EFFECT OF DIFFERENT SUBSTRATES AND CONCENTRATIONS ON THE PERFORMANCE OF DOUBLE CHAMBER MICROBIAL FUEL CELL

BY

Zia Ullah

Reg. No. 00000117746

DEPARTMENT OF ENVIRONMENTAL ENGINEERING
INSTITUTE OF ENVIRONMENTAL SCIENCES AND ENGINEERING (IESE)
SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING (SCEE)
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY (NUST)
ISLAMABAD, PAKISTAN

2017

EFFECT OF DIFFERENT SUBSTRATES AND CONCENTRATIONS ON THE PERFORMANCE OF DOUBLE CHAMBER MICROBIAL FUEL CELL

BY

Zia Ullah

Reg. No. 00000117746

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Environmental Engineering

DEPARTMENT OF ENVIRONMENTAL ENGINEERING
INSTITUTE OF ENVIRONMENTAL SCIENCES AND ENGINEERING (IESE)
SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING (SCEE)
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY (NUST)
ISLAMABAD, PAKISTAN

2017

APPROVAL SHEET

Certified that the contents and forms of the thesis entitled “Effect of different substrates and concentrations on the performance of double chamber microbial fuel cell” submitted by Mr. Zia Ullah have been found satisfactory for the requirement of the degree.

Supervisor: _____

Dr. Zeshan

Assistant Professor

Member: _____

Dr. Mohammad Arshad

Associate Professor

Member: _____

Dr. Zeeshan Ali Khan

Assistant Professor

Annex A To NUST Letter No 0972/102/Exams/Thesis-Cert Dated__ 2017

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis written by Mr. **Zia Ullah**, (Registration No.**00000117746**) of **IESE (SCEE)** has been vetted by undersigned, found complete in all respects as per NUST Statutes/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

Signature: _____

Name of Supervisor: _____

Date: _____

Signature (HOD): _____

Date:_____

Signature (Dean/Principal) :_____

Date:_____

DECLARATION

I, Mr. Zia Ullah, hereby declare that this research work entitled "*Effect of different substrates and concentrations on the performance of double chamber microbial fuel cell"* is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged / referred.

Zia Ullah
Reg.No. 00000117746

ACKNOWLEDGEMENTS

All praise to **ALLAH ALMIGHTY** who blessed me with talent and courage to accomplish this project, and who fetch me to this stage and helped me to come up with this effort. Indeed I could have done nothing without Your priceless help and guidance, so indeed none be worthy of praise but You.

I would like to express special thanks to my supervisor **Dr. Zeshan** for his help throughout my thesis. His important guidance, innovative suggestions and kind behavior were source of motivation during the study. I am grateful to all my teachers who taught me throughout my academic career and for their kind support. I am grateful to **Dr. Zeeshan Ali Khan** and **Dr. Mohammad Arshad** in particular for their kind help and facilitation throughout the project. I would thank all the laboratory staff and technicians for their help, support and cooperation. I render my special regards to Mr. Mohammad Basharat (lab technician) for his valuable assistance during research period.

I am profusely thankful to my beloved parents for allowing me to realize my own potential. All the support they have provided me over the years was the greatest gift anyone has ever given me. Heartfelt gratitude to my brothers Rizwan Ullah, Imran Ullah, Engr. Kaleeem Ullah, Dr. Mujahid Ulllah and sisters, Dr. Sonia in particular for their continuous support and prayers.

I owe special thanks to my best friends Bilal Siddiq and Junaid Khalid, who were always around at hard times and provided their unequivocal support throughout research, for which my mere expression of thanks likewise does not suffice. I would also like to pay thanks to my colleagues of environmental engineering, for their tremendous support and cooperation. Finally, I am thankful to all the individuals who have backed me and rendered valuable assistance to my study.

Zia Ullah

Dedicated to my exceptional parents, adored siblings and respectable teachers whose tremendous support and cooperation led me to this wonderful accomplishment.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

ARB	Anode respiting bacteria
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CE	Coulombic efficiency
CEM	Cation exchange membrane
DCMFC	Double chamber microbial fuel cell
HRT	Hydraulic retention time
IEM	Ion exchange membrane
g/L	Gram per liter
mV	Millivolt
MFC	Microbial fuel cell
Mol	Mole
mM	Milli mole
mW	Milliwatt
mW/m^2	Milliwatt per square metre
mA/m^2	Milliampare per square metre
OCV	Open circuit voltage
OLR	Organic loading rate
SCMFC	Single chamber microbial fuel cell
WWTP	Wastewater treatment plant

ABSTRACT

Microbial fuel cell (MFC) provides new opportunities for energy generation and wastewater treatment through conversion of organic matter into electricity by electrogenic bacteria. This study investigates the effect of different types and concentrations of substrates on the performance of double chamber microbial fuel cell (DCMFC). Three mediators-less laboratory scale DCMFCs were used in this study, which were equipped with graphite electrode and cation exchange membrane. The MFCs were fed with three different types of substrates (glucose, acetate and sucrose) at concentration of 1000 mg COD/L. The selected substrate (acetate) was studied for three different concentrations of 500, 2000 and 3000 mg/L of COD. Electricity generation and organic matter removal efficiency of MFCs were measured for evaluation of their performance. Results demonstrate that power production depends strongly on the types and concentrations of substrate used. The MFCs fed with acetate, sucrose and glucose produced maximum power density of 91, 64 and 51 mW/m^2 respectively. In contrast, COD removal efficiency of 79, 77 and 60% was recorded for MFCs fed with glucose, acetate and sucrose respectively. Coulombic efficiency (CE) for all substrates indicate that only 0.7-1.3% of COD was utilized in current production. The polarization curve shows that ohmic losses were dominant in DCMFCs established for all three substrates. The performance of MFC was found to be affected by the concentration of substrates. Polarization and power curves established for different concentrations of acetate show that maximum power density of 33, 110 and 114 mW/m^2 and internal resistance of and 871, 370 and 301 Ω are produced at concentrations of 500, 2000, and 3000 mg/L of COD respectively.

Chapter 1

INTRODUCTION

Over the past several decades, misuse and ever-growing demands of water resources have increased the risks of severe water stress and pollution in many parts of the world. The intensity and frequency of local water crises have been increasing, which result in serious implications for public health, food and energy security, environmental sustainability, and economic development (Connor, 2015). Pakistan is heading towards becoming a water-scarce country as per capita water availability has sharply declined (reduced from 5000 m^3 in 1950 to about 1000 m^3 in 2015). Water quality for both surface and ground water is deteriorating due to increase pollutants load and the increase in number of people living in areas with high risk of pollution (NRAW, 2016). One of the solutions to meet uprising water demand is water reclamation and reuse for non-potable purposes.

Wastewater treatment may be a potential direct source for obtaining freshwater. It is estimated that 99.9% of all wastewater is water and is recyclable (Virdis et al., 2008). Domestic, industrial and agricultural wastewaters have dissolved organics, which need removal before discharge into the environment (Angenent et al., 2004). The principal objective of wastewater treatment is generally to allow municipal and industrial effluents to be disposed of without danger to human health or unacceptable damage to the natural environment. Irrigation with treated wastewater is both disposal as well as effective form of utilization.

For wastewater treatment, the demands for energy in the present day are considerable. Aerobic treatment is conventionally used to remove these organic pollutants which is a very energy intensive process (Rozendal et al., 2008). An energy input of 0.3 kWh m^{-3} is required for aeration in conventional activated sludge (CAS) process and the plants operational processes needs twice of this amount. Recent technologies, such as membrane bioreactors (MBR), generally achieve higher effluent quality and better performances, but the problem is same as with the rest of technologies that is need of higher energy demands (-1 to -2 kWh m^{-3}) (McCarty et al., 2011). An outlook change is required from a conventionally energy

consuming treatment processes to enable energy recovery, as the basics of in practice wastewater treatment processes were established over a century ago. Some treatment plants, such as anaerobic digestion of organics for producing biogas, have become energy neutral, but these plants require very concentrated waste stream of organic matter (over 3000 g per m^3) and very precise operational conditions (large digester sizes and >20°C) (Logan & Rabaey, 2012).

While it takes energy to treat wastewater, it is also practicable to recover energy from the treatment process. Domestic wastewater has the potential to yield up to 2.2 $kWh^{-1}m^{-3}$ in aeration (Shannon et al., 2008). To take advantage of this energy potential, microbial fuel cells can be used, which have been shown to be able to use wastewater to generate electricity (Pandey et al., 2016).

Microbial fuel cells have been widely regarded as one of the promising approaches for sustainable energy production from variety of organic wastes and biomass, which utilize the catalytic activity of microorganisms to convert organic matters to electricity generation (Davis & Higson, 2007; Mohan et al., 2008). Searching new renewable energy resources to replace fossil fuels have been the focus in recent time. MFCs have attracted significant attention due to their potential to produce electrical power from a wide range of wastewater while achieving partial treatment (Colombo et al., 2017; Liu & Li, 2014; Sonawane et al., 2017b). However, their practical applications are confined by the low power density.

For a very long time, MFC has been considered as promising alternative technology for the biological reactor of conventional municipal wastewater treatment plants (Rodrigo et al., 2007). It has been considered applicable for highly-loaded industrial wastewater treatment as well (Cusick et al., 2010; Huang et al., 2009). Recently, processes using MFCs have received considerable attention to substitute for the CAS process because they can treat wastewater while generating electricity at the same time (Ahn & Logan, 2010; Feng et al., 2014; Ren et al., 2014; Zhang et al., 2013). MFCs have clearly an upper hand on the existing wastewater treatment system and can replace these energy-intensive activated sludge treatment. It could degrade waste and at the same time reclaim energy for further use at the plant by implementing. It has been shown in the past that, MFCs can reduce 50-90% of the

organic matter by using wastewater as the anode effluent (Liu et al., 2004; Cusick et al., 2010; Min & Logan, 2004). At the small scale, the results were encouraging but the scale-up exposure of MFC is a huge obstacle at the present time and a lot of work has to be done in coming years to overcome it affectively (Virdis et al., 2008).

1.1 Significance of the research

Several structural and operational aspects have been studied to enhance the performance of MFC, including inoculums, electrode material, ion exchange membrane, solution ionic strength, temperature, design and configurations and use of various types of real wastewater or synthetic solutions as substrates (Catal et al., 2011; Cheng et al., 2006; Lee et al., 2015). The uses of synthetic solutions as substrates have a great significance because this kind of energy conversion devices are not the best choice for its environmental applications searched in the last years. In fact, the greater and real opportunities could have been the supply of power to remote applications with low requirement of energy. Within this context, yielding energy from different organic matters in a synthetic fuel, manufactured only for this purpose, can be a possible approach to optimize this technology. The synthetic wastewater used in MFC should be a solution which have not only a carbon source to provide energy but also nutrients in sufficient ratios for the metabolic requirements of microorganisms, so that it does not become the limiting components of the process, which is a case with different types of wastewater typically fed to these systems (Asensio et al., 2016; Rodrigo et al., 2009).

Various organic substrates can be utilized as potential fuels for MFCs. Obviously, the simpler and easily biodegradable molecule results in the more effective process (Asensio et al., 2016; Virdis et al., 2010). An initial hydrolysis and fermentation step is required for complex substrate to break macromolecules to simpler ones and then convert them to other readily biodegradable substrates like acetate, which will be further degraded by anode respiring bacteria (ARB) (Kiely et al., 2011; Lalaurette et al., 2009). The redox mediators and/or redox transfer enzymes involved in the metabolism of simple substrates like alcohol and sugar proceeds through very different pathways and these differences should reflect on the performance and efficiency of MFC.

It is important to know which of these substrates delivers a higher efficiency. Adding to that, the effect of substrate concentration also plays an important role in evaluating the performance of MFC. Many researchers have been focusing on the use of pure cultures and various MFCs system operating mainly with pure substrates as a sole carbon source. The growth medium having necessary micronutrients and a carbon source (pure substrate) is generally stated as synthetic wastewater. It is interesting to explore the new dimensions of this technology with different types of synthetic wastewater and in various concentrations, to determine the fuel with best performance.

1.2 Objective of the study

With this background, the main purpose of the present study has been

- To study the effect of different substrates on the performance of double chamber microbial fuel cell.
- To find the effect of different concentrations of substrate on the performance of double chamber MFC

1.3 Scope of the study

The scope of study include

- Construction of three lab-scale double chamber MFCs.
- Use of synthetic wastewater and anaerobic sludge as inoculum
- Use of glucose, acetate and sucrose as a sole carbon source (substrate)
- Use of three different concentrations for best performing substrate
- To analyze MFC performance in terms of electricity generation, COD removal, coulombic efficiency and internal resistance.

Chapter 2

LITERATURE REVIEW

2.1 Microbial fuel cells - an overview

The first experimental proof of bioelectricity was found by Luigi Galvani in the late eighteenth century, who observed electric response for the first time by connecting frog legs to a metallic conductor (Piccolino, 1998). Michael C.Potter constructed the first documented MFC in 1911, to further explore the bioelectricity potential. He established the flow of current between two electrodes submerged in a sterile medium and in bacterial culture (Lewis, 1966). In 1931, a potentiostat-poised half-cell was operated by Barnett Cohen and achieved a current of 0.2 mA by applying 0.5 V. He demonstrated that the capability of this device could be enhanced by introducing benzoquinone or potassium ferricyanide as artificial electron mediators in the anaerobic compartment. Research on MFCs became popular in the 1960s, as the interest in converting organic waste into electric energy increased significantly (He & Angenent, 2006).

The space programme of USA NASA showed interest in of biological fuel cell during 1960s, as organic waste could be converted into electrical energy in space ships by this technology (Putnam, 1971). As a result, for a time this technology received some consideration, but soon further sources of energy, for example photovoltaic panels replaced it (Davis & Higson, 2007). In the early '90s, low power densities of reactor (less than 1 W m^{-3}) and the need for high concentrations of buffer solution and highly concentrated feed media characterized the research at the time (Logan, 2008), and it was thought that addition of chemical mediators like neutral red was the only way to carry electrons to exogenous electrodes from inside the cell. Later research proved that wastewater can be used as a suitable replacement and that these toxic mediators aren't necessary (Kim et al., 1999).

However MFC technology, due to comparatively short and interrupted development period is still in its infancy. Only very few microbial fuel cells systems with reactor volumes bigger than 1L have been tested, not like several conventional fuel cells (inorganic) which have now achieved an advanced state in their process of development.

2.2 Working principle

The MFCs are devices that convert chemical energy of feedstock into electrical energy through the metabolic activity of microorganisms. Usually, it comprises of two compartments; an anode which is anaerobic and a cathode which is aerobic. Both the compartments are parted by an ion-permeable membrane. In the anode compartment, substrate (fuel) is oxidized by microorganisms and release electrons, protons and CO_2. Electrons produced in the anode compartment then flow to the cathode compartment by means of an external electric circuit due to electrophilic attraction from electrode in cathode chamber, whereas protons produced in the anode migrate through the ion permeable membrane (proton exchange membrane) to cathode. The electrons and protons then combine with final electron acceptor (oxygen) and complete the circuit with this reduction reaction (Figure 2.1) (Chae et al., 2007; Lee et al., 2008). The amount of electrons coursing through the resistor in outer circuit is the current being generated. Other chemicals like sulphate or nitrate can also replace oxygen and serve as electron acceptors.

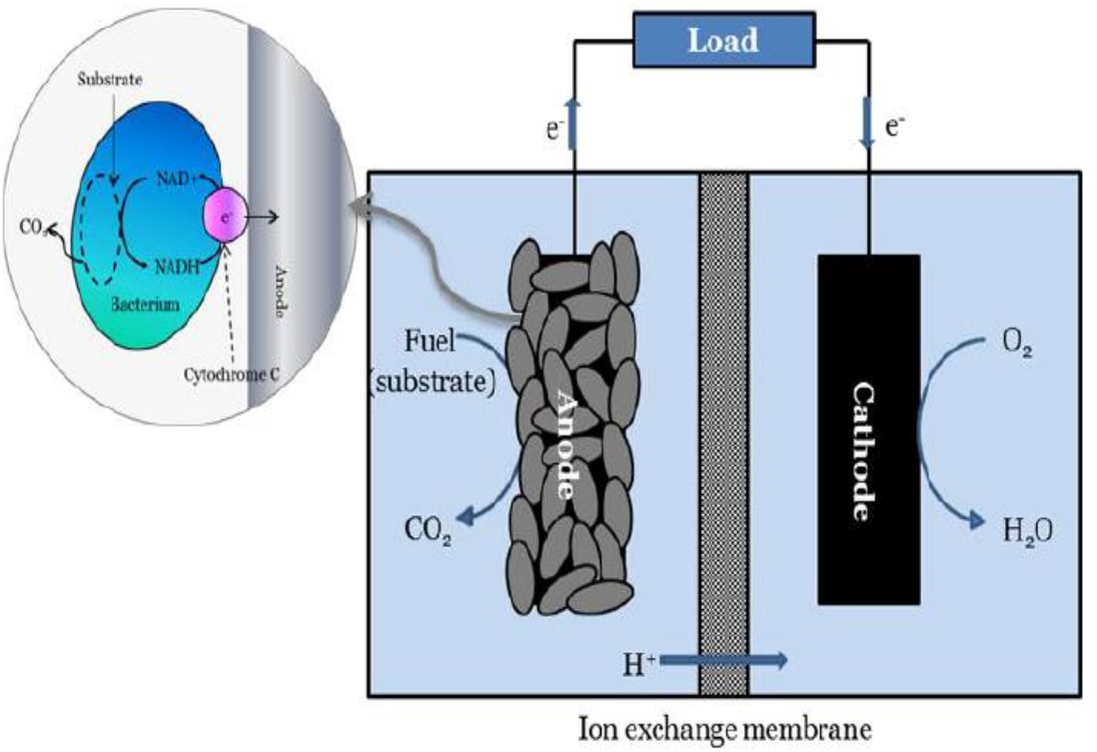


Figure 2.1: Schematic diagram of a microbial fuel cell

Table 2.1 shows some of the electron donors and acceptors that can be used in MFC and their reactions taking place in corresponding chambers.

Table 2.1: Some examples of electron donors and acceptors

Electrode	Electron donor	Reaction
Anode	Glucose	$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$
	Acetate	$C_2H_3O_2^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$
	Butyrate	$C_4H_8O_2 + 2H_2O \rightarrow 2C_2H_4O_2 + 4H^+ + 4e^-$
	Citrate	$C_6H_5O_7^{3-} + 11H_2O \rightarrow 6H_2CO_3 + 15H^+ + 18e^-$
	Glycerol	$C_3H_8O_3 + 6H_2O \rightarrow 3HCO_3^- + 17H^+ + 14e^-$
Electrode	**Electron acceptor**	**Reaction**
Cathode	Oxygen	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$
	Permanganate	$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O$
	Ferricyanide	$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$
	Nitrite	$NO^{2-} + 2e^- + 2H^+ \rightarrow N_2 + H_2O$
	Nitrate	$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$

2.3 Components and materials

2.3.1 Anode

Microorganism in anode compartment attaches to anode (electrode), degrade the substrate and donate the electron to circuit. In the past, different kinds of materials have been studied for this particular electrode in order to improve the performance of MFCs in terms of stability, easier operation and power output. A good anode material is one which has large surface area for microbial attachment and good current collection capability as well as high electrical conductivity for the charge transfer. Since the anodes become biotic, they should be inert to biochemical reactions, as well as non-toxic to microorganisms. Carbon is the most

versatile and handy electrode material, available as compact graphite rods, plates, or granules, as fibrous material (paper, fibers, felt, cloth, foam) and as glassy carbon (Logan et al., 2006). The simplest materials for anode electrodes are graphite rods or plates as they are easy to handle, relatively inexpensive, and have a defined surface area. Considerably larger surface area is accomplished with graphite felt electrodes (Aelterman et al., 2008). Anode modification has been considered an effective approach in order to enhance the anode performance. However, for wastewater treatment, long-term stability is a crucial requisite hence more consideration should be given to this aspect. An increasing threat of fouling anode structure because of biofilm growth also needs to be considered.

2.3.2 Cathode

The electrons and protons combine with oxygen at cathode (electrode) as electrons are sucked from anode due to electrophilic attraction. The choice of the cathode material to be used greatly affects MFC performance, and is varied according to its application. Oxygen is the most suitable electron acceptor due to its availability, sustainability. low cost, high oxidation potential and the lack of a chemical waste product (as the only end product is water). Pt catalysts are usually used for open-air (gas diffusion) cathodes or dissolved oxygen to increase the rate of oxygen reduction (Liu & Logan, 2004). Ferricyanide is very prevalent as an electron acceptor in experiments due to its good performance in MFCs (Park & Zeikus, 2003). Ferricyanide has the utmost benefit of low overpotential with a cathode of plain carbon, which results in working potential of cathode close to its OCV. Still, the insufficient reoxidation by oxygen, which requires the catholyte to be replaced regularly is the greatest disadvantage (Rabaey et al., 2005).

2.3.3 Ion exchange membrane (IEM)

An IEM is mainly used to separate the anode and cathode chambers physically while letting the protons flow to cathode at the same time. Its use, on the other hand, has its own drawbacks. It adds to the internal resistance of MFCs significantly and delays the transfer of proton between two chambers. Moreover, it increases the overall cost of microbial fuel cell (Rozendal et al., 2006). However it has also been reported that oxygen and substrate diffusion would increase in the absence of IEM, which will cause decrease in bacterial

catalytic activity and as a result CE will be reduced. In this context, the use of separator is very necessary (Zhang et al., 2010). The most commonly used Ion exchange membranes are proton exchange membranes (PEMs), Nafion® in particular (Dupont Co., USA). Ultrex CMI-7000 (Membranes International Incorp., Glen Rock, NJ) are better alternatives to Nafion and are considerably more cost-effective than Nafion and well suited for MFC applications as well (Logan et al., 2006).

2.4 Electron transfer mechanism

The transfer of electron from bacteria to electrode (anode) can take place through several mechanisms, illustrated as follows:

2.4.1 Direct electron transfer (Fig. 2.2A)

In this mechanism, electrons are transferred to electrode directly by the cell outer- membrane proteins. The membrane of a bacterial cell is not typically very conducive for electron transfer. The latest genetic characterization and biochemical studies showed that the enzymes on the respiratory chain of bacteria (outer-membrane cytochromes), might be involved in the electron transfer. For this electron transfer mechanism, direct contact of cytochromes is needed with the electrode (Magnuson et al., 2001).

2.4.2 Mediated electron transfer (Fig. 2.2B)

Adding artificial mediators to anode feed, such as methylene blue and neutral red can achieve efficient electron transfers, which are proficient in crossing the cell membranes and taking up electrons from intracellular electron carriers by, leaving the cell in the reduced form and then releasing the electron onto the electrode surface (Rabaey et al., 2004). However, due to toxicity and the cost of many synthetic mediators, the microorganism that handover electron through this method is not appropriate for wastewater treatment. More importantly, rapid loss of mediators occurs in a continuous-flow system.

2.4.3 Electron transfer via bacterial nanowires (Fig. 2.2C)

The current revelation of bacterial nanowires showed that the conductive, pilus like structures developed on the cell membrane may permit the direct reduction of a distant electron acceptor and straightforwardly involved in extracellular electron transfer. Several

types of bacteria such as *Shewanella oneidensis* MR-1, G. *sulfurreducens* PCA, the thermophilic fermentative bacteria *Pelotomaculum thermopropionicum* and a phototrophic cyanobacterium *Synechocystis* PCC6803 have been identified with these nanowires (Gorby et al., 2006).

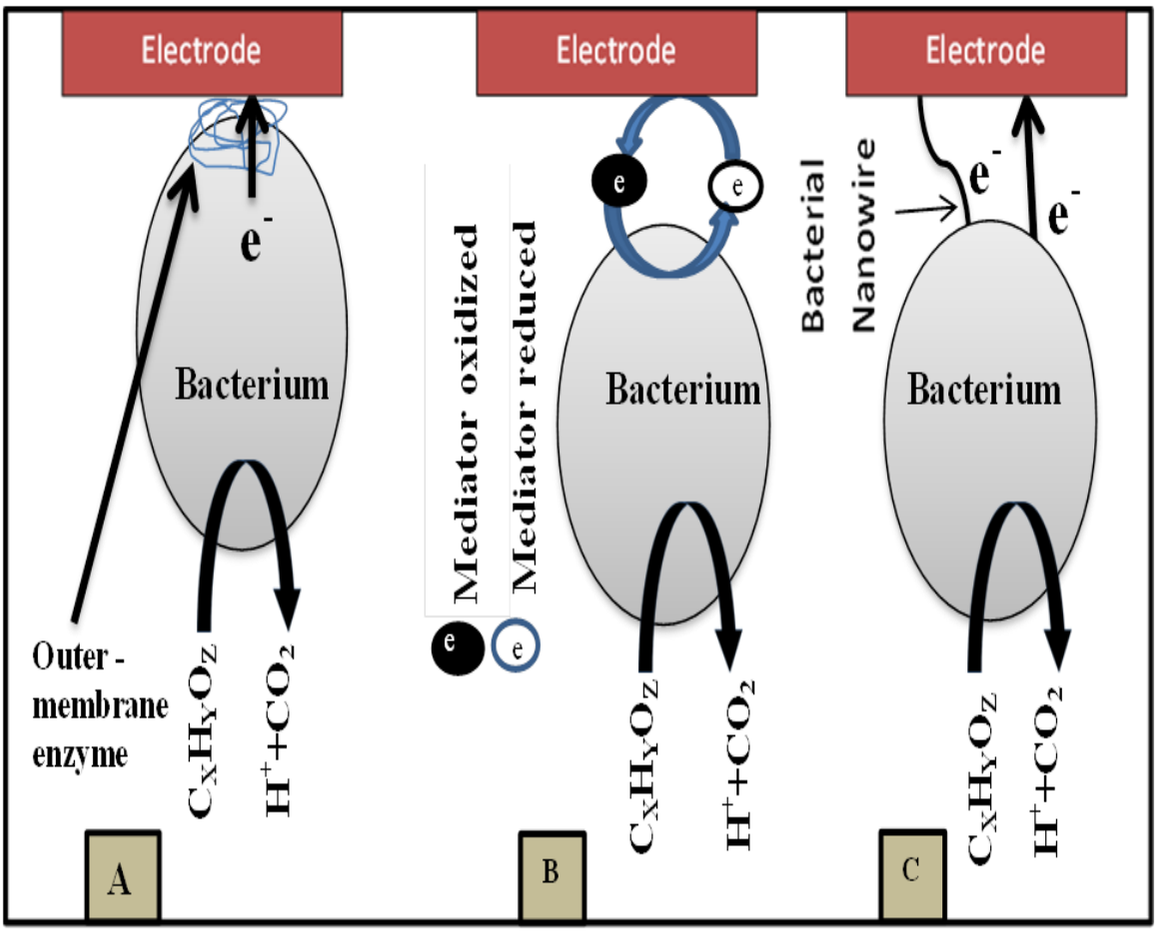


Figure 2.2. Electron transfer mechanisms: (a) direct electron transfer (b) electron transfer via mediators; (c) via pilus-like nanowires (Khanal, 2011).

2.5 MFC design

The MFCs are being fabricated in a variety of structural design, and various types of MFCs are generally assessed by stability, longevity, power output and CE. Moreover, the materials cost and feasibility of scaling up the structural design also needs to be taken into consideration in the real application.

2.5.1 Double chamber MFCs

The conventional design of MFCs comprises of two chambers, anaerobic (anode) and aerobic (cathode) chamber, which are joined by a bridge and both the chambers are separated by a CEM. This typical double chamber design is commonly run in fed-batch and batch mode. The main purposes of CEM, such as Nafion 117, are to allow protons flow from anode to cathode while physically separate the liquids in each chamber (Logan et al., 2006). The simplest form of DC-MFC is an H-type cell which consists of two glass bottles that have been attached to a duct at the lowest end. These two ducts are clamped together with some form of IEM between them to connect the internal circuit of the cell. Through holes or septa drilled in the lids of these bottles, electrodes are inserted (Oh & Logan, 2006).

Figure 2.3: H-cell MFC design (Oh and Logan, 2006)

A slightly more complex design, typically of rectangular construction involves a two chambered MFC with both chambers being connected by external bolts. The cathode is aqueous in this system as well. The advantages of this design over an H-cell are the ease of adding ports for continuous flow and the increased area for ionic transfer. The disadvantages are the difficulty involved in assembly as well as the need to sparge the cathode.

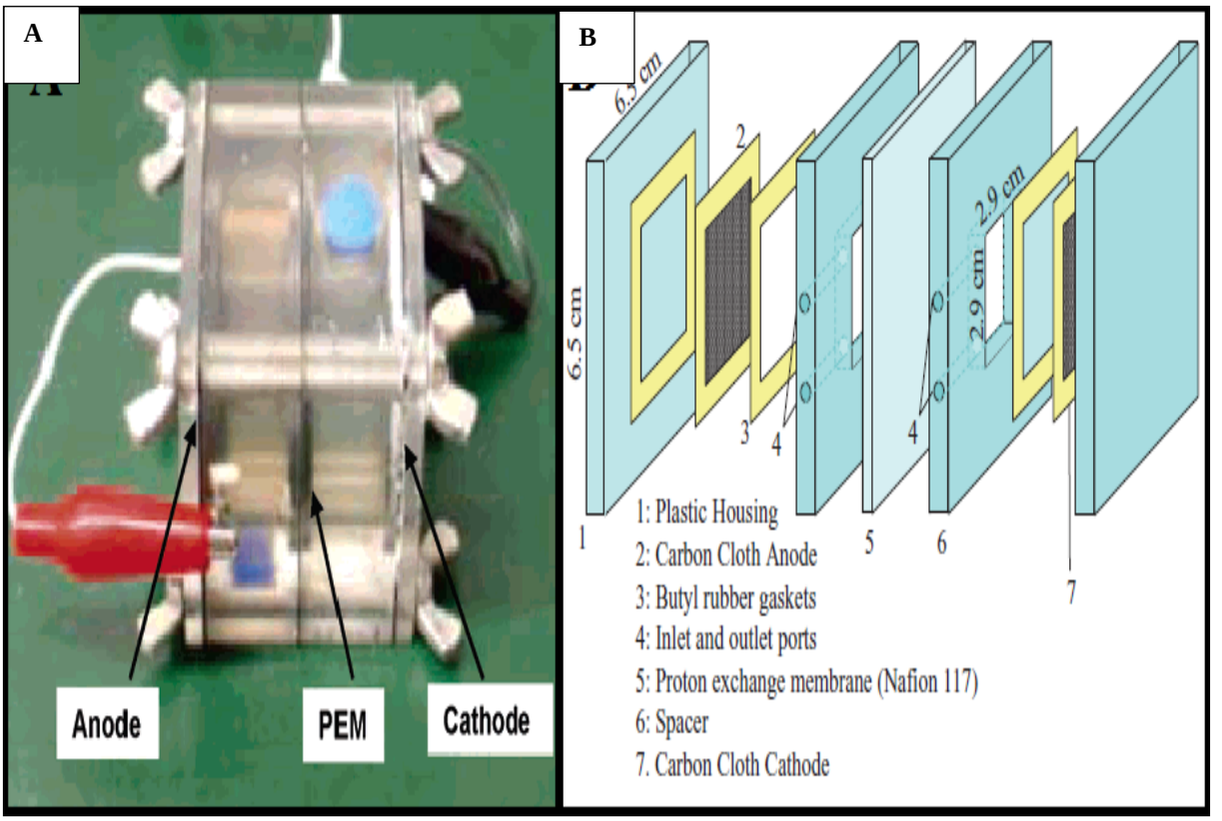


Figure 2.4: (A) 2-chambered MFC with aqueous cathode (Kim et al., 2007). (B) Typical schematic for a 2 chambered MFC (Nevin et al., 2008)

2.5.2 Single chamber microbial fuel cell (SCMFC)

Many researchers have chosen to use SCMFCs with air cathode due to their practical implementation characteristics and simple and economic design. SCMFCs can be fabricated by eliminating the cathode compartment and keeping the cathode in direct contact with air. The SCMFC provides advantages of simple scale up over the two chamber system (Liu & Li, 2014; Liu & Logan, 2004).

However, the major challenge for a membrane-less MFCs is much lower coulombic efficiency than that of MFC containing a membrane due to the consumption of substrate to oxygen diffused through the cathode when a mixed culture is used (Liu & Logan, 2004). These problems could be resolved by development of new separators, which can diminish the oxygen diffusion without affecting the power density and increasing the internal resistance of cell.

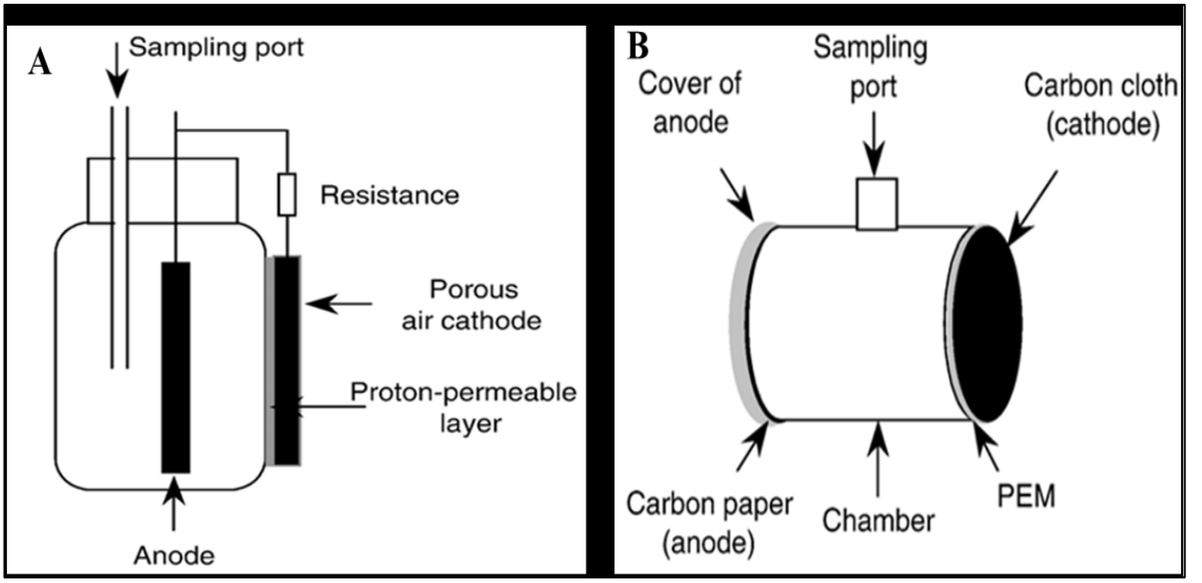


Figure 2.5: Some single chambered MFCs as reported in Liu and Logan (2004)

2.5.3 Other designs of MFCs

Voltage produced by MFC is still limited and cannot surpass a theoretical OCV of 1.14 V even neglecting the whole internal losses produced in a system. Other optimized structural designs of MFCs were fabricated. For example, stacked MFCs (Aelterman et al., 2006) and tubular/up flow architecture (Logan et al., 2008). Tubular MFCs operate in continuous flow mode normally. The flow first move through an anaerobic compartment and after that directly up into the aerobic compartment in the same column. This type of MFCs have quite a few advantages over typically used designs, such as combining the benefits of the two-chamber MFC with up flow anaerobic sludge blanket system and a higher affinity for oxygen with cathode (Lovley, 2006). These advantages give rise to improving both wastewater treatment and electricity generation. Using the same idea of connecting several MFCs to enhance the voltages, several MFCs in parallel and series can be connected to

improve voltage or current production. Aelterman constructed a stacked MFC, which was used to observe its performance connected in parallel or series. The separated MFCs were electrically connected by means of copper wires attached to the electrodes and tightened with the help of screw bolts. The result obtained showed that higher maximum bioeletrochemical reaction rate could be achieved with the parallel-connected system. The COD removal can be enhanced compared to a single cell by applying it to wastewater treatment application. The main purpose of all optimizations of MFCs' architecture or configuration is to increase the cell power output and reduce the internal resistance.

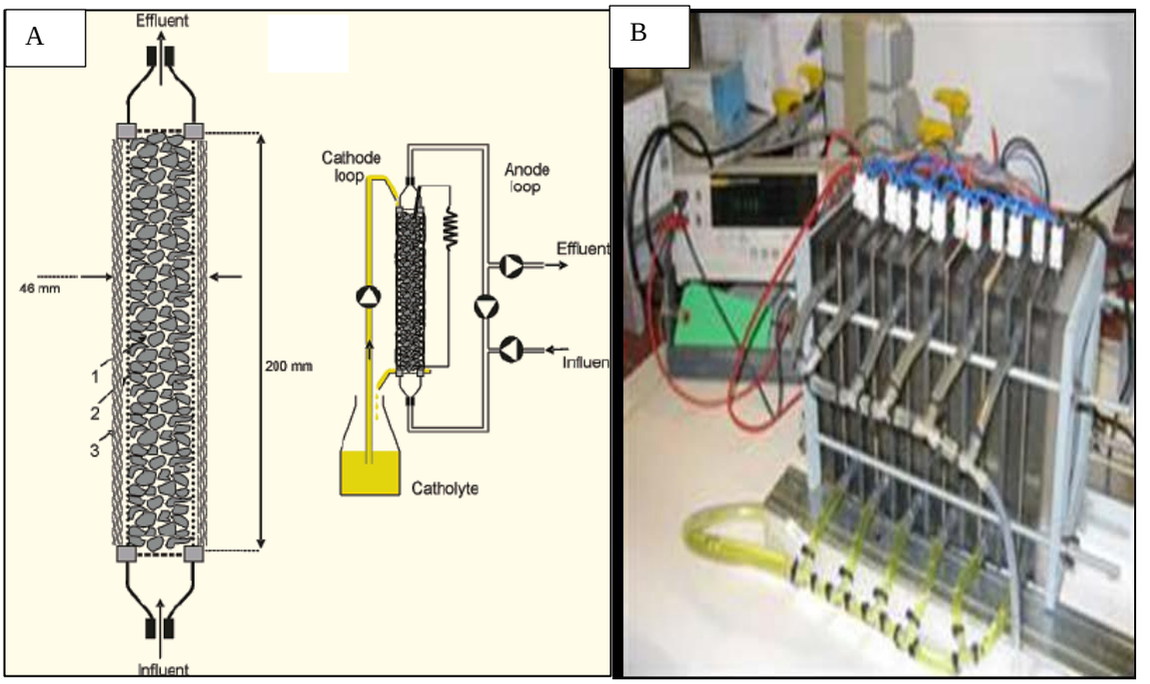


Figure 2.6: (A) Upflow, tubular MFC, with outer cathode and inner graphite bed anode (Rabaey et al., 2005) (B) Stacked MFC, in which one reactor block have 6 separate MFCs (Aelterman et al., 2006)

2.6 Factors affecting performance of MFCs

The degradation of organic matters by microorganisms in anode chamber is one of the key processes for electricity generation in MFC. Generally high internal resistances of MFCs limit the power output. There are many other external and internal factors associated with its performance, such as reactor design, separator materials, electrode, catalysts, substrate type, substrate concentration, electron acceptor, hydraulic retention time (HRT), feed pH, and

temperature that increase the internal resistance of the MFC. Most of the factors have been addressed in previous sections, so this section summarizes only the various operational factors which affect performance of MFC.

2.6.1 Temperature and pH

Temperature and pH are two factors that are normally kept constant during the MFC run, which help the biofilm in adapting to a determined set of conditions in order to survive and maintain a stable community. Always changing these factors lead to changes in the microbial community as the biofilm have to constantly adapt the new conditions and a very unstable electricity production is achieved. In order to optimize the electricity generation, the optimal values of temperature and pH must be found and should be applied right from the start of the MFC run. Several researchers have studied the effect of these two factors (Ali et al., 2015; Min et al., 2008). For temperature, a range between 10-50 ^{0}C has been described as "livable" for the biofilm while values between 30 ^{0}C and 35 ^{0}C have been termed as optimum (Sun et al., 2014). Regarding the pH, (Sun et al., 2014) refers the value between 7-9 as the ideal for biofilm formation and MFC performance.

2.6.2 Hydraulic retention time

Hydraulic retention time (HRT) is another important variable in MFC, particularly for wastewater treatment perspective. It affects both COD/BOD removal and power generation in the process. Power generation increased by 60% with an HRT increased from 4.2 to 15.6 h for an MFC using glucose as substrate, operated in a flow-through mode. The COD removal efficiency remained higher than 89% at all HRTs. At lower HRTs of 3.4–4.6 h, COD removal of only 40–50% was achieved for domestic wastewater (Cheng et al., 2006).

2.6.3 Ionic strength

Ionic strength affects the solution conductivity and thus the internal resistance, which thereby affects the performance of MFC. However, in generating electricity from municipal wastewater and saline industrial wastewaters in cities seawater is used for toilet flushing, use of MFCs may be highly effective. (Liu et al., 2005b) reported that power production increased up to 85% when NaCl (300 mM) was added to the solution in the anode chamber, due to the

reduction of internal resistance. It should be noted by the reader that it may not be practical to enhance MFC performance in this manner.

2.6.4 Substrate type

Substrate type is also one of the important factors affecting the performance of MFC. Various organic substrates can be utilized for electricity generation in MFCs. Different types of substrate influences the bacterial biofilm growth and the MFC performance including the power density and coulombic efficiency (Pandey et al., 2016). Several types of substrates including known chemicals, and mixture of chemicals, and real wastewater have been used in the past to generate electricity. The detail description of different types of substrates used in MFC has been given below in section 2.8.

2.6.5 Substrate concentration

Substrate concentration effect on MFC is directly associated with the microbial community in the anode chamber. Different microbial communities can be established, depending on the inoculum used and optimal values of substrate concentration can vary, due to which it is so difficult to find out an optimal range for this parameter. (Ghoreyshi et al., 2011) worked on the influence of date syrup and glucose concentration in MFCs inoculated with Saccharomyces cerevisiae concluded that the optimum concentration for both substrates was 3 $g.L^{-1}$ and that high concentrations of substrates may have a significant role in the performance of MFC. However, the results cannot be inferred to different case studies without making further investigation.

2.7 Evaluation of MFC performance

Generally, there are two key features while considering the performance of an MFC; how well it is capable to utilize a given feedstock and the amount of power it can produce. Although computing power output is straightforward in an MFC, reporting data to the research community is not that simple. Because of the variation of MFC operational conditions, reactor shapes and designs and compartment materials used by researchers, still unanimously acceptable standard parameters are needed. For instance, at larger scale, power density is usually considered as a source of showing the power output competency of MFC.

Table 2.2: Key parameters evaluating the MFC performance

Parameter	**Unit**	**Calculation/measurement**
Open circuit voltage	V	OCV(VO/C), voltage at infinite resistance
Voltage	V	Measured between two ends under the applied external resistance (R_{EXT})
Current	A	$I = V/R$ R is the loaded external resistance value in ohms (Ω).
Current density	A/m^2, A/m^3	$C.D = I/A$, $C.D = I/V$ Where A is total/projected surface area of anode/cathode (m^2), and V is the total reactor/ anodic/ cathodic volume (m^3).
Power density	W/m^2, W/m^3	$PD = P/A$, $PD = P/V$ A and V are the same as above.
Coulombic efficiency	%	$CE = \int I(t)\,dt / (F.b.Va.\Delta S)$ Where t is time (s), F is Faraday's constant (96,485C/mol-e-), b is the number of electrons produced per mol of oxygen (4 mol-e-), V_a the liquid volume (L) and ΔS is the substrate consumption in terms of COD (mol O2/L).
COD removal efficiency	%	$[(COD_{in} - COD_{out})/CODin].100$ Where CODin (mg/L) is the initial concentration and COD_{out} (mg/L) is the concentration after treatment.
Internal resistance	Ω	Calculated from the slope of the polarization curve

However it can be normalized by various factors such as total size of anode or cathode or membrane (Gajda et al., 2013; Oh & Logan, 2006). Sometimes power density is also expressed in terms of liquid volume of anode, cathode or both (Capodaglio et al., 2013). Although researchers working on MFC agree with the need for the same standard in this aspect, uniform parameter has not been established yet which are universally agreed by. It is difficult to evaluate power performance of different systems without presenting full information of reactor and component dimension in various parameters and may lead to overestimation by reporting data in this way. Table 2.2 summarizes the commonly used parameters for evaluating the MFC performance.

2.7.2 COD removal

The COD of wastewater can be removed in an MFC through conversion to electrical current, aerobic oxidation and/or through sulfate and nitrate reduction, or biomass. The sources of oxygen in MFC include that initially contained in the influent wastewater and/or that diffused from air to the anode chamber of MFC through the cation exchange membrane and/or through the air cathode in single chamber MFC. For substrates that are readily biodegradable, for instance simple sugars and volatile fatty acids, the COD removal efficiency is high when compared to complex substrates, more than 90 % in few cases (Catal et al., 2008; He et al., 2006; Liu et al., 2005b; Liu & Logan, 2004; Yang et al., 2012). For complex substrates such as domestic and industrial wastewater, the COD removal efficiencies range from 40 to 95% (Jiang et al., 2013; Mansoorian et al., 2013; Rabaey et al., 2005).

2.7.3 Coulombic efficiency

Coulombic efficiency is used to assess the recovery of electron from the organic matter in the form of current. Coulombic efficiency can be evaluated as a ratio of total recovered current (coulombs) which is obtained by integrating the current over time to the possible theoretical current that can be produced. In other words, it gives the information about the amount of COD utilized in current generation. The CE is normally lower than the COD removal rate as only certain amount of COD converts to electricity generation (Khanal, 2011). The rest of COD is utilized in other processes including the substrate utilization for

bacterial growth, fermentation, methanogenesis and transfer of electron from organic matter to other electron acceptors, which includes oxygen, sulfate and nitrate in solution (Prestigiacomo et al., 2016). The calculation of CE has been given in section 3.5.

2.7.4 Polarization and power curves

Polarization and power curves are powerful tools for the analysis and characterization of MFCs giving information about internal resistance and maximum power output. Polarization is the change of cell electrode potential from its equilibrium state due to a flow of current. It characterizes a great tool for the examination and classification of microbial fuel cell. To obtain polarization curve, a variable resistor box is used to set variable external loads. Using a periodical decrease or increase of the load, the voltage is measured and the current is calculated using Ohms law (Watson & Logan, 2011). The curves can usually be divided in three zones (Logan et al., 2006).

1. There is an initial steep decrease of the voltage when no or very less current is flowing. The activation losses are dominant in this zone. It is mainly due to the activation energy required for oxidation/reduction reaction. These losses can be reduced by improving electrode catalysis, increasing the electrode surface area, through establishing an enriched biofilm on the electrode, and increasing the operating temperature.
2. The voltage then falls more slowly and is fairly linear with current. In this zone the ohmic losses are dominant. Ohmic losses occur mainly due to resistance to electron flow through the electrical circuit (including electrodes), the medium in the anode chamber and the resistance to ion transport across the CEM.
3. There is a rapid fall of the voltage at higher currents. in this zone the mass transport effects (concentration losses) are dominant. These losses occur when the electron flow is limited due to insufficient mass transport to the anode and primarily occur at high currents.

A power curve that describes the power as the function of the current is calculated from the polarization curve. Power curve helps in finding the maximum achievable power in a microbial fuel cell (Wang et al., 2015). The correspondent power curve starts at zero and the power increases with current from this point onward to a maximum power point. The power

then drops beyond this to the point where no more power is produced (short circuit conditions) due to the electrode overpotentials and increasing ohmic losses. According to fuel cell theory, maximum power density occurs at point where total internal resistance is equal to the applied external resistance (Logan et al., 2006; Park & Ren, 2012) .

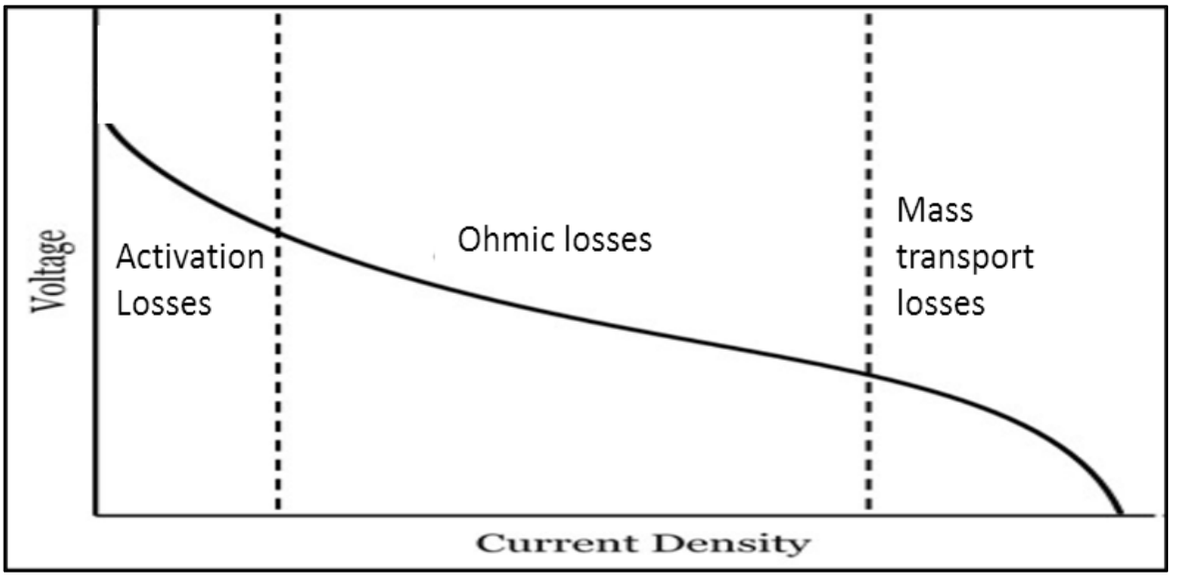


Figure 2.7: polarization curve showing different types of losses (Kunik, 2015)

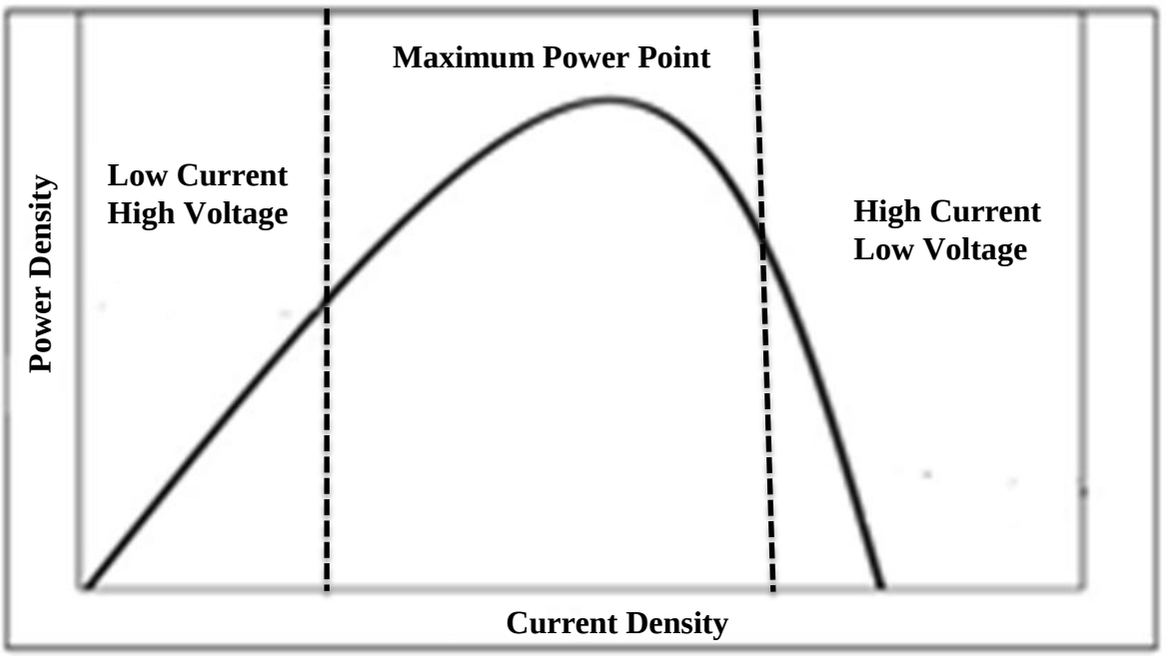


Figure 2.8: Idealized power curve for a typical MFC. Adapted from (Wang et al., 2015)

2.8 Recent advances in the use of wastewaters as substrates in MFCs

Researchers across the world have explored several wastewaters types with different designs of MFC and operational conditions, and reported on various parameters such as COD removal rate, CE and the effects of substrate concentration on the maximum voltage and power output. As is known, bacteria have very particular ranges of pH and temperature where they can flourish and achieve maximum performance. Thus, these parameters not only affect the metabolism of microorganism but also the biofilm formation and composition, which in turn have the influence on electric production and consequently the MFC performance. However, the situation for substrate is little different. The types of substrates have a huge influence in MFCs performance, mainly for the reason that bacteria are generally specialized in a particular organic compound that can be metabolized with maximum efficiency. The substrate concentration optimal range is not yet known and can also effect the growth of biofilm and the MFC performance. An overview of both low and high molecular weight substrates highlighting mainly different wastewaters as potential feed sources with respect to latest developments, existing challenges, and future perspectives is presented.

2.8.1 Complex or undefined wastewater substrates

Due to the potential of wastewater treatment and energy recovery with MFCs, several wastewater types have been used as substrates till now. Some real wastewater used as substrates are deliberated under specific categories in subsequent sub-sections.

An Enormous amount of food wastes are produced throughout the world which is rich in carbohydrate content. Around 27% of overall municipal solid waste consists of food waste. Worldwide, wastage of approximately one third of edible part of food produced (estimated 1.3 billion ton per year) for human consumption is a concern (Gustavsson et al., 2011). Several researchers have been encouraged by this to investigate food wastes as potential substrates in MFCs.

Venkata Mohan and coworkers evaluated the feasibility of composite vegetables waste as substrate in single chambered mediator-less MFC (Mohan et al., 2010). The MFC used in this study resulted in Pd_{max} of 57mWm^{-2} with effective COD removal of 62%. The efficiency

of substrate removal and catholytes on bioelectricity generation were assessed by using food-processing wastewater as anolyte by researchers (Sangeetha & Muthukumar, 2011). Salt bridge dual chambered, MFC in this case produced Pd_{max} of 123.8 mWm^{-2}, current density of 54.3 mAm^{-2} and 98.9% of COD removal. Synthetic wastewater with potato extracts (starch) was studied by Herrero-Hernandez and his coworkers (Herrero-Hernández et al., 2013). The MFC with titanium mesh electrodes resulted in Pd_{max} of 502mWm^{-2} and significant COD reduction of 61%. Fogg et al.,(2015) lately demonstrated the utilization of pomace as a substrate for current generation in MFC and reported Pd_{max} of 132mWm^{-2}.

The use of domestic wastewater for energy recovery is one of the major focuses of interest among researchers community. About a decade ago, domestic wastewater was assessed as potential substrate using SCMFCs (Liu et al., 2004; Min & Logan, 2004). For instance, using domestic wastewater, Pd_{max} of 26 mWm^{-2} was achieved while 80% of COD (initial COD of 200–300 mg l^{-1}) was removed (Liu et al., 2004). Air–cathode MFCs fed with domestic wastewater of 345 mg l^{-1} COD produced 22.5Whm^{-3} corresponding COD removal of 83% and 18% CE (Cusick et al., 2010).

2.8.2 Simple or defined substrates

Various organic substrates can be utilized for electricity generation in MFCs. Major metabolic fuels – carbohydrates, amino acids and fatty acids are the monomers of all high molecular weight and complex wastewaters. Definitely, carbohydrate is the most abundant group of these organics. Fuel and energy generation from lignocellulosic biomass such as woody biomass and agricultural residues has drawn significant attention because of their abundance and readily availability (Petrus & Noordermeer, 2006). Many researchers have been focusing on the use of pure cultures and various MFCs system operating mainly with pure substrates as a sole carbon source. The growth medium having necessary micronutrients and pure substrate (carbon source) is generally spoken of as synthetic wastewater. It is interesting to explore the new dimensions of this technology with different synthetic fuels and in various concentrations, to determine the fuel with best performance (Asensio et al., 2016; Pandey et al., 2016). Catal and his coworkers worked on electricity production from six different types of hexoses (glucose, fucose, galactose, fructose, mannose and

rhamnose,), three pentoses (ribose, xylose and arabinose), and three different types of sugar derivatives (gluconic acid, galacturonic acid and glucuronic acid) in air cathode SCMFCs by using a mixed bacterial culture as a source of inoculum. The Pd_{max} of 2770 mWm^{-2} was achieved with glucuronic acid, and then trailed by xylose and glucose. Lowest Pd of about 1240mWm^{-2} was produced by Mannose. The COD removal of 80% whereas CE ranging from 22% to 34% was obtained during the process (Catal et al., 2008) . Liu et al. reported Pd_{max} of 305mWm^{-2} and 506mWm^{-2} with butyrate and acetate fed MFCs, respectively (Liu et al., 2005a).

Energy conversion efficiencies have also been evaluated with the most commonly used fermentable (glucose) and non-fermentable (acetate) types of substrates in several studies. Min and Logan reported the maximum power density of 212 and 286 mWm^{-2} by using substrates of glucose and acetate respectively, with ferricyanide as catholyte and continuous mode of operation (Min & Logan, 2004). In one other study, Rabaey et al. reported 90 and 66 Wm^{-3} with acetate and glucose fed MFCs, respectively (Rabaey et al., 2005). Using batch fed mode, the Pd_{max} of 360 and only 9.8 mWm^{-2} were reported in the acetate-fed and glucose-fed MFCs, respectively (Lee et al., 2008). The main reason in attaining low power density with fermentable substrates is the existence of high concentration of non exoelectrogenic microbes in anode biofilms.

Chapter 3

MATERIALS AND METHOD

The experimental setup used for this research consist of construction of three lab-scale MFCs, which was run in two phases, first for three different types of substrates followed by different concentrations of one substrate. Inoculum and synthetic wastewater was prepared for all three substrates. Performance parameters were compared for each case. The following sections show the detail description of each step.

3.1 MFCs construction

Three identical lab-scale dual chamber MFCs were constructed from transparent acrylic sheets with a working volume of 1.9L for each chamber. MFCs were constructed in an H-shaped design with both chambers being separated by cation exchange membrane (CEM) (CMI-7000, Membranes International, Inc.) as shown in Figure 3.1.

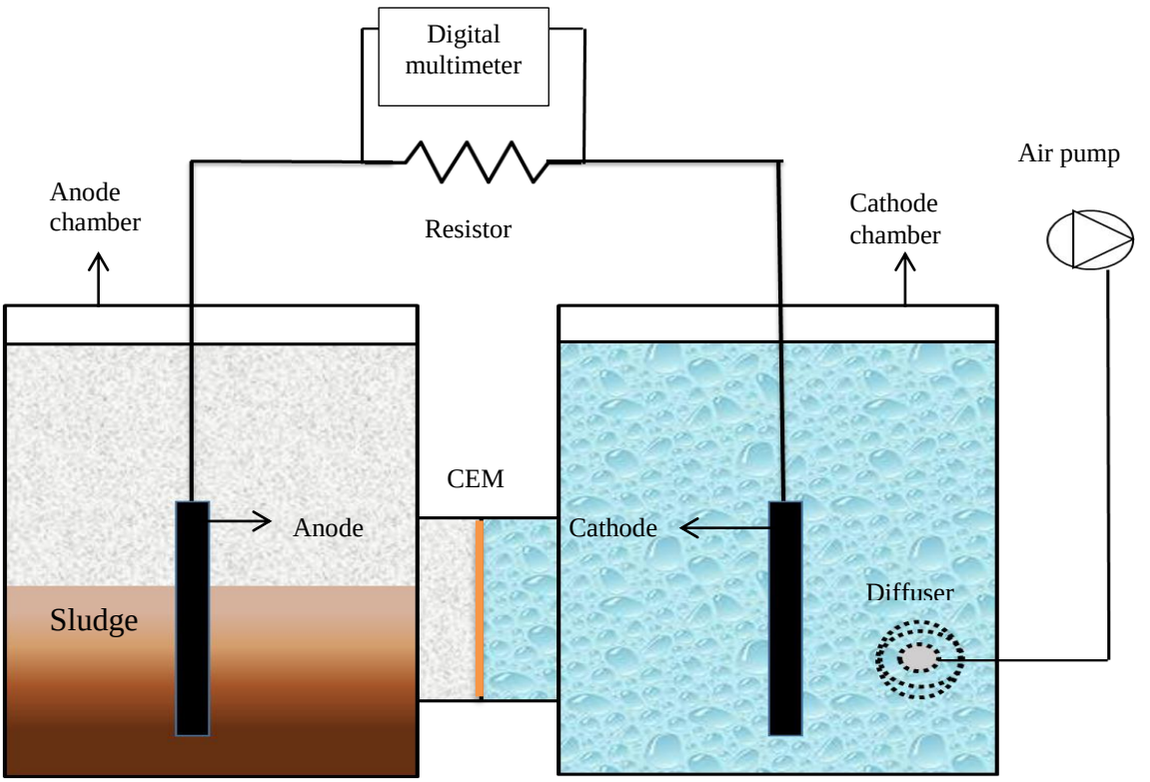


Figure 3.1: Schematic diagram of double chamber MFC used in the stud

The CEM surface area was 64cm^2, and it had been soaked in 5% NaCl solution for 12 hours before use to allow for hydration and expansion. The reason of using CMI-7000 is its excellent proton conductivity, thermal and chemical stability with much less water permeability (Khanal, 2011). Two uncoated rods of graphite were used as electrodes for each chamber. The effective length and diameter of graphite rods were 7.3 and 4.5 cm, respectively resulted in a surface area of 22 cm2. The graphite rods were abraded by sand paper before the installation to enhance the bacterial attachment. A copper wire was connected with each electrode and extended outside the MFC system to simply develop an electrical circuit for electrons transport.

Table 3.1: Technical specification of cation exchange membrane (CMI-7000)

Functionality	Strong acid cation exchange membrane
Functional Group	Sulphonic Acid
Ionic Form as Shipped	Sodium
Color	Brown
Standard Thickness (mm)	0.45±0.025
Electrical Resistance (Ohm.cm^2),0.5 mol/L NaCl	<30
Permselectivity (%)	94
Maximum Current Density (Ampere/m^2)	<500
Total Exchange Capacity (meq/g)	1.6±0.1
Water Permeability (ml/hr/ft^2) @5psi	<3
Thermal Stability (^{o}C)	90
Chemical Stability Range (pH)	1-10

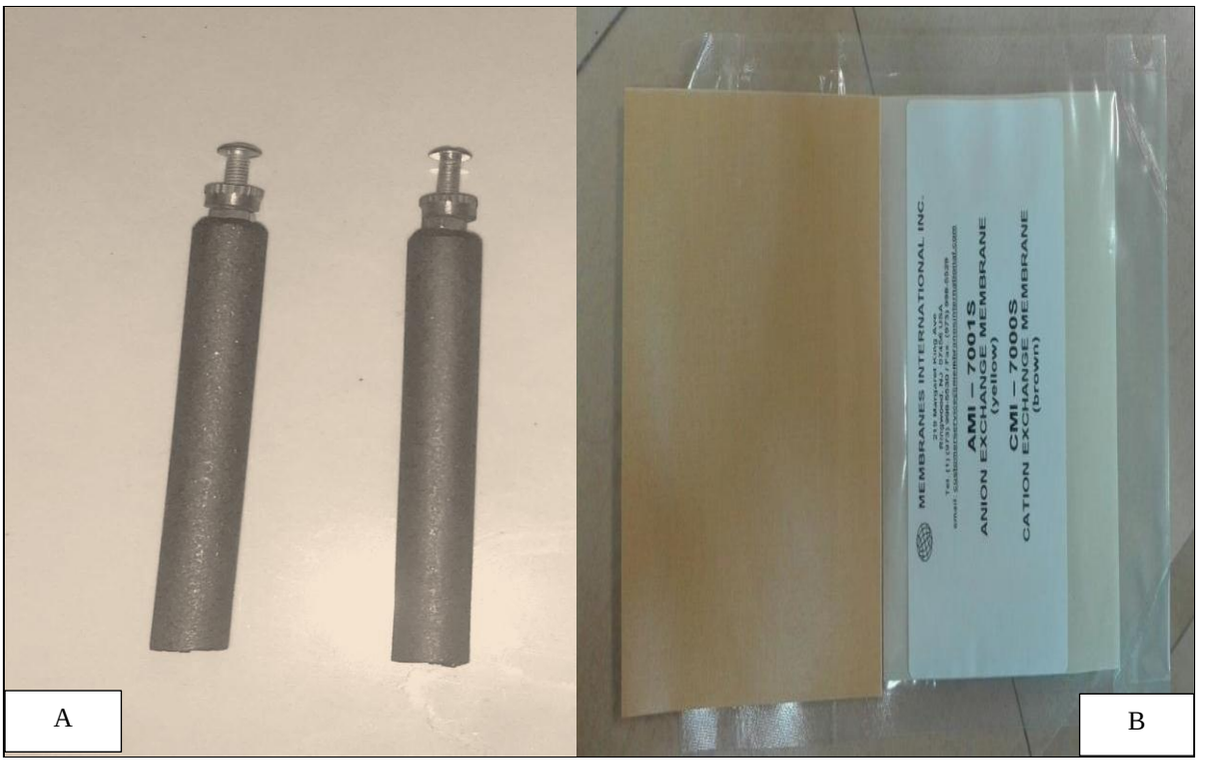


Figure 3.2: graphite rods (A) and cation exchange membrane (B), used in the study

3.2 MFC Inoculation and synthetic wastewater

Activated sludge from a wastewater treatment plant (NUST-MBR, Pakistan) was used as the inoculum for the anodic compartment. Before the use of activated sludge in MFCs, it was placed in the three different containers for three days in a 1:2 without aeration to favor the establishment of a mixed culture of anaerobic microorganisms. No synthetic wastewater was feed to the culture during this period (Asensio et al., 2016). After this period, synthetic wastewater with different carbon based substrates were fed for two month to the MFCs for acclamation purposes. Inorganic compounds were same in all cases.

The synthetic wastewater fed to each MFC consist of glucose, sucrose or sodium acetate as a sole carbon source in same concentration of 1000 mg/L COD in first experiment. In the second experiment, synthetic wastewater fed to each MFC consist of acetate as a sole carbon source but with different concentrations of 500mg/L, 2000 mg/L and 3000 mg/L COD as shown the Table 3.2. The synthetic wastewater prepared in phosphate buffer of 50 mM (pH 7) has the inorganic composition, given in the Table 3.3.

Table 3.2: type of fuel used in the synthetic wastewater and COD mg/ L

Chemical	**formula**	**COD (mg/L)**
Glucose	$C_6H_{12}O_6$	1000
Sucrose	$C_{12}\ H_{22}O_{11}$	1000
Sodium Acetate.trihydrated	$CH_3COONa.3H_2O$	1000
Sodium Acetate.trihydrated	$CH_3COONa.3H_2O$	500
Sodium Acetate.trihydrated	$CH_3COONa.3H_2O$	2000
Sodium Acetate.trihydrated	$CH_3COONa.3H_2O$	3000

Table 3.3: Inorganic compounds in wastewater composition

Chemical	**Formula**	**Quantity (mg/L)**
Ammonium Chloride	NH_4CL	191
Potassium Di-Hydrogen Phosphate	KH_2PO_4	87
Manganese chloride	$MnCl_{2..}4H_20$	02
Calcium chloride	$CaCl_2$	10
Ferric Chloride	$FeCl_3$	03
Magnesium sulphate	$MgSO_4.7H_20$	10
Sodium bicarbonate	$NaHCO_3$	To adjust pH

3.3 Operation of MFCs

It is worth to mention that anode chamber of each MFC was sparged with N2 gas at the start of every batch which reduces the chances of electron loss to O2. MFCs were operated in batch mode with hydraulic retention time (HRT) of 96 hours. All the tests were conducted in a 30 ^oC temperature-controlled water bath. Cathode compartment was filled with 100 mM phosphate buffer solution of pH 7 and was continuously aerated with fishery pump (3.5L/min) to supply oxygen.

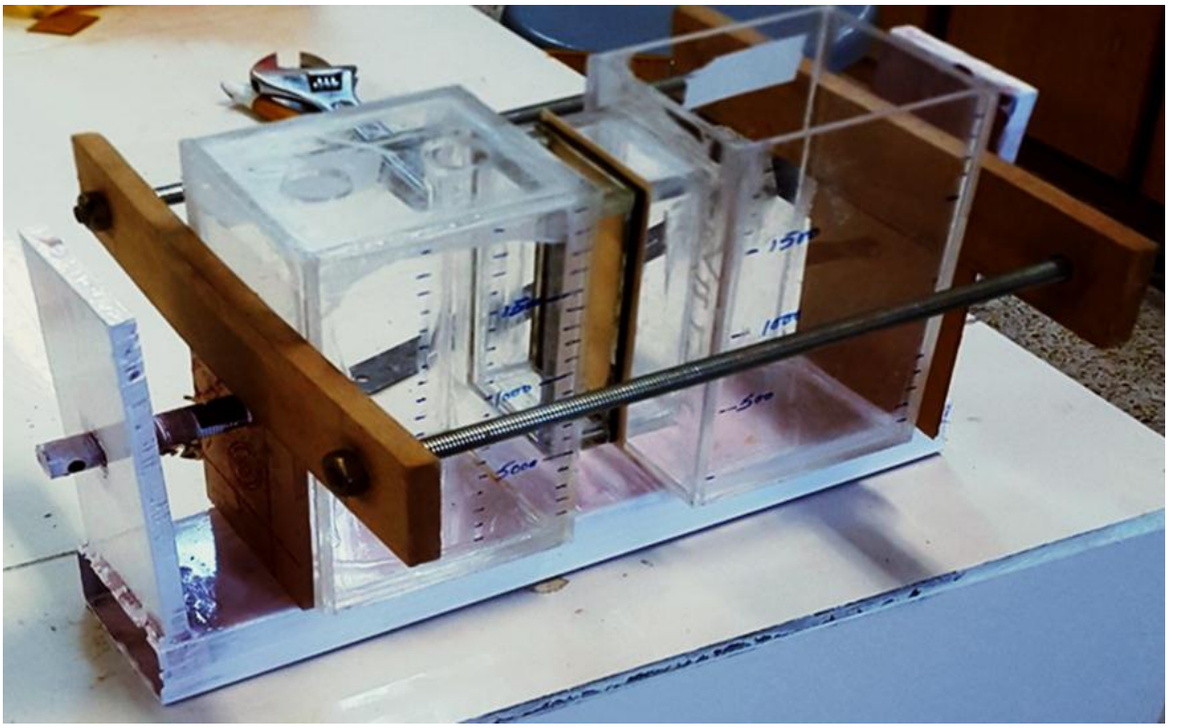


Figure 3.3: Pictorial view of double chamber MFC used in the study

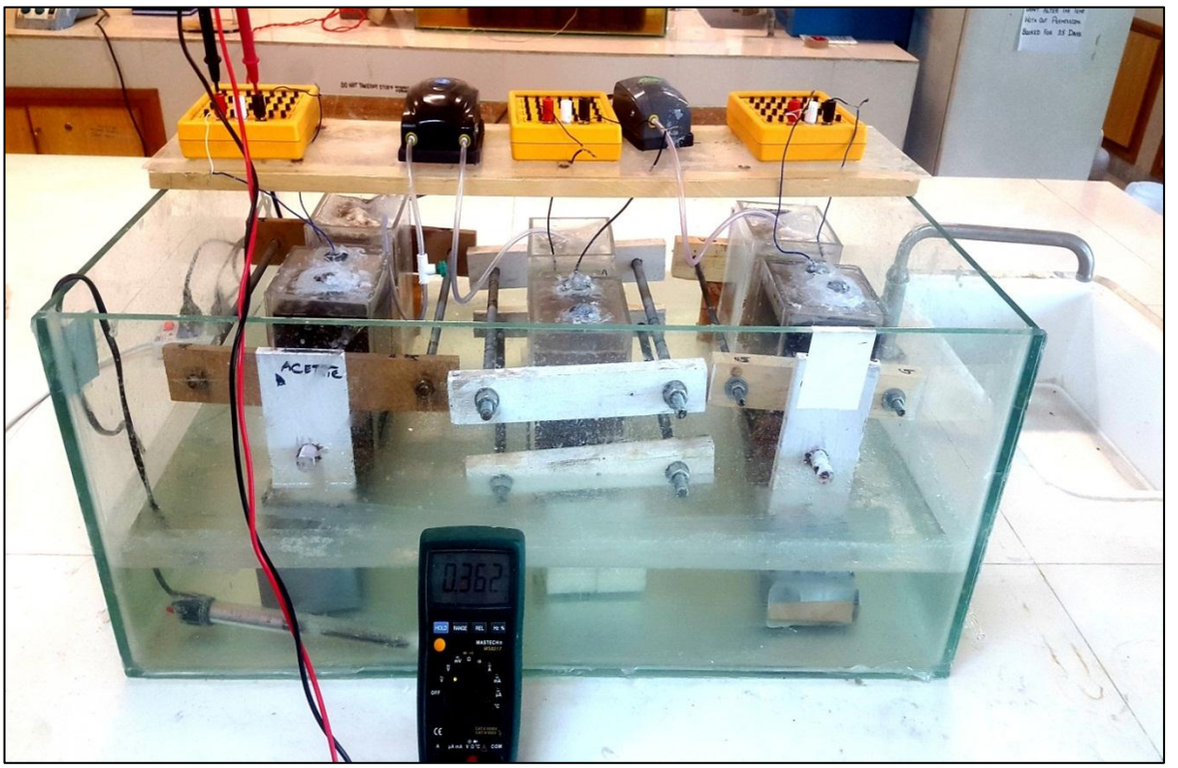


Figure 3.4: Complete assembly of apparatus

3.4 Electrochemical and chemical measurements

A digital multimeter (Mastech-8217) was used to continuously monitor the value of the cell potential (V). Chemical oxygen demand (COD) was determined using closed reflux titrimetric method (APHA., 2005) and pH was measured with Hach multimeter (Model-156). Power generation of the MFCs at different external loads was determined using polarization

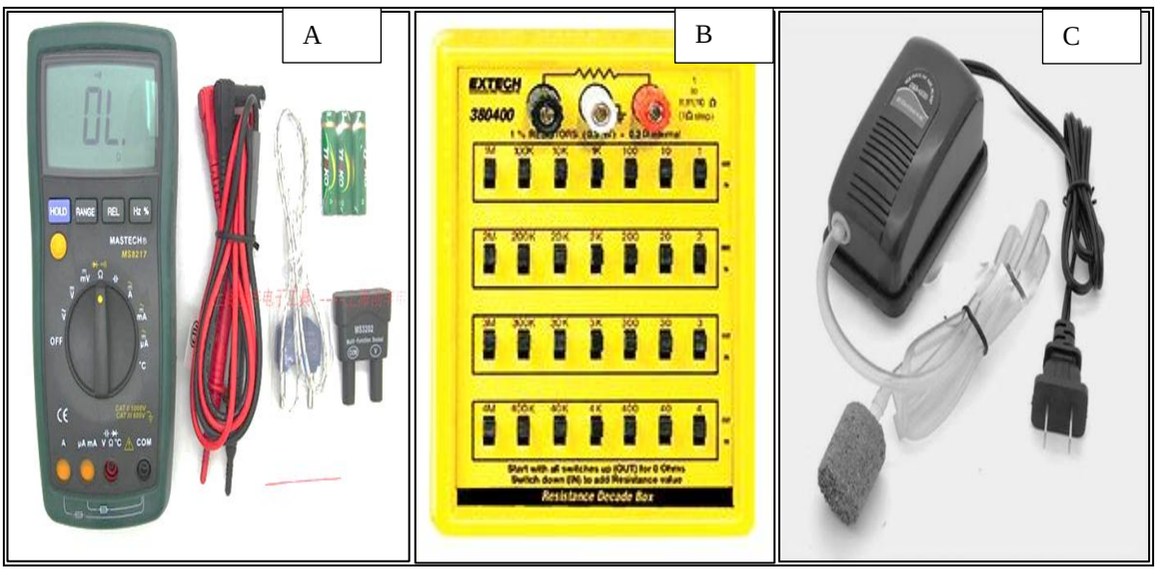


Figure 3.5: Digital Multimeter (A), Decade **resistance box (B), Air pump (C), used in the study**

measurements. Polarization curves were obtained by varying the resistance across the cell from 100 kΩ to 10 Ω in decreasing order using decade resistance box (Extech-380400**).**

3.5 Formulas used for calculations

Current and power output of the cell was determined from Ohm's law (Eqs. 1 and 2).

$$I = V/R_{ext} \quad (1)$$

$$P = V^2/R_{ext} \quad (2)$$

Where V is the voltage drop across a resistor (V), R_{ext} is the external resistor (Ω), I is the current (A) and P is the power output (W).

Current and power densities were obtained by normalizing Eqs. (1) and (2) with anode surface area.

$$C.D = V/(R_{ext}.\ A) \quad (3)$$

$$P.D = V^2/(R_{ext}.A) \quad (4)$$

Where C.D is the current density (A/m^2), P.D is the power density (W/m^2) and A is the area of anode electrode (m^2).

COD removal efficiency was calculated as stated in Eq. (5)

$$\text{COD removal}(\%) = (COD_{in} \_ COD_{out})/COD_{out}\ ) \times 100 \quad (5)$$

Where COD_{in} (mg/L) is the initial concentration and COD_{out} (mg/L) is the concentration after treatment.

Coulombic efficiency (CE) was determined by Eq. (6) as stated in (Montpart et al., 2015)

$$C.E = \int I\,(t)\,dt/(F.b.V.\Delta S) \quad (6)$$

Where t is time (s), F is Faraday's constant (96,485C/mol-e-), b is the number of electrons produced per mol of oxygen (4 mol-e-), V the liquid volume (L) and ΔS is the substrate consumption in terms of COD (mol O_2/L).

Internal resistance is calculated fronm the slope of polarization curve, (Eqn 7).

$$R_{int} = \Delta V/\Delta I \quad (7)$$

Where Δ Vis change in potential, Δ I is change in current and R_{int} is the internal resistance.

Chapter 4

RESULTS AND DISCUSSION

This study was conducted in two phases. The first phase aimed at finding out the effect of different types of substrate whereas the second phase consists of evaluating the effect of different concentrations of substrate on the performance of DCMFC. In order to evade problems in data comparison, all the three MFCs were operated under the same conditions for each phase.

4.1 Effect of different substrates on the performance of DCMFC

To find the effect of different substrates on the performance of DCMFC, three types of substrate that are glucose, acetate and sucrose were used. Initially all MFCs were operated under open circuit condition to assess their performance when no load is applied. After that, MFCs were operated for different concentrations under close circuit condition at fixed load of 1000 Ω.

4.1.1 Open circuit voltage (OCV) from different substrates

During the first phase of the operation, the reactors were operated at open circuit condition. The OCV was recorded at a time interval of 2 h and the recoded data were averaged for every 24 h. the variations in the OCVs with time represent three distinct phases, as shown in Figure 4.1. The upsurge during the initial stage of the operation indicates the formation of the microbial community. This phase is followed by a fairly steady phase where the microbial growth in system saturates the anode and maximum OCVs were achieved in the three reactors. As we have used already acclimatized sludge, so the first stage lasted for short time and peak of the phase come quite quickly. The maximum OCVs of 710 mV, 721 mV, and 781 mV across anode and cathode were obtained for the reactors with a substrate of glucose, sucrose and acetate, respectively, during the second phase of operation. Conventionally, OCV of MFC systems ranges from 0.5 to 0.8 V (Khanal, 2011). The third phase shows the decline in the performance which is the indication of substantial decrease in nutrients concentration (Sonawane et al., 2017a).

Another very interesting point under open circuit condition is the study of the organic load consumption. Fig. 4.2 shows the amount of COD at the start and end of batch for all three substrates. With the same initial COD of 1000 mg/L, final COD of 350 mg/L, 400 mg/L , and 500 mg/L was achieved which corresponded to the removal efficiencies of 65%, 60%, and 50% for glucose, acetate and sucrose respectively.

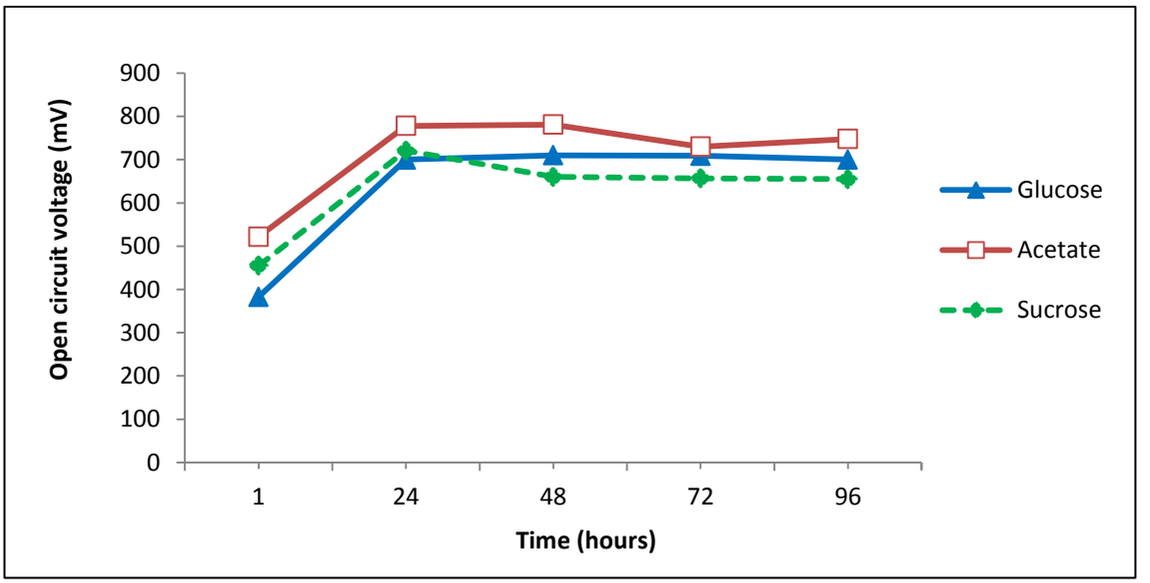


Figure 4.1: Variation in electrode potential with time under open circuit condition

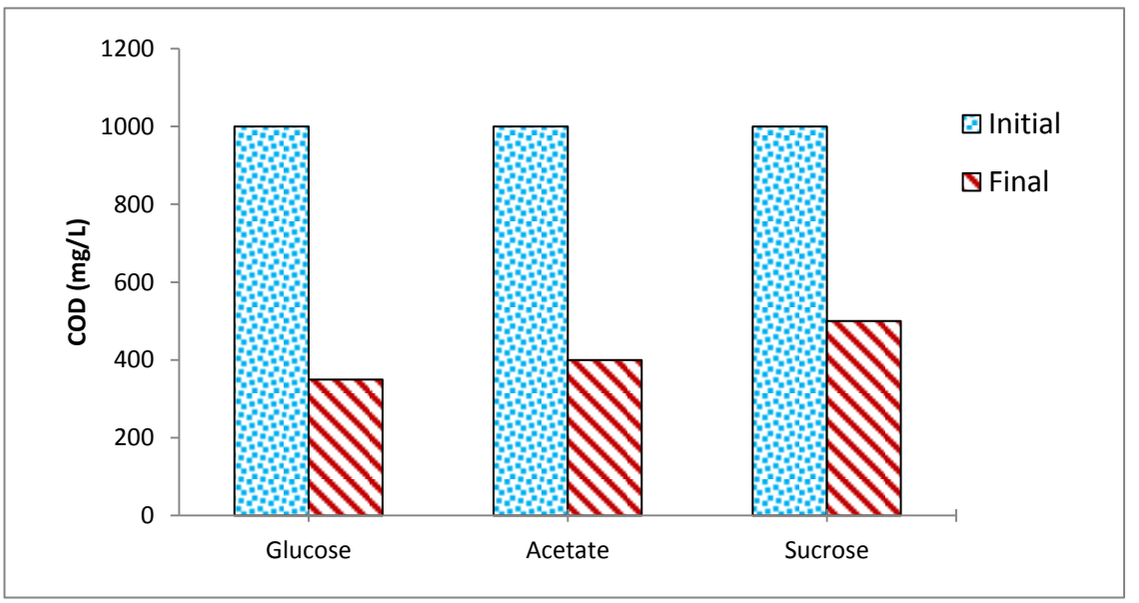


Figure.4.2: COD removal for different substrates under open circuit condition

The COD depletion rate was not very different from each other. Results from this study demonstrate that the MFC can enhance substrate consumption in close circuit as compared to the open-circuit condition, in which the normal anaerobic metabolism prevailed. In this study, the COD removal efficiency increased by 13% on average in close circuit as compared to open circuit for all substrates. Luo et al., (2009) earlier observed that degradation rate of phenol in the MFC increased about 15% with 1000 Ω resistor as compared to the open-circuit. This is easily explained in terms of the coexistence of electrogenic and non electrogenic microorganisms in the mixed biological culture contained in the anodic chamber (Lobato et al., 2013).

4.1.2 Voltage/power generation from different substrates

Following the OCVs measurement, resistor of 1000 Ω was connected between the anode and cathode to close the circuit and the voltage was recorded at a time interval of two hours. The recorded data was averaged for every 12 h unlike open circuit where it was recorded for 24 h. The system developed here was able to continuously generate electricity from the organic matter in the wastewater while accomplishing wastewater treatment. A similar trend to that of OCVs was observed, where maximum voltage was achieved after initial formation of microbial community and was followed by relatively steady state.

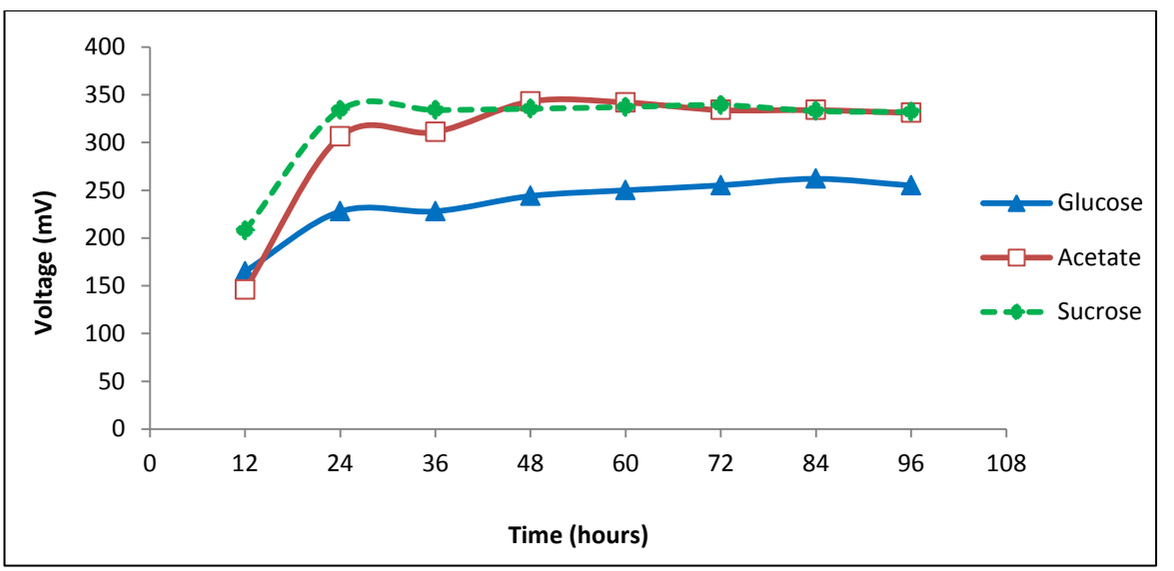


Figure 4.3: Variation in voltage generated with time for different substrates under close circuit

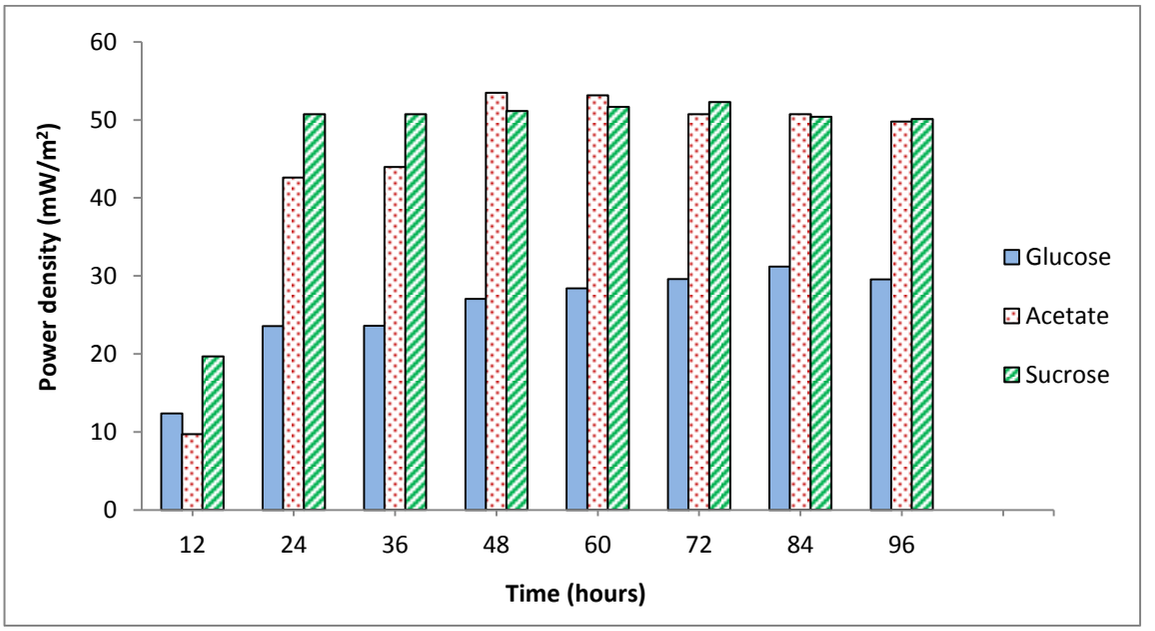


Figure 4.4: Power density vs. time for different substrates under close circuit

Maximum voltage of 262 mV, 343 mV and 339 mV (Figure 4.3) corresponding to maximum power densities of 31 mW/m^2, 53.4 mW/m^2 and 52.3 mW/m^2 (Figure 4.4) were achieved for glucose, acetate and sucrose respectively. Regardless of the generally similar trends, Figure 4.3 and 4.4 shows that patterns of voltage and power generation with time was distinctly different for fermentable and non-fermentable substrates. In the acetate-fed MFC, voltage and power rapidly reached a maximum value between 36 to 48 hours and then start to decline. In the glucose and sucrose-fed MFCs, voltage and power reached to maximum value rather slowly and lower maximum values are achieved (Lee et al., 2008).

4.1.3 COD removal and coulombic efficiency (CE) for different substrates

The COD removal and CE are two important parameters used in the evaluation of MFC performance. COD test is used to determine the availability of converting fuel (substrate) in the MFC, either into electricity generation, or through competitive reactions with other electron acceptors (e.g., oxygen, sulfate and nitrate) or growth of biomass. The CE was calculated based on the total substrate utilization into current. In this study, 60–80% of COD was removed for all substrates, however CE was only in the range of 0.7–1.3% (Figure 4.5) indicating that significant amount of electrons were lost.

The CE is not directly related to power density due to the fact that it is not a kinetic factor. Thus, due to space competition in the anode biofilm, low density of ARB could be the reason associated with low CE which may lead to low power density (Ismail & Habeeb, 2017). Also, COD removal is inversely proportional with CE. Previous studies have shown that lower coulombic efficiency with higher COD removal was potentially caused by non-electrogenic bacteria in the solution which utilizes the electron in other metabolic process. (Kim et al., 2008; Liu et al., 2005a; Strycharz-Glaven et al., 2011). Many factors could attribute to the loss of electron, including the substrate utilization for bacterial growth, fermentation, methanogenesis and transfer of electron from substrate to other electron acceptors, such as oxygen, sulfate and nitrate in solution (Prestigiacomo et al., 2016).

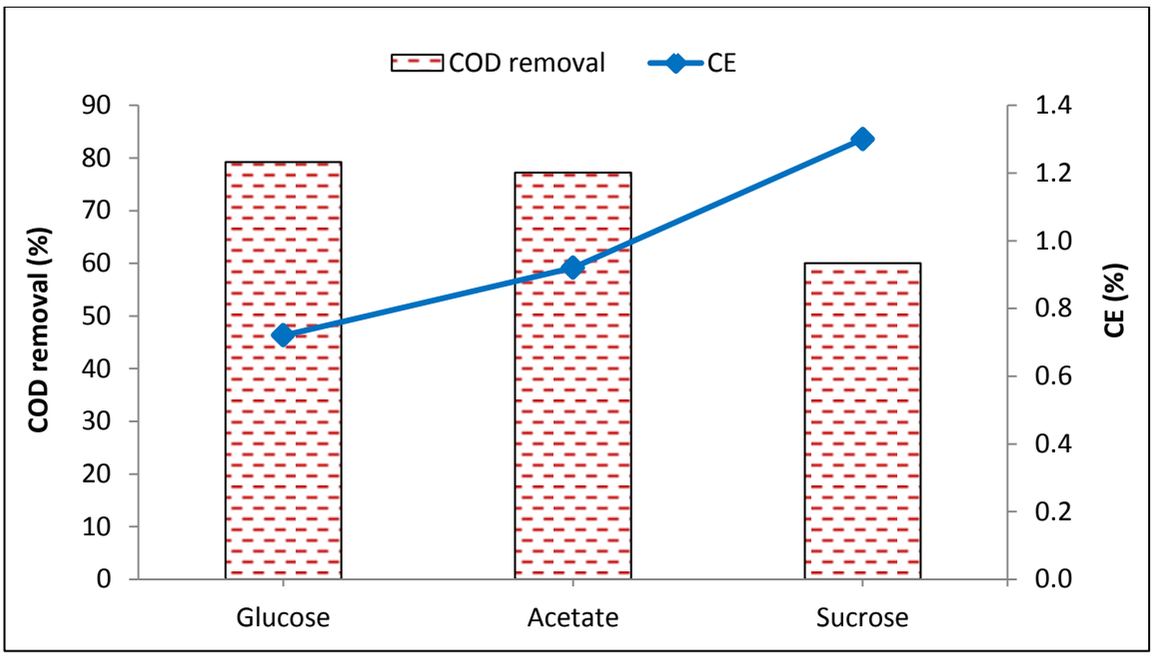


Figure 4.5: COD removal and coulombic efficiency for all substrates

4.1.4 Polarization curve for different substrates

Polarization curve was plotted as a factor of current or current density against electrode potential of MFC. The yielding data is obtained by varying the external load from 10 to 100,000 Ω in decreasing order after a steady state of operation as shown in Figure 4.6 which shows polarization curve as a factor of current density against potential at different resistance. Figure 4.6 clearly shows that potential decreases and current density increases

with increase in external resistance. Maximum current density of 378 mA/m^2, 863 mA/m^2 and 591 mA/m^2 were achieved for glucose, acetate and sucrose based MFCs, respectively. There was a sudden drop of cell potential at lower external resistance and relatively higher current in all polarization tests. Internal resistance was estimated from the slope of the curve (Logan et al., 2006), and it was observed to be 705 Ω, 472 Ω and 280 Ω for glucose, sucrose and acetate based MFCs, respectively. Polarization curve is usually divided into three regions which gives an idea about the type of loses in MFC. In the first region, there is an initial steep decrease of voltage due to the activation losses. Voltage then drops more slowly and fairly linear with current which is the indication of ohmic loses. In the third region, rapid fall of voltage at higher current occur due to the concentration losses (Rismani-Yazdi et al., 2008). In this study, the internal resistance is mainly due to the ohmic losses as the linear portion of voltage and current is dominant. The main reason for ohmic losses is possibly the uncoated electrode (Zhou et al., 2011).

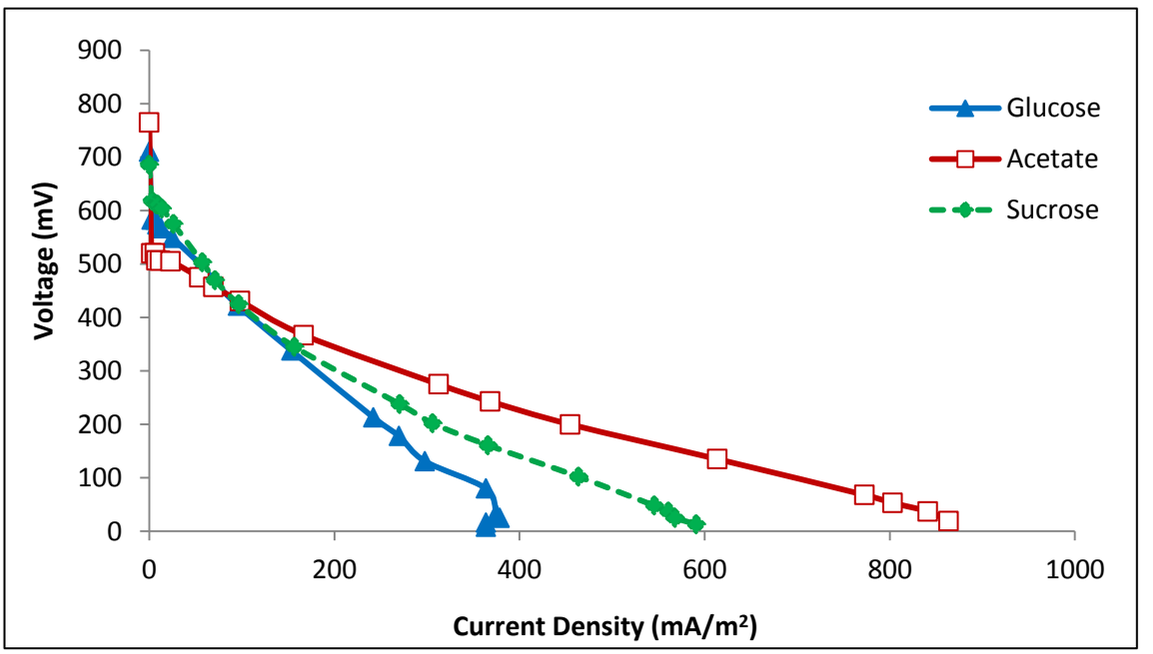


Figure 4.6: Steady-state polarization curve for all substrates

4.1.5 Power curve for different substrates

Power curve helps in finding the maximum achievable power in a microbial fuel cell. It is calculated from polarization data and plotted as a function of power density against current

density. Figure 4.7 shows power curve for all substrates, obtained during steady state. Figure shows that maximum power densities of 91, 64, and 51 mW/m^2 were achieved for acetate, sucrose and glucose, respectively. A higher power density in an acetate-fed MFC than a glucose-fed MFC was also found in three previous studies (Lee et al., 2008; Min & Logan, 2004; Rabaey & Verstraete, 2005), which reinforces the fact that non-fermentable compounds, especially acetate, are more efficient for power generation in MFCs. Moreover, greater the internal resistance, lower will be the power density because a substantial amount of electrons will be lost to overcome the internal resistance (Khater et al., 2015). A symmetrical semi-cycle power curve is obtained typically for a high internal resistance MFC which is limited by ohmic resistance rather than limited by mass transfer. In the case of a symmetrical semi-cycle power curve, the maximum power point will occur at a point where the $R_{int} = R_{ext}$ (Cheng et al., 2006).

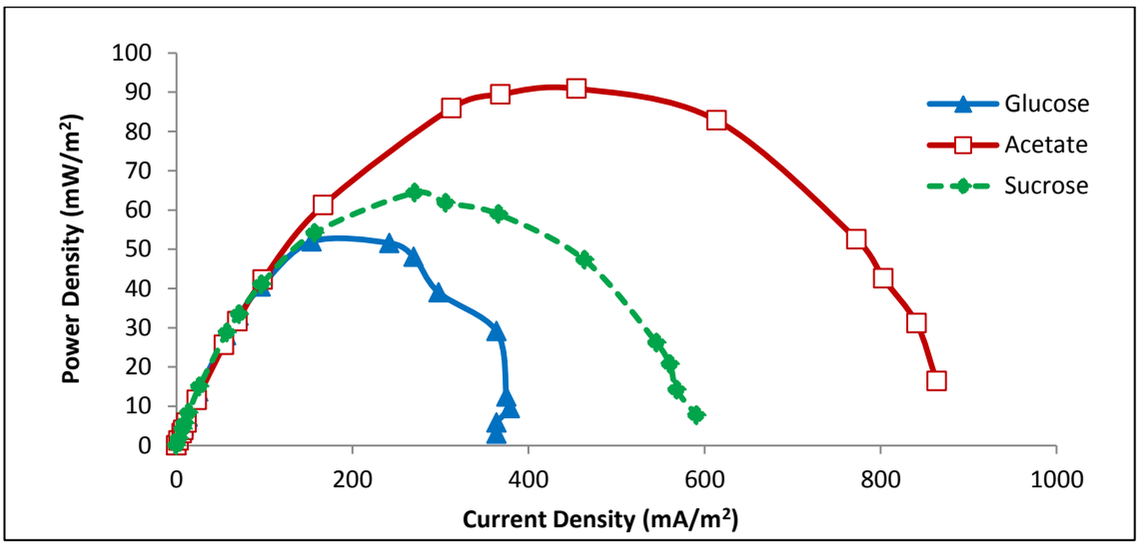


Figure 4.7: Steady-state power curve for all substrates

4.2 Effect of different concentration on performance of DCMFC

Following the different types of substrate, performance of DCMFC was evaluated for different concentrations of one substrate which is best among the three substrates, used in first phase. Acetate was selected based on the performance and was used with three different concentrations of 500 mg/L, 2000 mg/L and 3000 mg/L to assess the performance of DCMFC over different concentrations.

4.2.1 Voltage/power generation at different concentrations of acetate

Figure 4.8 (a) and (b) shows the voltage and power density generation for three different concentrations of acetate. The system was operated under the same close conditions of 1000 ohms. As shown in Figure, variation in voltage and power density is dependent on the concentration of acetate in MFC. The maximum voltages of 273 mV, 378mV and 435mV were achieved for MFCs operated at concentrations of 500, 2000 and 3000 mg/L of COD, respectively. The maximum power density of 34, 65 and 86 mW/m^2 were achieved for reactors operated at 500, 2000 and 3000 mg/L of COD, respectively.

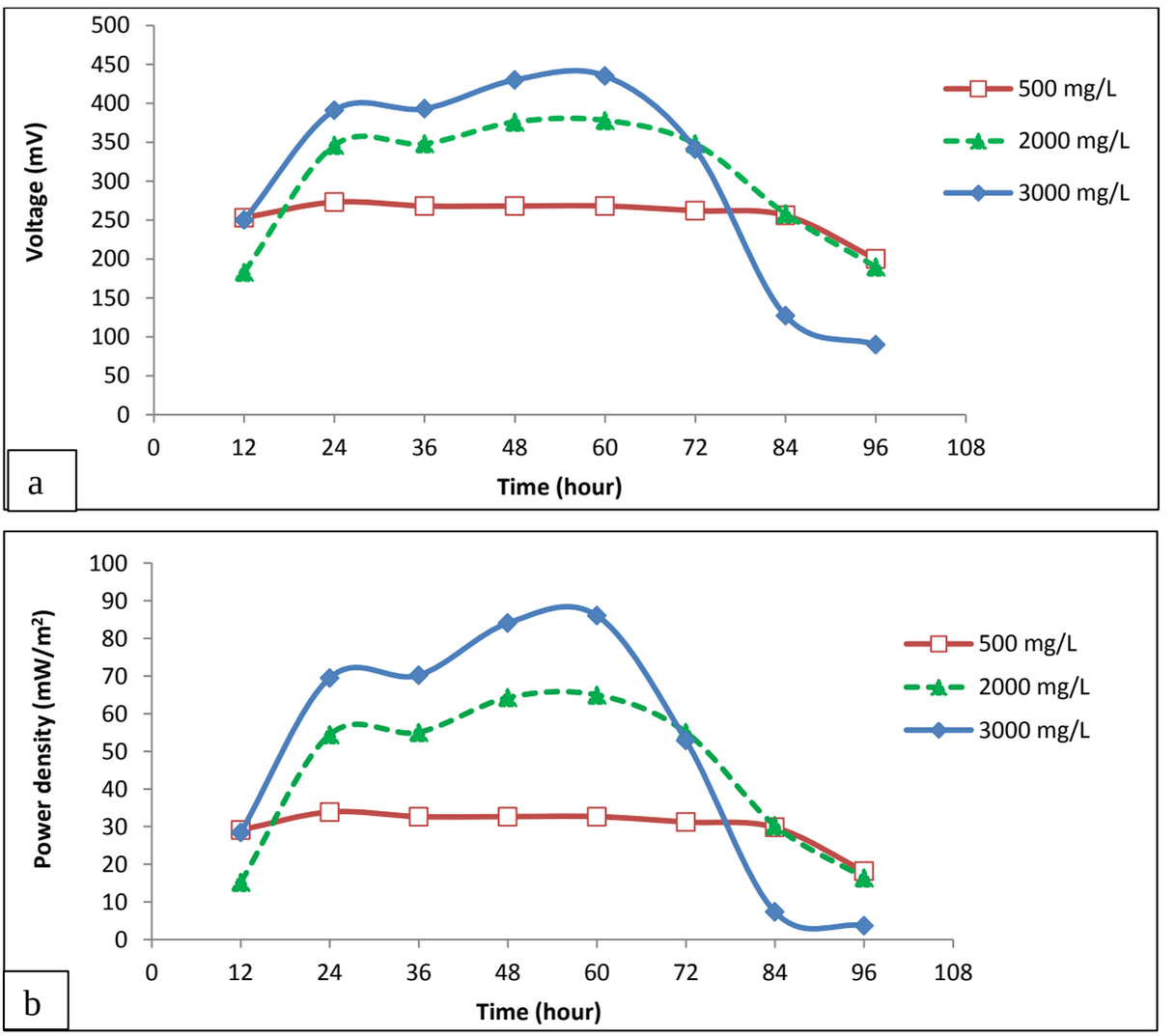


Figure 4.8(a). Variation in voltage generated with time for different concentrations of acetate (b) Variation in power density versus time for different concentrations of acetate.

The reactor operated at COD of 500 mg/L reached maximum output rather quickly that is within first 36 hours before it started to decline. While reactors with COD of 2000 mg/L and 3000 mg/L reached to its highest value during 50 to 60 hours on an average but to their maximum value, after which their output started to decrease.

Although it does give useful information about the pattern of voltage and power density, COD consumption and up to some extent the maximum value but the actual maximum value can be found from polarization and power curve with variable resistors. Results so far show that substrate concentrations have a substantial effect on maximum voltage and power generation. Results from this and previous studies show that electricity generation increases with substrate concentration (Asensio et al., 2016; Khater et al., 2015)

4.2.2 COD removal efficiency at different concentrations of acetate

Figure 4.9 shows the COD reduction profile of three concentrations of acetate with time. Results show that reactor fed with COD of 500, 2000 and 3000 mg/L ended up with 95, 420 and 678 mg/L with COD removal efficiency of 81, 79 and 77%, respectively. COD consumption rate of 101 mg/L/d for 500 mg/L COD, 395 mg/L/d for 2000 mg/L COD and 580 mg/L/d for 3000 mg/L COD during the four day batch.

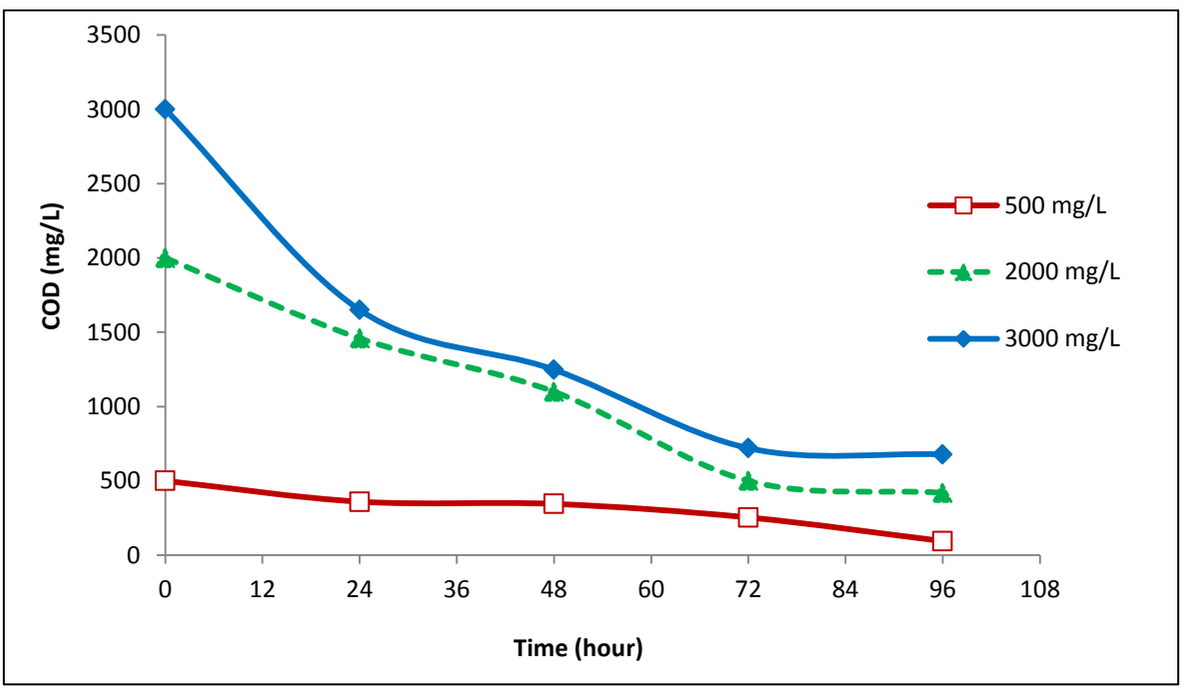


Figure 4.9: Variation in COD with time for three different concentrations

The results demonstrates that higher the concentration of substrate, the higher would be observed degradation rate, with a clear behavior of first order kinetic, which pointed out the linear dependence of the substrates consumption with the influent COD concentration (Asensio et al., 2016). It is important to mention that COD changes tell about the globalized metabolism of the bacteria that are present in the anode compartment but these do not inform about the behavior of electrogenic microorganisms, because both non-electrogenic and electrogenic microorganisms are expected to consume COD (Strycharz-Glaven et al., 2011).

4.2.3 Polarization curve for different concentrations of acetate

Polarization curve was obtained for all three concentrations in the same way as previously used for different types of substrates. The Figure 4.10 shows polarization curve for three different concentrations of substrates that is 500, 2000 and 3000 mg/L of COD. Maximum current density was found to be dependent of substrates concentration. Maximum current density of 284 mA/m^2, 659 mA/m^2 and 795 mA/m^2 were achieved for MFCs operated at concentration of 500, 2000 and 3000 mg/L of COD respectively. There was a sudden drop of cell potential at lower external resistance and relatively higher current in all polarization tests. Same three phases of voltage loss can be observed in the curves. In the first region, there is an initial steep decrease of voltage due to the activation losses. Voltage then dropped more slowly and fairly linear with current which was the indication of ohmic loses. In the third region, rapid fall of voltage at higher current occured due to the concentration losses (Logan et al., 2006).

Internal resistance, estimated from the slope of the curve was found to be 871 Ω for MFC with COD of 500 mg/L, 370 Ω for MFC with COD of 2000 mg/L and 301 Ω for MFC with COD of 3000 mg/L. It can be concluded from the results that internal resistance decreases with increase in COD. Khater et al. have similar type of observation for glucose used as a substrates in a single chamber MFC (Khater et al., 2015). Some other researcher have also reported that an increase in COD led to decrease in an internal resistance and increase in current production but high substrate concentrations were found to inhibit power generation in MFC (Khater et al., 2017; Liu et al., 2009; McLean et al., 2010).

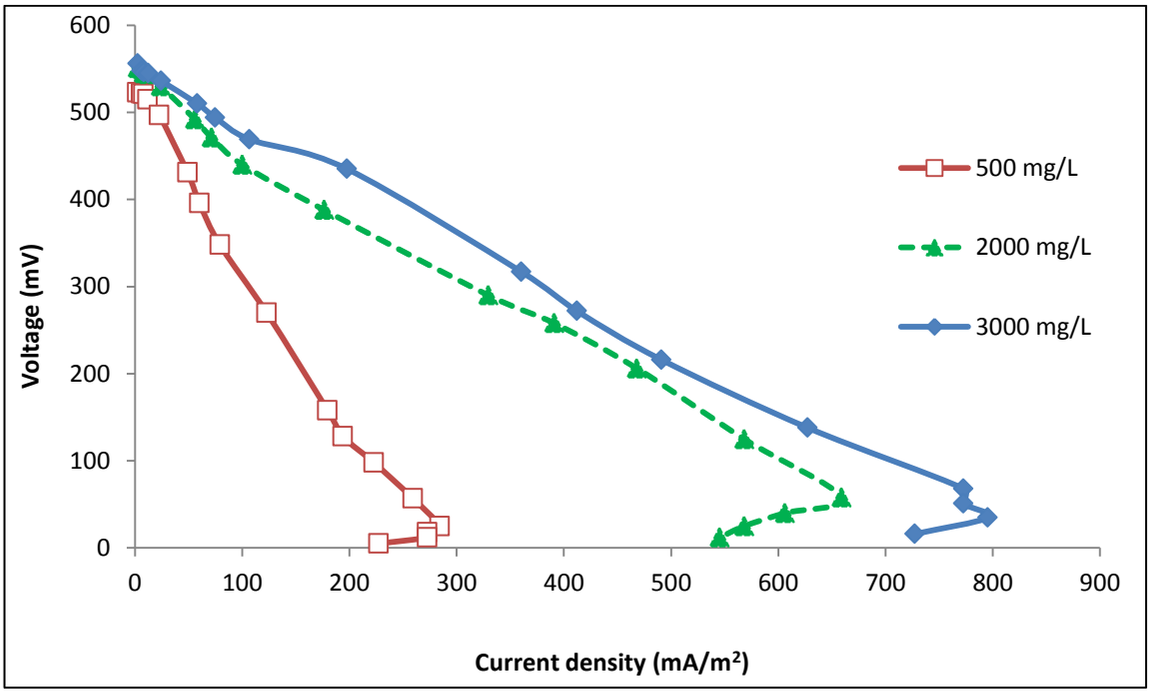


Figure 4.10: Steady-state polarization curve for different concentrations

4.2.4 Power curve for different concentrations of acetate

Figure 4.11 shows power curve obtained in steady state for all three concentrations of acetate. Figure shows that maximum power densities of 33, 101 and 114 mW/m^2 were achieved for MFCs operated at concentration of 500, 2000 and 3000 mg/L of COD. Results from this and previous studies show that maximum power density increases with substrate concentration. It also reinforce the fact that greater the internal resistance, lower will be the power density because a substantial amount of electrons will be lost to overcome the internal resistance (Nam et al., 2010). A symmetrical semi-cycle power curve is obtained typically for a high internal resistance MFC which is limited by ohmic resistance rather than limited by mass transfer. In the case of a symmetrical semi-cycle, the maximum power point will occur at a point where the $R_{int} = R_{ext}$ (Fan et al., 2008). In our case maximum power density point is not exactly equal but nearest to the external resistance. For MFC with COD of 500 mg/L, maximum power density of 33 mW/m^2 was achieved at external resistance of 1000 ohm which was corresponded to the internal resistance of 871 ohm. For MFC with COD of 2000 mg/L, maximum power density of 101 mW/m^2 was achieved at external resistance of 300 ohm which is corresponding to the internal resistance of 370 ohm. In the same way, for

COD of 500 mg/L, maximum power density of 114 mW/m^2 was achieved at external resistance of 400 ohm which was corresponding to the internal resistance of 301 ohm.

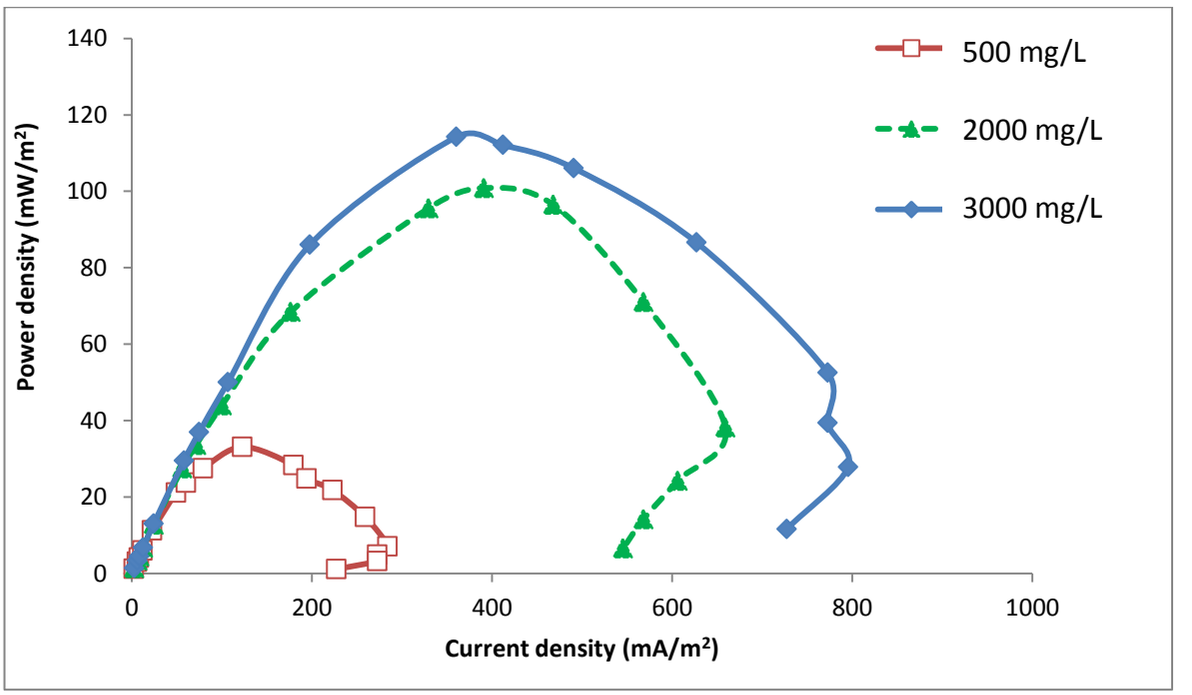


Figure 4.11: Steady-state power curve for different concentration

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

To evaluate the effect of different types of substrates and concentrations on the performance of microbial fuel cell, three different substrates were chosen: glucose, acetate and sucrose and three different concentrations of 500, 2000 and 3000 mg/L of COD for one selected substrate (acetate) were studied. The system developed here was able to continuously generate electricity from the organic matter in the wastewater while accomplishing wastewater treatment. The following conclusions can be drawn from this work

- There is a clear effect of the type of organic substrate on the performance of MFC. For the same organic load, acetate is the most efficient substrate in comparison to (single sugar) glucose and (double sugar) sucrose. Regardless of the generally similar trends, the patterns of voltage and power generation with time were distinctly different for fermentable and non-fermentable substrates. In the acetate-fed MFC, voltage and power reached to maximum point sooner and higher than glucose and sucrose-fed MFCs. COD removal of 60-80% with CE of 0.7-1.3 % indicate that electric current was the least significant electron sink in all MFCs. The polarization curve shows that ohmic losses were dominant.
- The performance of MFC was found to be affected by the concentration of substrates. For a system fed with different concentrations of acetate solution, electricity production increased with COD concentration. The MFC with 2000 mg/L of COD turned out to be the best concentration among all three, based on maximum power density, COD removal and internal resistance. COD removal efficiency of 77-81 % was achieved for different concentrations. Internal resistance decreased with increase in COD concentration.

5.2 Recommendations

Following recommendations are noteworthy for further study.

1. Ohmic losses were the main reason of high internal resistance which is mainly due to type of electrode, CEM and interconnections used. Using electrode with high surface

area or coating a catalyst will be able to reduce the internal resistance. For this purpose carbon brushes or coating of platinum catalyst on the electrodes are the best options.

2. Work on MFC design is also needed so that it can be used for batch mode as well as continuous flow mode.
3. CE can be improved by reducing the oxygen access from cathode chamber. Thus membrane with better proton permeability but less oxygen diffusion properties should be used.

REFERENCES

- Aelterman, P., Rabaey, K., Pham, H.T., Boon, N., Verstraete, W. 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environmental Science & Technology, 40(10), 3388-3394.
- Aelterman, P., Versichele, M., Marzorati, M., Boon, N., Verstraete, W. 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresource Technology, 99(18), 8895-8902.
- Ahn, Y., Logan, B.E. 2010. Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresource Technology, 101(2), 469-475.
- Ali, A.E.-H., Gomaa, O.M., Fathey, R., Kareem, H.A.E., Zaid, M.A. 2015. Optimization of double chamber microbial fuel cell for domestic wastewater treatment and electricity production. Journal of Fuel Chemistry and Technology, 43(9), 1092-1099.
- Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A., Domíguez-Espinosa, R. 2004. Production of bioenergy and biochemicals from industrial and agricultural wastewater. TRENDS in Biotechnology, 22(9), 477-485.
- APHA, AWWA, WEF, (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, Washington, DC.
- Asensio, Y., Fernandez-Marchante, C.M., Lobato, J., Canizares, P., Rodrigo, M.A. 2016. Influence of the fuel and dosage on the performance of double-compartment microbial fuel cells. Water Researcch, 99, 16-23.
- Capodaglio, A., Molognoni, D., Dallago, E., Liberale, A., Cella, R., Longoni, P., Pantaleoni, L. 2013. Microbial fuel cells for direct electrical energy recovery from urban wastewaters. The Scientific World Journal, 2013, 1-8.
- Catal, T., Kavanagh, P., O'Flaherty, V., Leech, D. 2011. Generation of electricity in microbial fuel cells at sub-ambient temperatures. Journal of Power Sources, 196(5), 2676-2681.

- Catal, T., Li, K., Bermek, H., Liu, H. 2008. Electricity production from twelve monosaccharides using microbial fuel cells. Journal of Power Sources, 175(1), 196-200.
- Chae, K.J., Choi, M., Ajayi, F.F., Park, W., Chang, I.S., Kim, I.S. 2007. Mass transport through a proton exchange membrane (Nafion) in microbial fuel cells. Energy & Fuels, 22(1), 169-176.
- Cheng, S., Liu, H., Logan, B.E. 2006. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. Environmental Science & Technology, 40(7), 2426-2432.
- Colombo, A., Schievano, A., Trasatti, S.P., Morrone, R., D'Antona, N., Cristiani, P. 2017. Signal trends of microbial fuel cells fed with different food-industry residues. International Journal of Hydrogen Energy, 42(3), 1841-1852.
- Connor, R. (2015). The United Nations world water development report 2015: water for a sustainable world (Vol. 1). UNESCO Publishing. ISBN-9231000713.
- Cusick, R.D., Kiely, P.D., Logan, B.E. 2010. A monetary comparison of energy recovered from microbial fuel cells and microbial electrolysis cells fed winery or domestic wastewaters. International Journal of hydrogen energy, 35(17), 8855-8861.
- Davis, F., Higson, S.P. 2007. Biofuel cells—recent advances and applications. Biosensors and Bioelectronics, 22(7), 1224-1235.
- Fan, Y., Sharbrough, E., Liu, H. 2008. Quantification of the internal resistance distribution of microbial fuel cells. Environmental science & technology, 42(21), 8101-8107.
- Feng, Y., He, W., Liu, J., Wang, X., Qu, Y., Ren, N. 2014. A horizontal plug flow and stackable pilot microbial fuel cell for municipal wastewater treatment. Bioresource technology, 156, 132-138.
- Fogg, A., Gadhamshetty, V., Franco, D., Wilder, J., Agapi, S., Komisar, S. 2015. Can a microbial fuel cell resist the oxidation of Tomato pomace? Journal of Power Sources, 279, 781-790.
- Gajda, I., Greenman, J., Melhuish, C., Ieropoulos, I. 2013. Photosynthetic cathodes for microbial fuel cells. international journal of hydrogen energy, 38(26), 11559-11564.

- Ghoreyshi, A., Jafary, T., Najafpour, G., Haghparast, F. 2011. Effect of type and concentration of substrate on power generation in a dual chambered microbial fuel cell. World Renewable Energy Congress-Sweden; 8-13 May; 2011; Linköping; Sweden. Linköping University Electronic Press. pp. 1174-1181.
- Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S. 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences, 103(30), 11358-11363.
- Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R., Meybeck, A. 2011. Global food losses and food waste. FAO Rome. 2011, 1-38.
- He, Z., Angenent, L.T. 2006. Application of bacterial biocathodes in microbial fuel cells. Electroanalysis, 18(19-20), 2009-2015.
- He, Z., Wagner, N., Minteer, S.D., Angenent, L.T. 2006. An upflow microbial fuel cell with an interior cathode: assessment of the internal resistance by impedance spectroscopy. Environmental Science & Technology, 40(17), 5212-5217.
- Herrero-Hernández, E., Smith, T., Akid, R. 2013. Electricity generation from wastewaters with starch as carbon source using a mediatorless microbial fuel cell. Biosensors and Bioelectronics, 39(1), 194-198.
- Huang, L., Cheng, S., Rezaei, F., Logan, B.E. 2009. Reducing organic loads in wastewater effluents from paper recycling plants using microbial fuel cells. Environmental Technology, 30(5), 499-504.
- Ismail, Z.Z., Habeeb, A.A. 2017. Experimental and modeling study of simultaneous power generation and pharmaceutical wastewater treatment in microbial fuel cell based on mobilized biofilm bearers. Renewable Energy, 101, 1256-1265.
- Jiang, H.-m., Luo, S.-j., Shi, X.-s., Dai, M., Guo, R.-b. 2013. A system combining microbial fuel cell with photobioreactor for continuous domestic wastewater treatment and bioelectricity generation. Journal of Central South University, 20(2), 488-494.
- Khanal, S.K. 2011. Anaerobic biotechnology for bioenergy production: principles and applications. John Wiley & Sons. New York, USA. ISBN-1119949424.

- Khater, D., El-khatib, K., Hazaa, M., Hassan, R. 2015. Electricity generation using Glucose as substrate in microbial fuel cell. Journal of Basic and Environmental Sciences, 2, 84-98.
- Khater, D.Z., El-Khatib, K.M., Hassan, H.M. 2017. Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation. Journal of Genetic Engineering and Biotechnology, 15(1), 127-137.
- Kiely, P.D., Regan, J.M., Logan, B.E. 2011. The electric picnic: synergistic requirements for exoelectrogenic microbial communities. Current Opinion in Biotechnology, 22(3), 378-385.
- Kim, B.H., Kim, H.J., Hyun, M.S., Park, D.H. 1999. Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology, 9, 127-131.
- Kim, I.-S., Chae, K.-J., Choi, M.-J., Verstraete, W. 2008. Microbial fuel cells: recent advances, bacterial communities and application beyond electricity generation. Environmental Engineering Research, 13(2), 51-65.
- Kim, J.R., Cheng, S., Oh, S.-E., Logan, B.E. 2007. Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environmental Science & Technology, 41(3), 1004-1009.
- Kunik, E. 2015. A technical and cultural feasibility assessment of household microbial fuel cells for use in Hanan'g District, Tanzania, Michigan Technological University. Houghton, Michigan, United States.
- Lalaurette, E., Thammannagowda, S., Mohagheghi, A., Maness, P.-C., Logan, B.E. 2009. Hydrogen production from cellulose in a two-stage process combining fermentation and electrohydrogenesis. International Journal of Hydrogen Energy, 34(15), 6201-6210.
- Lee, H.S., Parameswaran, P., Kato-Marcus, A., Torres, C.I., Rittmann, B.E. 2008. Evaluation of energy-conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. Water Research, 42(6-7), 1501-10.
- Lee, K.-y., Ryu, W.-s., Cho, S.-i., Lim, K.-h. 2015. Comparative study on power generation of dual-cathode microbial fuel cell according to polarization methods. Water Research, 84, 43-48.

- Lewis, K. 1966. Symposium on bioelectrochemistry of microorganisms. IV. Biochemical fuel cells. Bacteriological Reviews, 30(1), 101.
- Liu, B., Li, B. 2014. Single chamber microbial fuel cells (SCMFCs) treating wastewater containing methanol. International Journal of Hydrogen Energy, 39(5), 2340-2344.
- Liu, H., Cheng, S., Logan, B.E. 2005a. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environmental Science & Technology, 39(2), 658-662.
- Liu, H., Grot, S., Logan, B.E. 2005b. Electrochemically assisted microbial production of hydrogen from acetate. Environmental Science & Technology, 39(11), 4317-4320.
- Liu, H., Logan, B.E. 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental Science & Technology, 38(14), 4040-4046.
- Liu, H., Ramnarayanan, R., Logan, B.E. 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environmental Science & Technology, 38(7), 2281-2285.
- Liu, Z., Liu, J., Zhang, S., Su, Z. 2009. Study of operational performance and electrical response on mediator-less microbial fuel cells fed with carbon-and protein-rich substrates. Biochemical Engineering Journal, 45(3), 185-191.
- Lobato, J., del Campo, A.G., Fernández, F.J., Cañizares, P., Rodrigo, M.A. 2013. Lagooning microbial fuel cells: a first approach by coupling electricity-producing microorganisms and algae. Applied Energy, 110, 220-226.
- Logan, B.E. 2008. Microbial Fuel Cells. John Wiley & Sons.
- Logan, B.E., Call, D., Cheng, S., Hamelers, H.V., Sleutels, T.H., Jeremiasse, A.W., Rozendal, R.A. 2008. Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environmental Science & Technology, 42(23), 8630-8640.
- Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. 2006. Microbial fuel cells: methodology and technology. Environmental Science & Technology, 40(17), 5181-5192.

- Logan, B.E., Rabaey, K. 2012. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. Science, 337(6095), 686-690.
- Lovley, D.R. 2006. Bug juice: harvesting electricity with microorganisms. Nature reviews. Microbiology, 4(7), 497.
- Luo, H., Liu, G., Zhang, R., Jin, S. 2009. Phenol degradation in microbial fuel cells. Chemical Engineering Journal, 147(2-3), 259-264.
- Magnuson, T.S., Isoyama, N., Hodges-Myerson, A.L., Davidson, G., Maroney, M.J., Geesey, G.G., Lovley, D.R. 2001. Isolation, characterization and gene sequence analysis of a membrane-associated 89 kDa Fe (III) reducing cytochrome c from Geobacter sulfurreducens. Biochemical Journal, 359(1), 147-152.
- Mansoorian, H.J., Mahvi, A.H., Jafari, A.J., Amin, M.M., Rajabizadeh, A., Khanjani, N. 2013. Bioelectricity generation using two chamber microbial fuel cell treating wastewater from food processing. Enzyme and Microbial Technology, 52(6), 352-357.
- McCarty, P.L., Bae, J., Kim, J. 2011. Domestic wastewater treatment as a net energy producer–can this be achieved?. Environmental Science and Technology, 45 (17), 7100–7106.
- McLean, J.S., Wanger, G., Gorby, Y.A., Wainstein, M., McQuaid, J., Ishii, S.i., Bretschger, O., Beyenal, H., Nealson, K.H. 2010. Quantification of electron transfer rates to a solid phase electron acceptor through the stages of biofilm formation from single cells to multicellular communities. Environmental Science & Technology, 44(7), 2721-2727.
- Min, B., Logan, B.E. 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environmental Science & Technology, 38(21), 5809-5814.
- Min, B., Román, Ó.B., Angelidaki, I. 2008. Importance of temperature and anodic medium composition on microbial fuel cell (MFC) performance. Biotechnology Letters, 30(7), 1213-1218.

- Mohan, S.V., Mohanakrishna, G., Sarma, P. 2010. Composite vegetable waste as renewable resource for bioelectricity generation through non-catalyzed open-air cathode microbial fuel cell. Bioresource Technology, 101(3), 970-976.
- Mohan, S.V., Raghavulu, S.V., Sarma, P. 2008. Biochemical evaluation of bioelectricity production process from anaerobic wastewater treatment in a single chambered microbial fuel cell (MFC) employing glass wool membrane. Biosensors and Bioelectronics, 23(9), 1326-1332.
- Montpart, N., Rago, L., Baeza, J.A., Guisasola, A. 2015. Hydrogen production in single chamber microbial electrolysis cells with different complex substrates. Water Resarch, 68, 601-15.
- Nam, J.-Y., Kim, H.-W., Lim, K.-H., Shin, H.-S. 2010. Effects of organic loading rates on the continuous electricity generation from fermented wastewater using a single-chamber microbial fuel cell. Bioresource technology, 101(1), S33-S37.
- Nevin, K.P., Richter, H., Covalla, S., Johnson, J., Woodard, T., Orloff, A., Jia, H., Zhang, M., Lovley, D. 2008. Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells. Environmental Microbiology, 10(10), 2505-2514.
- NRAW (2016). National Research Agenda on Water 2016-25. Pakistan Council of Research in Water Resources (PCRWR)/US-Pakistan Center for Advance Studies in Water (USPCAS-W) Joint Report, PP.43, Islamabad, Pakistan.
- Oh, S.-E., Logan, B.E. 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. Applied Microbiology and Biotechnology, 70(2), 162-169.
- Pandey, P., Shinde, V.N., Deopurkar, R.L., Kale, S.P., Patil, S.A., Pant, D. 2016. Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery. Applied Energy, 168, 706-723.
- Park, D.H., Zeikus, J.G. 2003. Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnology and Bioengineering, 81(3), 348-355.

- Park, J.-D., Ren, Z. 2012. Hysteresis-controller-based energy harvesting scheme for microbial fuel cells with parallel operation capability. IEEE Transactions on Energy Conversion, 27(3), 715-724.
- Petrus, L., Noordermeer, M.A. 2006. Biomass to biofuels, a chemical perspective. Green Chemistry, 8(10), 861-867.
- Piccolino, M. 1998. Animal electricity and the birth of electrophysiology: the legacy of Luigi Galvani. Brain Research Bulletin, 46(5), 381-407.
- Prestigiacomo, C., Fernandez-Marchante, C.M., Fernández-Morales, F.J., Cañizares, P., Scialdone, O., Rodrigo, M.A. 2016. New prototypes for the isolation of the anodic chambers in microbial fuel cells. Fuel, 181, 704-710.
- Putnam, D.F. 1971. Composition and concentrative properties of human urine. NASA-Langley Reserach Center,112, Washington DC, USA.
- Rabaey, K., Clauwaert, P., Aelterman, P., Verstraete, W. 2005. Tubular microbial fuel cells for efficient electricity generation. Environmental Science & Technology, 39(20), 8077-8082.
- Rabaey, K., Verstraete, W. 2005. Microbial fuel cells: novel biotechnology for energy generation. Trends in Biotechnology, 23(6), 291-298.
- Ren, L., Ahn, Y., Logan, B.E. 2014. A two-stage microbial fuel cell and anaerobic fluidized bed membrane bioreactor (MFC-AFMBR) system for effective domestic wastewater treatment. Environmental Science & Technology, 48(7), 4199-4206.
- Rismani-Yazdi, H., Carver, S.M., Christy, A.D., Tuovinen, O.H. 2008. Cathodic limitations in microbial fuel cells: an overview. Journal of Power Sources, 180(2), 683-694
- Rodrigo, M., Canizares, P., Lobato, J., Paz, R., Sáez, C., Linares, J. 2007. Production of electricity from the treatment of urban waste water using a microbial fuel cell. Journal of Power Sources, 169(1), 198-204.
- Rodrigo, M.A., Cañizares, P., García, H., Linares, J.J., Lobato, J. 2009. Study of the acclimation stage and of the effect of the biodegradability on the performance of a microbial fuel cell. Bioresource Technology, 100(20), 4704-4710.

- Rozendal, R.A., Hamelers, H.V., Buisman, C.J. 2006. Effects of membrane cation transport on pH and microbial fuel cell performance. Environmental Science & Technology, 40(17), 5206-5211.
- Rozendal, R.A., Hamelers, H.V., Rabaey, K., Keller, J., Buisman, C.J. 2008. Towards practical implementation of bioelectrochemical wastewater treatment. Trends in Biotechnology, 26(8), 450-459.
- Sangeetha, T., Muthukumar, M. 2011. Catholyte performance as an influencing factor on electricity production in a dual-chambered microbial fuel cell employing food processing wastewater. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 33(16), 1514-1522.
- Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Mariñas, B.J., Mayes, A.M. 2008. Science and technology for water purification in the coming decades. Nature, 452(7185), 301-310.
- Sonawane, J.M., Adeloju, S.B., Ghosh, P.C. 2017a. Landfill leachate: A promising substrate for microbial fuel cells. International Journal of Hydrogen Energy,42 (37), 23794-23798
- Sonawane, J.M., Yadav, A., Ghosh, P.C., Adeloju, S.B. 2017b. Recent advances in the development and utilization of modern anode materials for high performance microbial fuel cells. Biosensors and Bioelectronics, 90, 558-576.
- Strycharz-Glaven, S.M., Snider, R.M., Guiseppi-Elie, A., Tender, L.M. 2011. On the electrical conductivity of microbial nanowires and biofilms. Energy & Environmental Science, 4(11), 4366-4379.
- Sun, G., Thygesen, A., Ale, M.T., Mensah, M., Poulsen, F.W., Meyer, A.S. 2014. The significance of the initiation process parameters and reactor design for maximizing the efficiency of microbial fuel cells. Applied Microbiology and Biotechnology, 98(6), 2415-2427.
- Virdis, B., Rabaey, K., Rozendal, R.A., Yuan, Z., Keller, J. 2010. Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells. Water Research, 44(9), 2970-2980.
- Virdis, B., Rabaey, K., Yuan, Z., Keller, J. 2008. Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Research, 42(12), 3013-3024.

- Wang, H., Park, J.-D., Ren, Z.J. 2015. Practical energy harvesting for microbial fuel cells: a review. Environmental Science & Technology, 49(6), 3267-3277.
- Watson, V.J., Logan, B.E. 2011. Analysis of polarization methods for elimination of power overshoot in microbial fuel cells. Electrochemistry Communications, 13(1), 54-56.
- Yang, Q., Wang, X., Feng, Y., Lee, H., Liu, J., Shi, X., Qu, Y., Ren, N. 2012. Electricity generation using eight amino acids by air–cathode microbial fuel cells. Fuel, 102, 478-482.
- Zhang, F., Ge, Z., Grimaud, J., Hurst, J., He, Z. 2013. Long-term performance of liter-scale microbial fuel cells treating primary effluent installed in a municipal wastewater treatment facility. Environmental Science & Technology, 47(9), 4941-4948.
- Zhang, X., Cheng, S., Huang, X., Logan, B.E. 2010. The use of nylon and glass fiber filter separators with different pore sizes in air-cathode single-chamber microbial fuel cells. Energy & Environmental Science, 3(5), 659-664.
- Zhou, M., Chi, M., Luo, J., He, H., Jin, T. 2011. An overview of electrode materials in microbial fuel cells. Journal of Power Sources, 196(10), 4427-4435.

Appendix

CALCULATIONS

Calculations of Electrical parameters:

External resistance = 1000 ohm

Surface area of electrode = 22 cm^2

As Voltage was recorded directly by digital multimeter, the rest of the parameters were calculated as given below.

- **Current density**

$$\text{C.D} = V/(R_{ext}.\ A)$$

$$= 0.262\ \text{V}/(1000\ \Omega \times 0.0022\ \text{m}^2)$$

$$= 0.119\ \text{A/m}^2$$

$$= 119\ \text{mA/m}^2$$

- **Power output**

$$\text{P} = \text{V}^2/\text{R}_{\text{ext}}$$

$$= (0.262\ \text{V})^2/1000\ \Omega$$

$$= 0.069 \times 10^{-3}\ \text{W}$$

$$= 0.07\ \text{mW}$$

- **Power density**

$$\text{P} = \text{P}/\text{A}$$

$$= 0.07 \times 10^{-3}\ \text{W}/\ 0.0022\ \text{m}^2$$

$$= 0.031\ \text{W/m}^2$$

$$= 31\ \text{mW/ m}^2$$

- **Coulombic efficiency**

Data for glucose

Hours	Avg. Voltage (mV)	Avg. Current (mA)
0-12	165	0.17
12-24	227.8	0.23
24-36	228	0.23
36-48	244	0.24
48-60	250	0.25
60-72	255.2	0.26
72-84	262	0.26
84-96	255	0.26

$$\boldsymbol{C.E} = \int_{\mathbf{0}}^{\boldsymbol{t}} \mathbf{I}\,(\mathbf{t})\,\mathbf{dt})\,/(\mathbf{F.b.V.}\Delta\boldsymbol{S})$$

Where

$\int I = (0.17+0.23+0.23+0.24+0.25+0.26+0.26+0.26)$ mA

$= 1.9$ mA

F = Faraday's constant (96,485C/mol-e^-)

b = Number of electrons produced per mol of oxygen (4 mol-e-)

V= Liquid volume in anode (1.2 L)

ΔS = Substrate consumption in terms of COD (mol O_2/L)

$= 1000$ mg/L- 208mg/L

$= 792$ mg/L

$= 0.792$ g/L

$= 0.792$ g/L / 32 g

$= 0.025$ mol O_2/L

$C.E = \int_0^{12}(0.0019) / 96485\times4\times1.2\times 0.025$

$= 0.007$

$= 0.7\ \%$

➢ Polarization and Power curve

Step 1: Voltage recorded for different resistors in decreasing order during steady state

Step 2: Voltage converted to current by dividing on corresponding resistor

Step 3: Current converted to current density by diving on anode surface area i.e. 22 cm^2

Step 4: Power output is obtained by the product of voltage and current

Step 5: Power converted to current density by diving on anode surface area i.e. 22 cm^2

Data for glucose

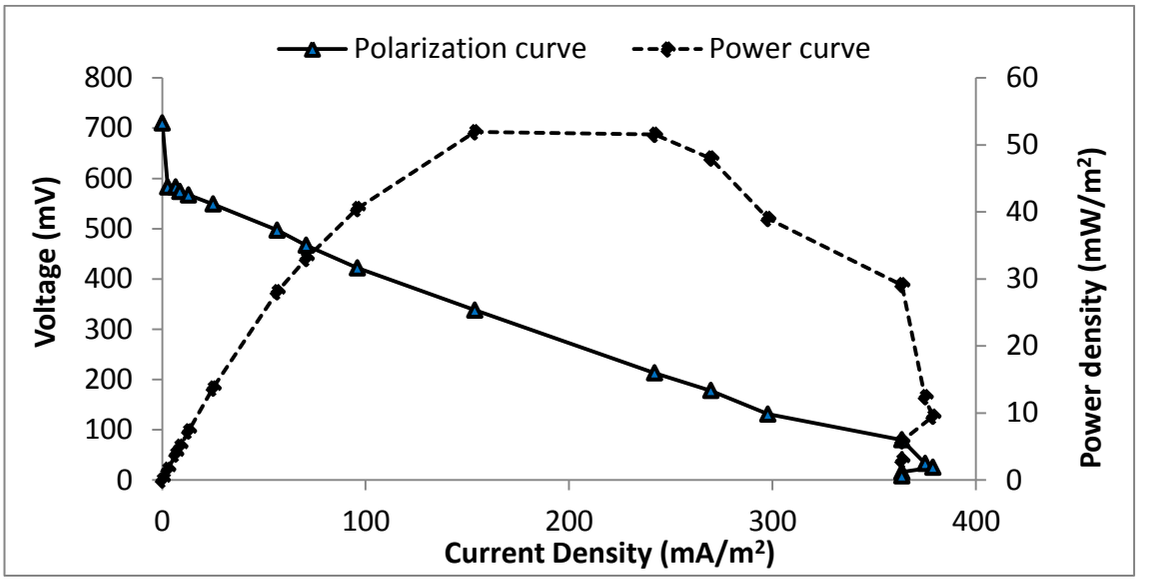
Resistor (ohm)	Voltage (mV)	Current(mA)	current density (mA/m^2)	Power (mW)	Power density (mW/m^2)
100000	583	0.006	2.65	0.003	1.545
40000	583	0.015	6.63	0.008	3.862
30000	574	0.019	8.70	0.011	4.992
20000	567	0.028	12.89	0.016	7.307
10000	549	0.055	24.95	0.030	13.700
4000	497	0.124	56.48	0.062	28.069
3000	467	0.156	70.76	0.073	33.044
2000	422	0.211	95.91	0.089	40.474
1000	338	0.338	153.64	0.114	51.929
400	213	0.533	242.05	0.113	51.556
300	178	0.593	269.70	0.106	48.006
200	131	0.655	297.73	0.086	39.002
100	80	0.800	363.64	0.064	29.091
40	33	0.825	375.00	0.027	12.375
30	25	0.833	378.79	0.021	9.470
20	16	0.800	363.64	0.013	5.818
10	8	0.800	363.64	0.006	2.909

Polarization Curve:

- Plotted as a factor of current or current density against electrode potential

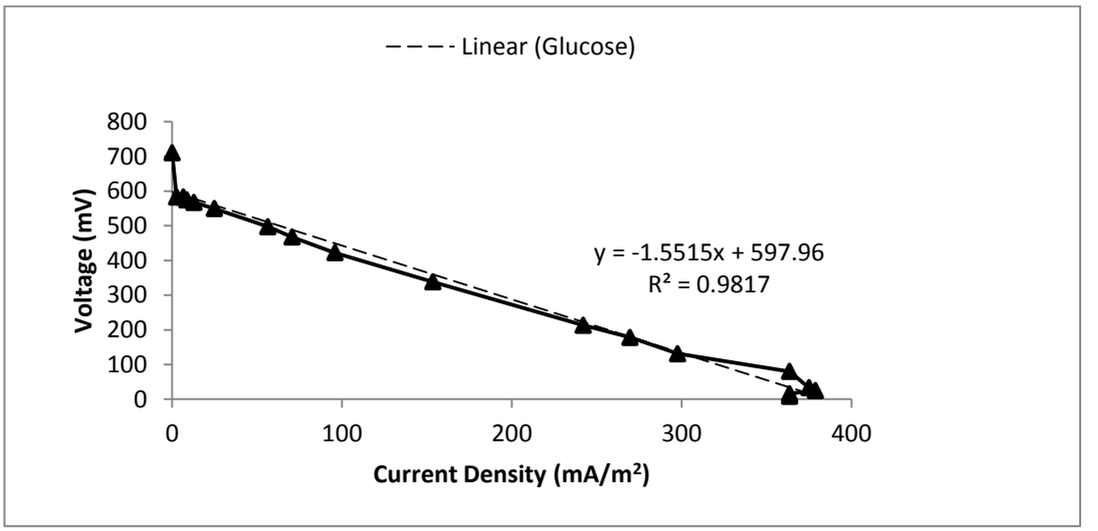
Power curve:

- Plotted as a function of current or current density against power density



Determination of Internal Resistance:

Internal resistance is calculated from the slope of linear portion of polarization curve, as given below.



$$R_{int} = 1.5515\ \Omega.m^2$$

$$= 1.5515\ \Omega / 0.0022$$

$$= 705\ \Omega$$

OR

$R_{int} = \Delta V / \Delta I$

$= 598 \text{ mV} / 384 \text{ mA.m}^{-2}$

$= 1.55 \ \Omega.\text{m}^2$

$= 1.55 \ \Omega / 0.0022$

$= 705 \ \Omega$

OR

The slope of VI curve directly as, R_{int} of 705 Ω in the case below.