period. This trend was observed for the runs without anode compartment stirring. The voltage produced and corresponding current and power density are illustrated in Figure 3.1 below.

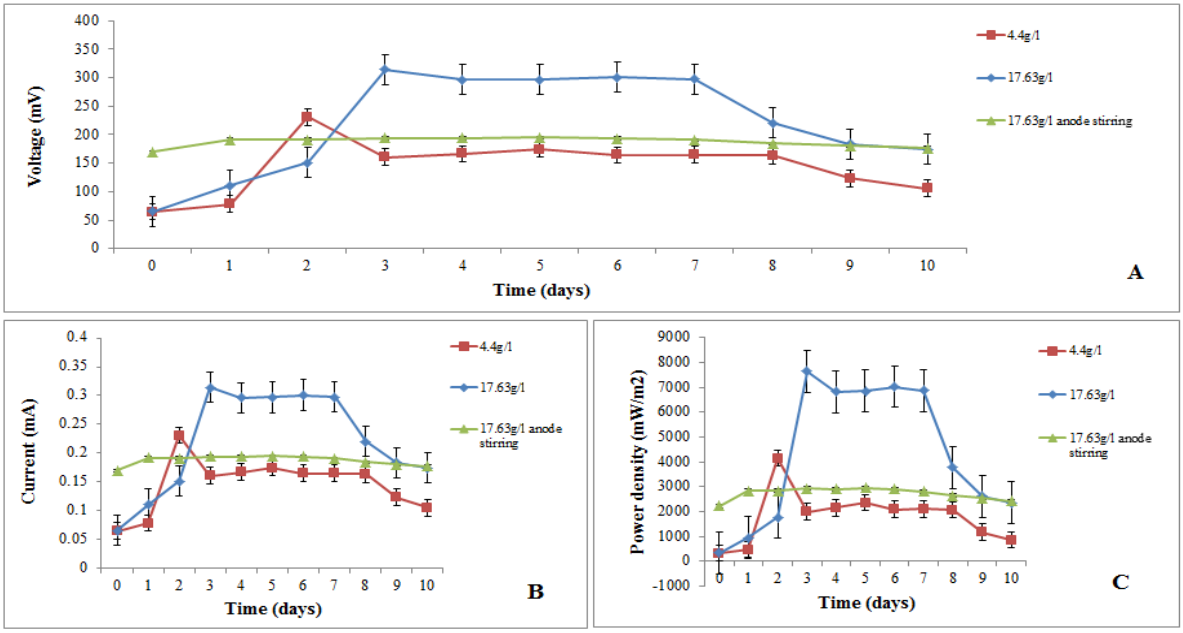

Figure 3.1: MFC-reactor voltage (A), current (B) and power density (C) output profile with 1000Ω external resistance monitored over 10days. The concentration in the legend referes to the amount of sucrose (g/L) in the wastewater. The bars on each data point represent the mean values ± standard deviation

The 2day lag phase observed in this study was shorter than the 5-7 days lag phase reported by Elikkya and Matheswaran (2013). The difference in lag phase is due to the different adaptation time required by bacteria to acclimitise to anolyte conditions. The adaptation time varies with the type of substrate and inoculate used in a MFC system (Elikkya and Matheswaran 2013). Hu (2008) and Elikkya and Matheswaran (2013) observed that using isolates from wastewater decreases the lag phase because intrinsic bacteria do not require acclimitising to the susbtrate conditions (Elikkya and Matheswaran, 2013).

Since synthetic wastewater was used in this research, no intrinsic bacteria could be used, and thus the MFC was expected to have a lag phase. The relatively short lag phase suggested that the anaerobic bacterial consortium isolated from anaerobic sludge was able to adapt to the MFC environment during the development phase (Chapter 1). This observation is supported by work done by Kassongo and Togo (2010) which demonstrated that pre-incubation of bacteria in the wastewater prior to use in MFC decreased the lag phase and improved MFC output (Kassango and Togo, 2010).

Effect of anode stirring

It was observed that the nutrients and organic substrate settle at the bottom of the anode compartment within minutes of wastewater feeding in batch mode (Figure 3.2). This meant that a nutrient gradient was created between the bottom and top part of the anode chamber. This observation prompted an experiment to investigate the effect this concentration gradient had on electrical energy generation when the MFC-reactor is run in batch mode (Chapter 3 materials and methods).

During this experiment (cycle 3), it was observed that, in contrast to the voltage output profile of the non-stirred anode compartment, the run with the stirred anode had a lag phase of a day after which stable voltage of 195 ± 3mV was produced for 7days. In addition, the increase in voltage before the stable state and the decrease thereafter was not as sharp as in the non-stirred runs. It was also worth noting that there was a small difference between the initial (169mV) and the stable (195±3mV) voltage produced in this run (Figure 3.1).

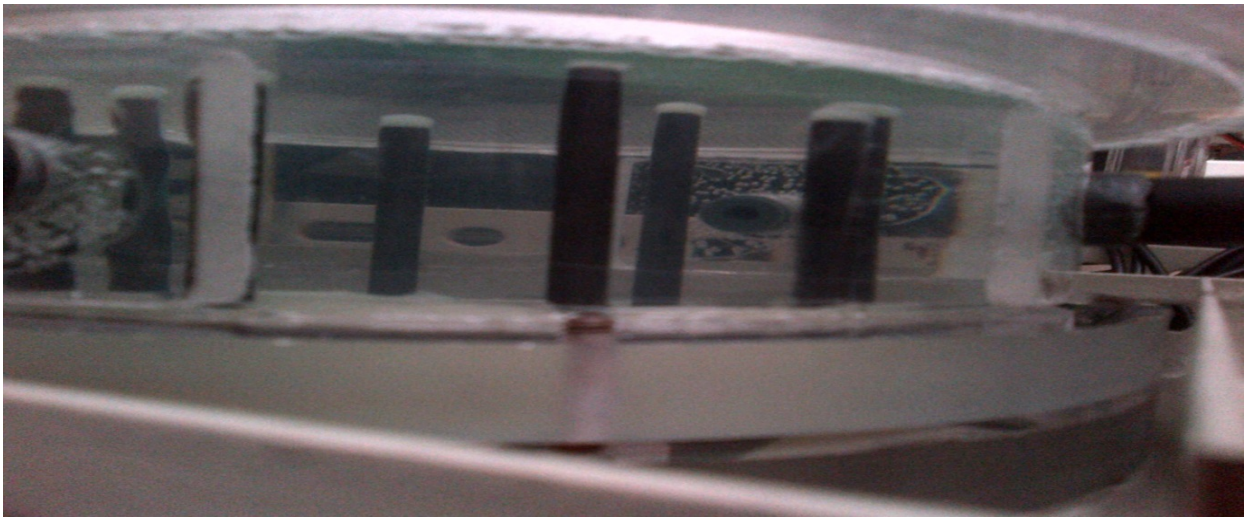

Figure 3.2: Photograph illustrating a bed of nutrients and organic substrate at the bottom of the anode compartment after batch feeding. The black rods are the anode electrodes, the nutrients also settle on the electrodes.

Comparing the voltage output profile of the anode stirring and none stirring cycle suggests that the substrate is not consumed at the same rate under these anode operating conditions and this may be due to the difference in substrate availability rather than concentration since the same concentration was used both cycles. This speculation is based on the observation that there was no initial peak, followed by a decrease in voltage output before a stable voltage was produced. Also, when the anode was stirred, the MFC produced a stable voltage longer than when the anode was not stirred (Figure 3.1).

Effect of resistance on performance

The effect varying external resistance has on voltage, current and power density production when operated in batch feed was observed. It was found that voltage was directly proportion to, and thus increased with increasing external resistance (Figure 3.3 A). This observation was also made by Wen et al. (2010) and supports the view that a MFC acts like a chemical fuel cell which depicts the same relationship between voltage and resistance (Ohm's Law).

In contrast to the resistance/voltage relationship, it was noted that current and current density decreased with increasing external resistance (Figure 3.3 B). This is also a characteristic of a

chemical cell and the trend reported by numerous other studies in MFC research (Feng *et al*., 2008; Hu, 2008). Interestingly, a completely different trend was observed for the relationship resistance had with power density. As illustrated in Figure 3.3 (C), initially, power density increases with external resistance in the range between 10 and 1000Ω where it peaks. Power density then starts to decrease with further increases in resistance between 1000 and 9000Ω (Figure 3.3 C).

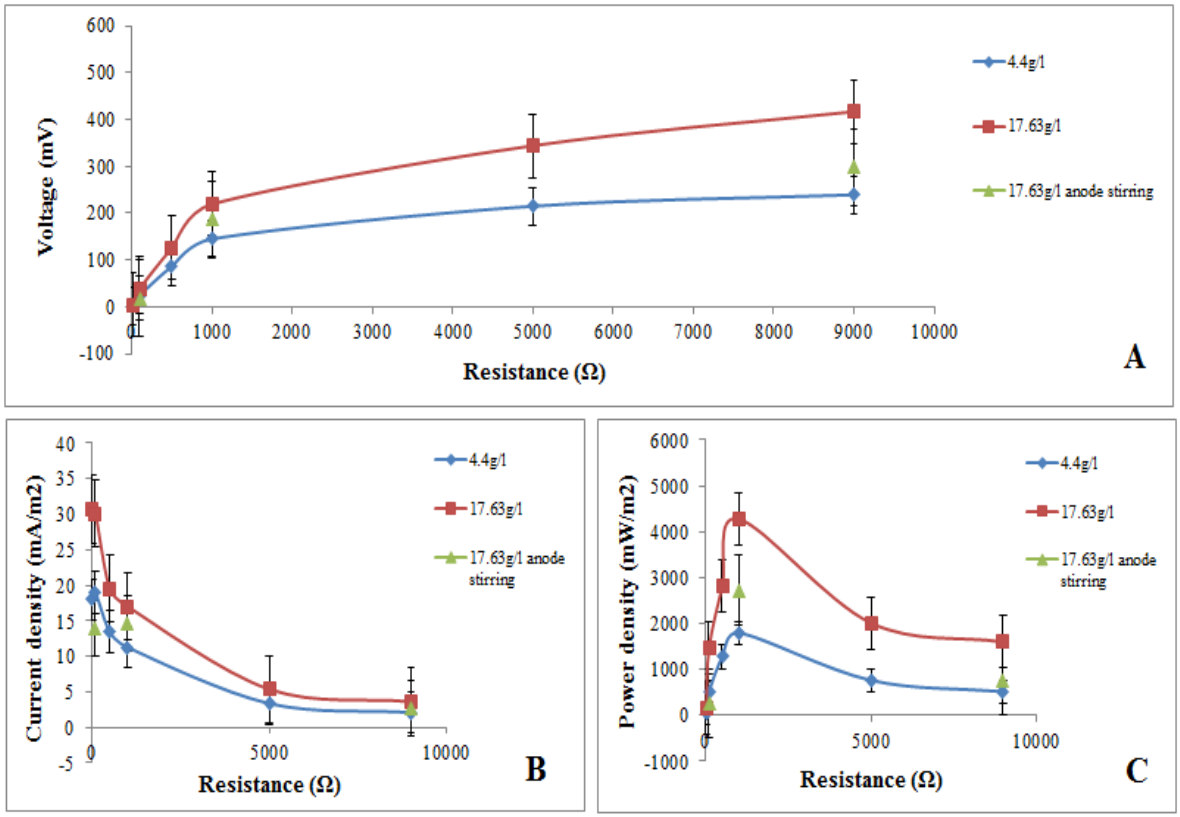

Figure 3.3: Effect of external resistance on voltage (A), current density (B) and power density (C).

The lowest voltage was produced when an external resistance of 10Ω was used and the highest was observed when 9000Ω was used in all 3runs of the batch cycle (Figure 3.3 A). Dissimilarly, the highest current and current density was produced when the external resistance was 10Ω and the lowest when 9000Ω was used (Figure 3.3 B). The highest power and power density was produced when 1000Ω external resistance was used and the lowest when both 9000 and 10Ω were used (Figure 3.3 C).

Effect of sucrose concentration

Results from this study indicate that the MFC-reactor produces more voltage, current, current density and power density when a higher concentration of organic substrate is used (Figure 3.1). Maximum voltage of 164.55±2mV was produced during steady state when wastewater-sucrose concentration of 4.4g/L was used compared to 301±3mV and 190.5±4mV observed when 17.63g/L was used in non-stirred and stirred batch respectively (Figure 3.1). Figure 3.4 below illustrates the average current and power density produced when the two concentrations were used.

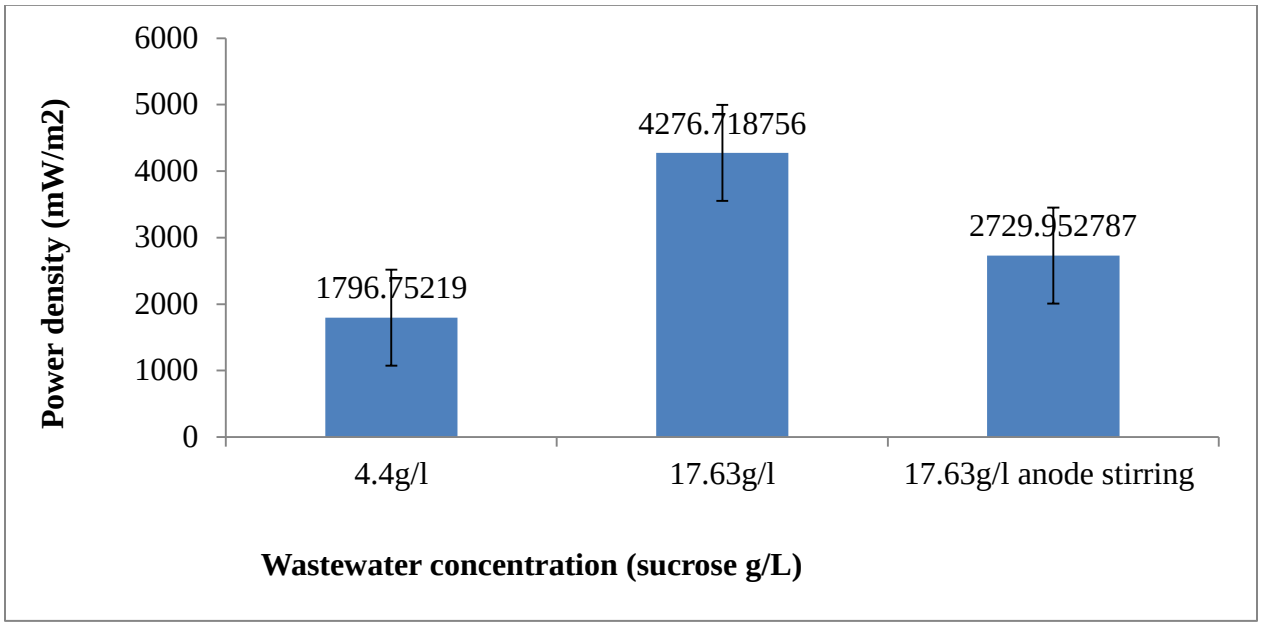

Figure 3.4: Average power density produced when wastewater of 4.4 and 17.63g/L sucrose was used to feed the reactor in batch mode. The error bar indicates the mean of each data point ± standard deviation

The run fed with wastewater-sucrose concentration of 17.63 g/L operated without anode stirring produced a maximum power density of 4276.71mW/m^2. This was much higher compared to the 2729.95mW/m^2 and 1796.75mW/m^2 obtained from the anode stirring and 4.4g/L run (Figure 3.4). Although wastewater with the same sucrose concentration (17.63g/L) was used in the anode compartment stirring and non-stirring runs, more power was produced when there was no mixing in the anode compartment.

Effect of feeding rate

Results from the batch dynamics experiments (setion 3.3.1) suggest that more voltage is produced when the MFC-reactor is operated with wastewater-sucrose concentration of 17.63g/L and an external resistance of 1000Ω (Figure 3.4 and 3.3 C). These two parameters were thus selected to investigate the performance of the MFC in continuous mode.

The voltage increased steadly for 15days of continuous feeding at 1.2ml/min untill a maximum steady state was reached on day 20. This voltage was maintained for 25days untill the feeding rate was decreased to 0.6ml/min on day 41 (Figure 3.5). The MFC-reactor produced a maximum and sustained voltage of 536±5mV with a feeding rate of 1.2ml/min. This was higher than the 323±2mV recodered from day 44 when a steady state for feeding at 0.6ml/min was established following a decrease in feeding rate.

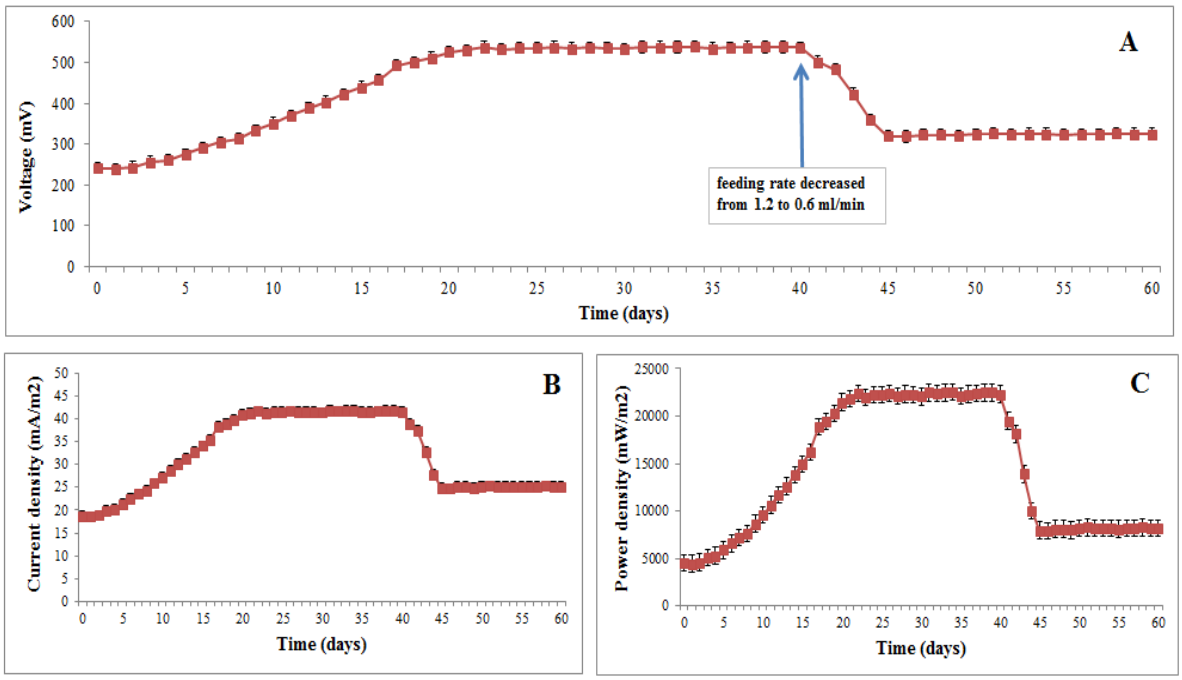

Figure 3.5: Output profile of the MFC-reactor illustrating voltage (A), current density (B) and power density (C) produced when wastewater (17.63g/L) was fed continuously at the rate of 1.2 and 0.6ml/min with 1000Ω external resistance. The error bars represent the mean of each data point ± standard deviation

The results of this study indicate that the MFC-reactor can be operated as a continuous system and further, that it can produce a maximum and sustained power when operational parameters are kept constant. Additionally, the results show that a steady state of power production can be re-established after 4days following a decrease in feeding rate (Figure 3.5). Table 2 summarizes the output of the MFC and compares it to other work reported in literature.

Table 2: Power density output of MFC used for treating wastewater

Reference	COD (mg)	inoculum	feeding mode	Pd (mW/m^2)
This study	2000	anaerobic sludge	batch and continuous	2000 and 2904.01
Jang *et al*., 2006	300	anaerobic sludge	continuous	1.3
Elakkiya *et al*., 2013	3200	diary waste isolate (aerobic)	batch	192.161
Al-Sheri *et al*., 2011		sewage inoculate	batch	1190.47

The MFC used in this study had a power output in the upper range of that reported for other MFCs (Table 2). This may be attributed to the larger working volume (8L) and the high wastewater COD concentration used compared to many MFC designs used to date (Table 2).

Conclusions

Based on the results presented in this chapter, the following conclusions can be drawn:

a). The MFC-reactor can be operated in batch and continuous mode.

b). Higher output (voltage, current and power density) is produced at a higher concentration of sucrose.

c). External resistance is directly proportional to voltage production and inversely proportional to current and current density

d). Higher power density is produced at 1000Ω external resistance

Chapter 4: Selecting optimal operational parameters for bioremediation

4.1 Introduction

The most desirable characteristic of MFC technology is the ability to degrade a wide variety of wastewater as a substrate (Pant *et al*., 2010). This is particularly important to a number of industries including: food processing, agriculture, paper production, textile and brewing industries. The latter industries consume large amounts of water and release wastewaters that contains high concentrations of soluble organic matter (COD) and is sometimes toxic (Huang and Logan, 2008).

Such industrial wastewater is difficult to purify with currently available conventional water treatment technologies (Du *et al*., 2010). With the growing commitment of governments to promote sustainable production globally, an increasing number of countries have implemented tax fees similar to the South African carbon tax law that requires companies to pay penalties for releasing toxic, difficult to remediate, and wastewater with high COD concentrations into the municipal wastewater streams (Huang and Logan, 2008).

It follows then that industry is in need of finding more efficient methods of treating their wastewater (Huang and Logan, 2008). These methods should be aligned with the view of reducing, reusing or recycling electrical energy and water for sustainable production (Pant *et al*., 2010). The use of MFC has successfully demonstrated the ability to produce bio-electricity that can reduce the huge amount of electrical energy input required for wastewater treatment while it simultaneously removes the waste content in water and thus remediating it making it recyclable with conventional wastewater technologies (Huang and Logan, 2008).

Bioremediation efficacies of more than 80% have been reported in for MFC in literature for studies using various wastewaters as substrate to date. The energy outputs related to this is projected not to be sufficient to power the fluid recirculation that is used to pump the fluid in up-flow systems that show the most potential for scaling (Du *et al*., 2010). The primary function of MFC technology is therefore currently more accepted as wastewater treatment that can co-power itself rather than as energy production systems (Du *et al*., 2010). This chapter thus investigates

the efficacy of the MFC to remediate sucrose based synthetic wastewater under various operating conditions.

Objectives:

a) assess bioremediation efficiency under batch feeding
b) monitor bioremediation efficiency during continuous feeding
c) assess the effect of substrate concentration on remediation efficacy

4.2 Materials and Methods

Chemical Oxygen Demand analysis

A colorimetric assay was performed to determine the COD of the media based on the protocol by LaPara, *et al*., (2000) as follows; a digestion solution and a catalyst solution were prepared. The digestion solution comprised 2.6 g potassium dichromate ($K_2Cr_2O_7$) and 8.33 g mercuric sulphate ($HgSO_4$) dissolved in 42 ml 95-99 % H_2SO_4 , to which 208 ml dH_2O was carefully added to complete the solution. The catalyst solution consisted of 5.06 g silver sulphate (Ag_2SO_4) added to 500 mL 95-99 % H_2SO_4.

Potassium hydrogen phthalate (PHP) was used to construct a standard curve for the assay, initially prepared by dissolving 765 mg PHP into 1 L dH_2O, which is equivalent to 900 mg COD/L. This was then serially diluted to the following concentrations (mg COD/L): 50; 100; 250; 500; 750; 900.

To determine COD of the media for the standard, two millilitres of each standard or 10-fold diluted samples of the influent and effluent was dispensed into test tubes containing 1.5 mL digestion solution and 3.5 mL catalyst solution. These were mixed briefly by votexing and placed onto a heating block (HI839800 COD Reactor, Hanna Instruments) at 150 °C for two hours. After removal from the heating block, the samples were left to cool. Absorbance was then measured using a spectrophotometer at 600 nm.

The COD removal efficiency (bioremediation efficiency) was calculated as the change in concentration over time for example: $\Delta \mathbf{tCOD} = \mathbf{(tCOD_t - tCOD_0)/t}$ and was expressed as a percentage decrease in COD concentration over time. When the MFC was operated in batch

(cycle 1 -3) the COD of the media was analysed before feeding at the final concentration when the cycle was stopped (after 10days). The final sample from the batch experiments was taken from the feeding plot. In contrast to this, the effluent sampling pot at the top was used to sample the effluent every third day during continuous feeding (cycle 4).

Results and Discussion

The MFC had the best remediation efficacy when it was operated in continuous feeding mode (73.3%) compared to when it was operated in batch mode (Figure 4.1). In batch mode, the best remediation occurred when the anode was stirred (58%) compared to when it was not (50.2%), this is illustrated in Figure 3.1. These results are agree with those obtained by Elakkiuyu *et al*., (2013) of diary wastewater at pH 7 obtained a remediation potential of 89%. The same study also found that remediation efficiency of 91% and 90% obtained when the anode is operated at acidic pH using anaerobic and aerobic bacteria respectively (Elakkiuyu *et al*., 2013).

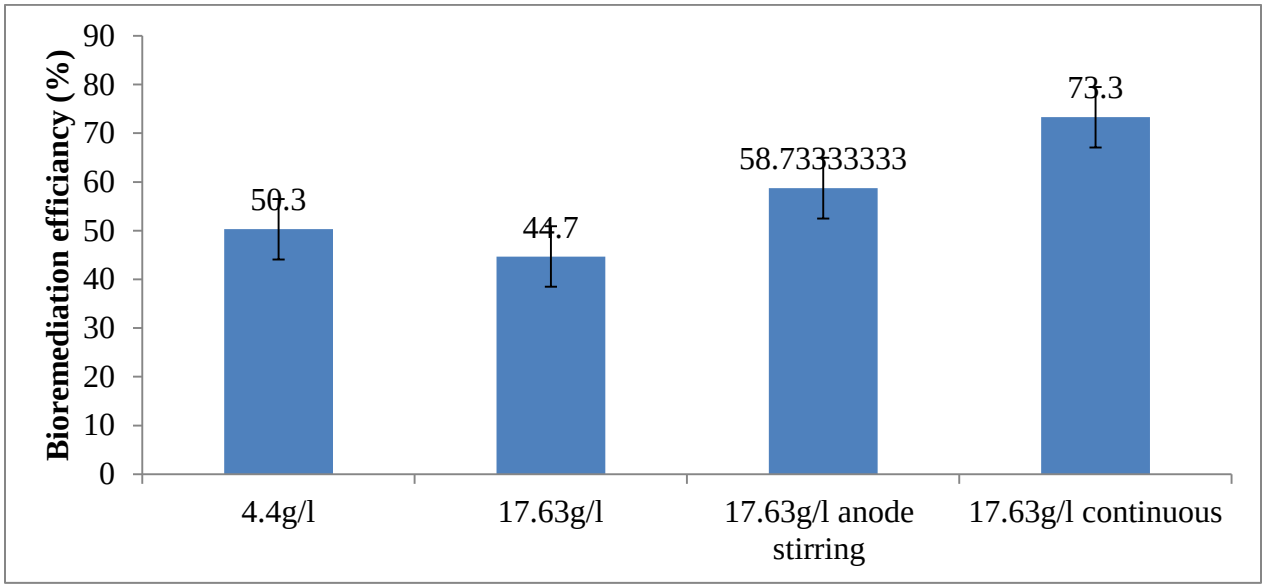

Figure 4.1: Bioremediation efficiency (%) of the MFC reactor when operated in batch and continuous mode at different substrate concentration (4.4 and 17.63g sucrose). The error bars represent the mean ± standard deviation of each data point.

Unexpectedly, the least bioremediation efficiency (44.7%) was not obtained when low substrate concentration was used (Figure 4.1). This observation is not consistent with the biochemistry law

of mass transfer which suggests that when the low substrate is available to a large number of bacteria, the substrate will be depleted rapidly.

The MFC achieved relatively low bioremediation efficiencies compared to other studies that used MFC for wastewater remediation which reported up to 2-fold higher than the observed efficiency. The low efficiency may be due to the strength of the synthetic water used (2000mgCOD) compared to other studies (Table 3).

Table 3: Bioremediation efficiencies of wastewater treating MFC

Reference	substrate type	remediation efficiency	biofilm development	pH
This study	sucrose SW	73%	yes	7.4
Elakkiya, (2013)	dairy waste	89%	yes	7
		90%	yes	5
		86%	yes	9

The bioremediation efficacy of the MFC increased significantly reaching a stable peak of 73% efficiency. This increase may be due to the activity of aerobic bacteria in the cathode chamber. Unlike in batch mode where the two chambers are separated for the duration of the experiment, during continuous feeding, the wastewater passes through both the chambers. The higher remediation efficiency observed in during continuous feeding thus suggesting that, the aerobic bacteria in the cathode chamber also aids in the degradation of substrate (Han *et al.*, 2011).

This suggestion is supported by another observation made in this study. The bioremediation seems to increase with the development of biofilm in both the anode and cathode chamber. The remediation efficiencies of the second replicate were generally higher than those of the initial efficiencies (results not shown). These results are consistent with those recorded by Hu, 2008 which led to him concluding that the development of aerobic in the cathode chamber increases both the power output and substrate degradation in MFC (Hu, 2008).

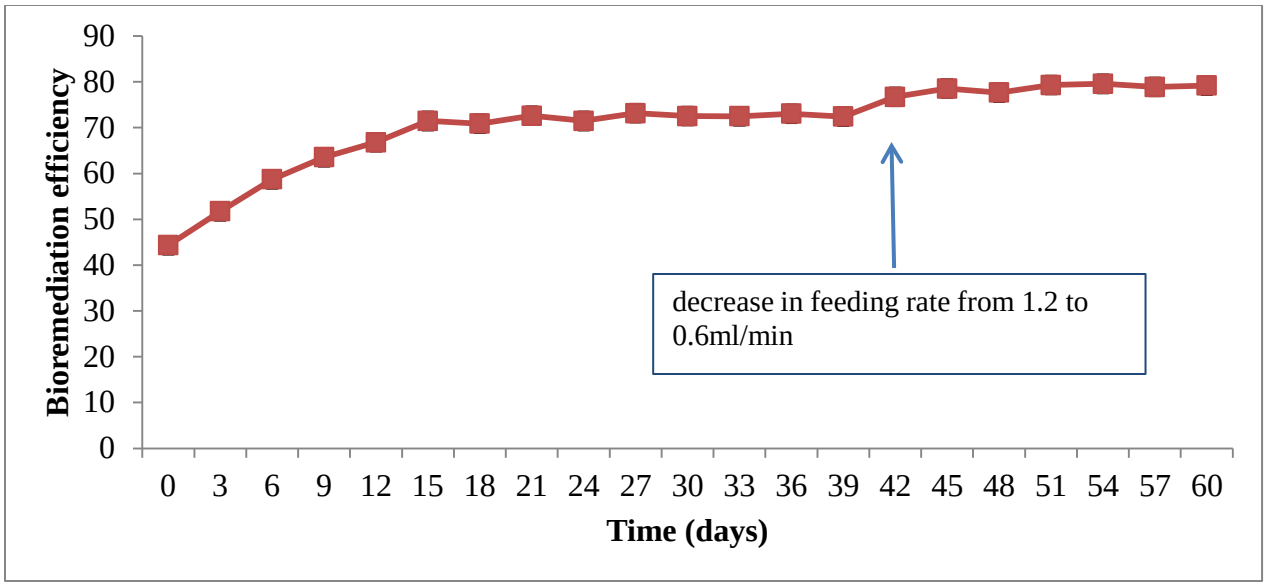

Figure 4.2: Development of bioremediation efficiency during continuous feeding mode with synthetic wastewater of 17.63g/L sucrose and external resistance of 1000Ω. The graph represents results of a single, continuous experiment.

Figure 4.2 illustrates the change in bioremediation efficiency with change in operational parameter i.e there was a gradual increase in efficiency during continuous feeding from 43% efficiency on the last day of batch operation (on day 0, the start of cycle 4) to a sustained efficiency of 78±5% when the feeding rate was 1.2ml/min. Another increase was observed when the feeding rate was reduced to 0.6ml/min on day 40.

Reducing the feeding rate of this allows the wastewater to flow through the MFC slower than when it is pumped up rapidly. The synthetic wastewater thus spends a longer time in the MFC in contact with both anaerobic and aerobic bacteria. The bacteria thus get more time to completely degrade the organic matter present in the wastewater.

Conclusions:

Based on the results presented in this chapter, the following conclusions can be drawn:

a) The MFC can be operated in both batch and continuous mode to remediate synthetic wastewater.

b) Different operating parameters impact bioremediation efficiency of the MFC.

c) The MFC can be operated continuously while maintaining stable bioremediation efficiency.

d) Development of a biofilm in both the anode and the cathode chamber improves the bioremediation efficacy of the synthetic wastewater.

5. Overall discussion and conclusion

The results presented here support the proof of concept made in the hypothesis. A double chamber MFC reactor was operated in both batch fed and continuous fed modes to successfully remediate the sucrose based synthetic wastewater using the bio-electrochemically active bacteria from anaerobic sludge while producing significantly high power density output.

Higher power density was produced when the MFC was operated in continuous mode (2904mW/m^2) compared to batch mode (2000mW/m^2). Bioremediation efficacy was also found to be higher (73%) during continuous feeding compared to (44.7, 50.2, 58 %) obtained when operated in batch mode. These results suggest that the design presented in this work has a potential for continuous operation in waste water treatment systems.

None of the MFC components required changing during operation due to bio-fouling. This indicates the suitability of the material used for constructing to be used sustainably in long term operation and this is due to the durable nature of the materials such as graphite rods used for both electrodes and the marble balls used in the membrane.

While the material such as plastic used for constructing the reactor is resistant to bio-fouling, it is a good surface for bacterial attachment, hence the development of a thick bacteria biofilm covering most of the internal surface of the MFC in both chambers. Advanced bacterial growth reaching biofilm attachment in the cathode, indicates that even though the cathode was not inoculated, the cathode conditions promote growth of aerobic bacteria that in turn catalyze the cathode reactions.

Conclusion

Proof of concept for an efficient and sustainable membrane to replace PEM in MFC design has been demonstrated in this work. Additionally, the exclusion of chemical mediators in this study has demonstrated that the use of chemical electron mediators can be replaced by allowing the development of a mixed culture bacteria biofilm in both the anode and cathode chamber and electrodes and thus, act as bio-catalyst to reduce activation energy limitation of electron transfer. The results illustrated that although the use of PEM and mediators still has higher power output,

they are not necessary in MFC design and can be replaced by chamber and more sustainable material such as perforated plates and marble to create an efficient barrier.

References

Aelterman, P., Rabaey, K., Clauwaert, P. and Verstrate, W. (2006). Microbial fuel Cells foe wastewater treatment. Water science and Technology. 54 (8): 9-15.

Aldrovandi, A., Marsil, E., Stante, L., Paganin, P., Tabacchoini, S., and Giordona, A. (2009). Sustainable power production in a membrane-less and mediator-less synthetic wastewater Microbial Fuel Cell. Bioresource technology. 100: 3252-3260.

Al-Shehri, A.N., Gherem, K.M. and Al-Garnis, S.M. (2011). A comparative study for electricity generation in microbial fuel cell reactor with and without mediators. Scientific Research and Essays. 6 (29): 6197-6202

Das, S. and Mangwani, N. (2010). Recent developments in Microbial Fuel Cells: A review. Journal of Scientific and Industrial Research. 69: 727-731.

Debabov, V.G. (2007). Electricity from Microorganisms. Microbiology. 77(2): 123-131.

Du, Z., Li, H. and Gu, T. (2007). A state of the art review on Microbial Fuel cells: A promising technology for wastewater treatment and bioenergy. Biotechnology Advances. 25: 464-482.

Elakkiyu, E. and Matheswaran, M. (2013). Comparison of anodic metabolisms in bioelectricity production during treatment of dairy wastewater in Microbial Fuel Cell. Bioresource Technology. 136:407-412

Feng, Y., Wang, X., Logan, B.E. and Lee, H. (2008). Brewery wastewater treatment using air-cathode Microbial Fuel Cells.Applied Microbiology and Biotechnology. 78: 873-880.

Ghangrekar, M.M. and Shinde, V.B. (2006). Performance of membrane-less microbial fuel cell treating wastewater and the effect of electrode distance and area on electricity production. Bioresource Technology. 36.3

Han, J.L., Liu, Y. Chang, C. T., Chen, B. Y., Chen, W.M. and Xu, H.Z (2011). Exploring characteristics of bio-electricity generating and dye decolorization of mixes and pure bacterial cultures from wine bearing wastewater treatment. Biodegradation. 22:321-333

Haung, L. and Logan B.E. (2008). Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Applied Microbiology and Biotechnology. 80: 439-355.

Hu, Z. (2008). Electricity generation by a baffle chamber membrane less microbial fuel cell. Journal of Power Sources. 179: 27-33.

Jang, J.K., Pham, T.H., Chang, S.I., Kang, K.H., Moon, H., Cho, K.S. and Kim, B.H. (2004). Construction and operation of a novel mediator-and membrane-less Microbial Fuel Cell.Process Biochemistry. 39: 1007-1012.

Jiang, D., Li,B., Jia, W., and Lei, Y. (2010). Effect of inoculum types on bacterial adhesion and power production in Microbial Fuel Cells. Applied Biochemistry and Biotechnology. 160: 182-196.

Kaekannetra, P., Chiwes, W. and Chiu, T.Y. (2011). Treatment of cassava mill wastewater and production of electricity through Microbial Fuel Cell technology.Fuel. 90: 2746-2750.

Kassongo, J. and Togo, C.A. (2010). The potential of whey in driving Microbial Fuel Cells.A duel prospect of energy recovery and remediation.African Journal of Biotechnology. 9 (46): 7885-7890.

Kim, B.H., Chang, I.S. and Gadd, G.M. (2007). Challenges in Microbial Fuel Cell development and operation. Applied Microbiology and Biotechnology. 76: 485-494

Kim, J.R., Min, B. and Logan, B.E. (2005). Evaluation of procedures to acclimate a microbial fuel cell for electricity production.Applied Microbiology and Biotechnology. 68. 23-30.

Larrosa-Gurrrro, A., Scott, K., Head, I.M., Mateo, F., Ginesta, A. and Godinez, C. (2010). Effects of temperature on the performance of Microbial Fuel Cells.Fuel. 89: 3985-3994.

Liu, H. and Logan, B.E. (2004). Electricity generation using an air-cathode, single cjamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental Science and Technology. 38: 4040-4046.

Liu, H., Ramnaraynan, R. and Logan, B.E. (2004 a). Production of Electricity during wastewater treatment using a single chamber MFC. Environmental Science and Technology. 38:2281-2285.

Liu, Z.D. and Li, H.R. (2007 b). Effects of bio and abio-factors on electricity production in mediator-less microbial fuel cell. Biochemical Engineering Journal. 36: 209-214.

Logan, B.E, Hanelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. and Rabaey, K. (2006 a). Microbial Fuel Cells: Methodology and Technology. Environmental Science and Technology. 40. (17): 5181-5192.

Logan, B.E. and Regan, J.M. (2006 b). Electricity producing bacterial communities in Microbial Fuel Cells.TRENDS in Microbiolgy.14 (12).513-518.

Logan, B.E. and Regan, J.M. (2006 c). Microbial Fuel Cells: Challenges and Applications. Environmental Science and Technology. 5172-5180.

Lovely, D.R. (2006). Microbial Fuel Cells: Novel microbial physiologies and engineering approaches. Current Opinion in Biotechnology. 17: 327-332.

Mardnpour, M.M., Esfahany, M.N., Behzad, T. and Sedaqatvand, R. (2012). Single chamber microbial fuel cell with spiral anode for dairy wastewater treatment. Biosensors and Bioelectronics. 38:264-269.

Mohan, Y., Kumar, S.M.M. and Das, D. (2008). Electricity generation using microbial fuel cells. International Journal of hydrogen energy. 33:423-426.

Mohan, Y., Raghavula, S.V. and Sarma, P.N. (2008). Biochemical evaluation of bioelectricity production processes from anaerobic wastewater treatment in a single chambered microbial fuel cell (MFC) employing glass wool membrane. Biosensors and Bioelectronics. 23:1326-1332.

Pant, D., Van Bogaert, G., Diels, L. and Vandroekhoven. (2010). A review of the substrates used in Microbial Fuel Cells (MFCs) for sustainable energy production. Bioresource Technology. 101: 1533-1543.

Park, D.H. and Zeikus, J.G. (2003). Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnology and bioengineering. 81: 348-355

Rabaey, K. and Verstraete, W. (2005). Microbial Fuel Cells: novel biotechnology for energy generation. TRENDS in Biotechnology. 23(6): 291-298.

Schwartz, K. (2007). Microbial Fuel Cells: Design elements and application of a novel renewable energy source. Basic Biotechnology eJounal. 2: 1-8.

Sharma, V and Kundu, P.P. (2010). Biocatalysts in microbial fuel cells. Enzyme and Microbial Technology. 47:179-188.

Sung, T.O., Jung, R.K., Giuliano, C.P., Tae, H.L., Changwon, K. and William, T.S. (2010). Sustainable wastewater treatment: How might Microbial Fuel Cells contribute. Biotechnology Advances. 28: 871-881.

Wen, Q., Wu, Y., Zhao, L.X., Sun, Q. and Kong, F.Y. (2010). Electricity generation and brewery wastewater treatment from a sequential anode-cathode microbial fuel cell. Biomedical and Biotechnology. 11 (2): 87-93.