NEUTRAL RED IMMOBILIZED GRAPHITE FELT ANODIC MICROBIAL FUEL CELL FOR WASTEWATER TREATMENT AND GENERATION OF ELECTRICITY

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Submitted to

Central Department of Biotechnology

Tribhuvan University, Kirtipur, Nepal

Bimala Dhakal

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List of Abbreviation and Acronym

AS- Activated Sludge

NH_4-N- Ammonia Nitrogen

BOD-Biochemical Oxygen Demand

BT- Biotechnology

CO_2-Carbondioxide

COD-Chemical Oxygen Demand

DET-Direct Electron Transfer

DNS - 3, 5-Dinitrosalicyclic acid

H_2O- Hydrogen oxide

"H" shape MFC-Hydrogen shaped MFC

H_2O_2-Hydrogen Peroxide

L- Liter

MET- Mediated Electron Transfer

MFC-Microbial Fuel cell

mA-Mill Ampere

mg- Milligram

ml- Milliliter

mV-Mill Volt

nm-Nanometer

NADH- Nicotinamide Adenine Dinucleotide

NO_3-N-Nitrite Nitrogen

Ω-Ohm

OCV-Open circuit voltage

PO_4- Phosphorus

PEM-Proton Exchange Membrane

H_2SO_4- Sulphuric Acid

TF- Trickling Filter

UV-Ultraviolet

V-Volt

Abstract

Microbial fuel cell technology can be a new approach for wastewater treatment as it produces sustainable clean energy by minimizing COD level.

In this research, a MFC was constructed by using graphite felt immobilized with neutral red as anode and a platinum coated platinum wire as cathode. Anolyte used was municipal wastewater and catholyte was phosphate buffer pH 7. The collected municipal wastewater contained 1.457mg/L Ammonical Nitrogen, 33.363mg/L COD, 0.537mg/L total Phosphorus, 0.105mg/L reducing sugar and 0.139 mg/L Nitrite nitrogen. The mixed culture of organism dominantly present in the wastewater was used in MFC. The COD of wastewater was found to reduce by 69.96% when MFC was run for 5 days. Similarly, the level of reducing sugar decreased by 90.38% after5 days. Constructed MFC showed an open circuit voltage of 513mV on the fifth day and remained almost constant after that day. The maximum power generated by MFC was found to be 24.45 $W/m^3$when 1% H_2O_2 was supplied as a source of oxygen in the cathode compartment. The result was found to be effective when cellulose acetate as proton exchange membrane was used compared with the Nafion membrane. The fuel cell showed a constant voltage rate when cheese whey was used as anolyte with *Saccharomyces Cerevisiae* as the organism indicating that there was no utilization of the feed.

Keywords: Microbial Fuel cell, Wastewater treatment, Electricity generation, mixed microbial culture.

CHAPTER ONE

INTRODUCTION

"Wastes are the materials that are not prime products for which the initial user has no further use in terms of his/her own purposes of production, transformation or consumption, and will be ready to dispose. Wastes may be generated during the extraction of raw materials, the processing of raw materials into intermediate and final products, the consumption of final products, and other human activities.

Wastewater is the water containing wastes from residential, commercial, and industrial processes. Municipal wastewater contains sewage, gray water (e.g., water from sinks and showers), and sometimes industrial wastewater. Large industries, such as refineries, also generate wastewater. In context of our country, major rivers are polluted due to the discharge of sewage directly into river. The wastewater of Bagmati river at Sundarighat, a very populated section of Kathmandu was found to contain 18mg/L of NH_4-N,1.7mg/L of PO_4-P,0.6mg/L of NO_3-N, 317mg/L of COD in the month of may which was found to vary in other months.(Green *et al*, 2003)

Wastewater treatment has been a problem globally as huge amount of investment and energy is consumed. Conventional Wastewater treatment process is very complicated which involves5 different steps i) Preliminary ii) primary iii) Secondary iv) Tertiary and v)Disinfection (Flörke, M., personal communication. December 5, 2013).

During Preliminary Treatment, the incoming raw sewage, or influent, is strained to remove all large objects that make their way into the sewer system. The main purpose of primary treatment is to produce both a generally homogeneous liquid capable of being treated biologically and a sludge that can be separately treated or processed. Primary clarifiers are usually equipped with mechanically driven scrapers that continually drive the collected sludge towards a hopper in the base of the tank from where it can be pumped to further sludge treatment stages. Primary treatment can reduce BOD by 20 to 30 % and suspended solids by up to 60 %. (Flörke, M.,personal communication. December 5, 2013).

Secondary treatment uses biological processes to catch the dissolved organic matter missed in primary treatment. Microbes consume the organic matter as food, converting it to carbon dioxide, water, and energy. Secondary treatment can remove up to 85 %-90% of BOD and total suspended solids. (Flörke, M., personals communication. December 5, 2013). The highest level of wastewater treatment is tertiary treatment, which is any process that goes beyond the

previous steps and can include using sophisticated technology to further remove contaminants or specific pollutants. Tertiary treatment is typically used to remove phosphorous or nitrogen, which cause eutrophication. All in all, tertiary treatment can remove up to 99% of all impurities from sewage, but it is a very expensive process.

The purpose of disinfection in the treatment of wastewater is to substantially reduce the number of microorganisms in the water to be discharged back into the environment and is almost always the final step in the treatment process regardless of the level or type of treatment used.

Chlorination remains the most common form of wastewater disinfection due to its low cost and long-term history of effectiveness. However chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual chlorine or chloramines (formed by the combination of chlorine and ammonia) may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated adding to the complexity and cost of treatment.

When Ultraviolet (UV) light is used instead of chlorine, the treated water has no adverse effect on organisms that later consume it. UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens making them incapable of reproduction. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement, and the need for a highly treated effluent to ensure that the target microorganisms are not shielded from the UV radiation.

Ozone is considered to be safer than chlorine because it is generated onsite as needed and does not have to be stored. Ozonation also produces fewer disinfection by-products. However, a disadvantage of ozone disinfection is the high cost of the ozone generation equipment and the requirements for special operators.

Some type of wastewater treatment discharge carbon emissions that have caused a hole that is still increasing in the ozone area. This has caused increased warming of our planet. The damage to our water system has been a result of pollution and the hole in the ozone layer. The hole in the ozone has caused ice burgs to melt. Because of the damage of ice in the Polar Regions, species are losing their environment and dying off.

Domestic or industrial wastewater, hold high energy density, theoretically higher than necessary for disposal, the development of techniques for capturing the energy contained in this biomass would provide a new source of electrical power which would reduce the

consumption of energy for wastewater treatment(Cheng & Logan, 2011).The latest need is to find replacement for conventional wastewater treatment as the present conventional bioreactors such as the Activated Sludge (AS) process or the Trickling Filter (TF) process used for wastewater treatment has several disadvantages as mentioned below.

1. Production of byproducts: Many unnecessary byproducts are formed in this type of treatment.
2. Need for aeration: Aeration in AS can consume 50% of the electricity used at a treatment plant and is costly in many other conventional treatment processes.
3. Excess solids production: In an aerobic system such as TF or AS, solids production is high whose treatment is expensive.
4. Potential for odor control: High surface areas needed in TFs exposed to air, and the flow of large amounts of air through the aeration basin in an AS process greatly increases the potential for odor generation to a surrounding community.

MFC an anaerobic process being able to overcome all these disadvantages are being recognized as a new method for wastewater treatment. A MFC is a bioreactor that converts the energy stored in chemical bonds in organic compound directly into current through direct catalytic reactions of microorganism under anaerobic conditions(Bond & Lovley, 2003).Microbes are the key players in MFCs, they oxidize organic substrates, such as glucose, those which are unable to oxidize glucose use complex carbohydrate such as lactose, sucrose and starch to produce ATP and generate electrical power (Rohan, *et al* 2003).

MFCs exhibit safe and quiet performance (Rabaey& Verstraete, 2005).MFCs have high conversion efficiency since MFCs can harvest up to 90% of the electrons from the bacterial electron transport system, which is up to 50% for typical fossil fuel power plants .MFCs operate efficiently at ambient temperature; electricity obtained from MFCs is sustainable. The fuel to electricity conversion by MFCs is not limited by the Carnot cycle because chemical energy from the oxidization of fuel molecules is converted directly into electricity instead of incurring partial heat losses and, theoretically a MFC is capable of energy efficiency far beyond 50% (Mathuriya& Yakhmi, 2014).

Different configurations of MFC i.e Single chambered MFC, Double chambered, Stacked MFC, Salt Bridge MFC, H-shape MFC and Up-flow MFC are being used. Amongst all, the double chambered MFC is most common in the laboratory. Up-flow mode MFCs are more suitable for wastewater treatment, compared to two-chamber MFC.

The demand for energy is increasing at an exponential rate due to the exponential growth of world population. The combined effect of the widespread depletion of fossil fuels and the gradually emerging consciousness about environmental degradation has given priority to the use of renewable alternative energy sources.

The use of fossil fuels causes damage to climate, environment and human health, also create economic problems to many nations which have resulted unbearable inequalities and political tensions internationally that led to wars (International Energy Agency, 2013).Biomasses represent the most used renewable vectors for producing electrical and thermal energy through traditional combustion technologies. Thus MFC is of vital importance, being capable to produce power along with wastewater treatment simultaneously. So in this research we developed a double chambered Microbial fuel cell with wastewater as the substrate using mixed microbial culture isolated from wastewater, capable to remove the chemical oxygen Demand and to generate electricity simultaneously.

Statement of the problem

From decades, Nepal has been facing a severe problem in sewage treatment since the domestic waste wastewater and a drainage supply from the local communities is directly discharged into rivers. There are few no of wastewater treatment plants that too are not functioning well. Thus, an appropriate technique of wastewater treatment that outdates the disadvantages of existing wastewater plant has become a necessity.

Rationale

Wastewater ranging from human feaces, manure sludge, cassava mill water, confectionery wastewater, fermented vegetable waste, domestic wastewater, paper industry wastewater is rich in organic carbon matter. These have to be removed before discharge; however the techniques are expensive and not feasible. Thus, there is an urgent demand of suitable wastewater treatment technique for countering such problems. In this study, we aimed to develop a MFC capable for treating the wastewater biologically decreasing the COD, reducing sugar, Phosphorus and Nitrogen.

Hypothesis

Microbial Fuel cell has been studied for the treatment of wastewater and generation of electricity in laboratories. Thus, MFC may possess the capability that can counteract the existing problems of wastewater and exploiting fossil fuels. The fuel cell can be constructed, optimized and analyzed for COD removal efficiency and current production.

OBJECTIVES

General objectives

- To develop a MFC for efficient wastewater treatment and study the generation of electricity.

Specific objective

- To isolate and characterize the organism present in wastewater.
- To determine the concentration of contaminants present in wastewater.
- To observe the COD and reducing sugar removal efficiency and measure the produced current by MFC.

CHAPTER TWO
LITERATURE REVIEW

2.1 Wastewater treatment

The most important application of MFCs is in wastewater treatment. Wastewater contains energy, in the form of biodegradable organic matter, that we expand energy to remove rather than trying to recover it. At a conventional wastewater treatment plant in Toronto, Canada, it was estimated that there was 9.3 times the energy in the wastewater, than was used to treat it. (Logan, 2008)

MFCs have been considered for treating waste water as early as 1991. Municipal wastewater contains a multitude of organic compounds such as acetate, propionate, and butyrate which can be thoroughly broken down toCO_2 and H_2O. MFCs using certain microbes have a special ability to remove sulfides as required in wastewater treatment (Rabaey *et al*., 2006). MFCs can enhance the growth of bioelectrochemically active microbes during wastewater treatment thus they have good operational stabilities. Up to 80% of the COD can be removed in some cases and a Coulombic efficiency as high as80% has been recorded (Rabaey *et al*., 2006) although the energy that could be captured from wastewater is not enough to power a city, it is large enough to someday power a treatment plant. With advances, capturing this power could achieve energy sustainability for the water infrastructure.

2.2 Working Principle of MFCs

A typical MFC consists of an anode, a cathode, a proton exchange membrane (PEM) and an electrical circuit(Logan, 2008) . In an MFC, bacteria present in the anode compartment uses organic substrates as fuels to produce electrons and protons through biological processes (Rabaey & Verstraete, 2005) (www.microbialfuelcell.org). These electrons are accepted by nicotinamide adenine dinucleotide (NADH) in the electron transport chain and subsequently transferred to terminal electron acceptors such as nitrate, sulphate and oxygen and then reaches the outer membrane proteins (Rega, 2006;Salgado, 2009). Bacteria then transfer electrons to anode through which electrons reach the cathode via an external electrical circuit, thus producing electric current, which is measured by a voltmeter or ammeter connected to the

device (Salgado, 2009). The protons generated are diffused through the PEM to the cathode and subsequently combine with the electrons and oxygen to form water. The cathode is exposed to oxygen whereas anode compartment is typically maintained under anaerobic conditions as electricity generation is inhibited by oxygen(Logan, 2008).The electrode reaction is the breakdown of the biodegradable substrate to carbon dioxide and water along with production of electricity using acetate as a substrate(Student,2010;Duetal,2007).

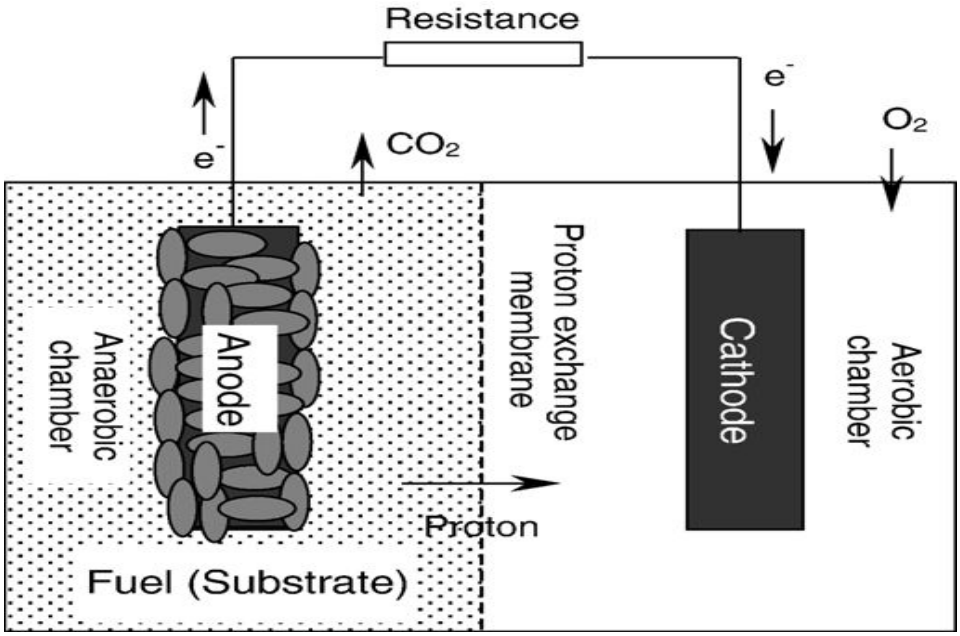


Figure 2.1:Schematic diagram of typical two-chamber Microbial fuel cell (Du *et al*, 2007))

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

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

Electrohydrogenesis has been developed recently as an electrolysis method for converting biodegradable material directly into hydrogen using modified microbial fuel cells. A microbial Electrolysis cell (MEC) operates in a manner similar to an MFC except that the cathode is sealed to exclude oxygen and an additional voltage is added to the circuit. In normal operating conditions, protons from the anodic reaction migrate to the cathode thereby combining with oxygen to form water. Hydrogen generation from the protons and the electrons produced by the metabolism of microbes in an MFC is thermodynamically unfavorable (Liu *et al*,2005). Applying an external potential to increase the cathode potential in a MFC circuit can overcome the thermodynamic barrier. In this mode, protons and electrons produced by the anodic reaction is combined at the cathode to form hydrogen. The concentration of wastewater is

usually evaluated on the basis of the amount of oxygen used to oxidize organic matter, in terms of biochemical oxygen demand (BOD) in a five-day biodegradation test, or via chemical oxygen demand (COD) in a chemical test that fully oxidizes all substances, organic and inorganic. On the basis of COD, it is easy to determine the potential for hydrogen production as one mole of COD indicates that one mole of O_2 is needed for the reaction. MECs can produce H_2 with a minimum external potential of only 110mV at neutral pH, much lower than the 1210mV required for the electrolysis of water at the same pH. This is because some of the MECs energy comes from the biomass oxidation process in the anode chamber. Also unlike MFCs, MECs don't require oxygen in the cathode chamber and so have greater cell efficiency as oxygen leakage to the anodic chamber (known to inhibit electricity generation is no longer an issue). Also importantly for MECs and MFCs alike, hydrogen can be accumulated and stored for later usage to counteract the inherent low power feature of MFCs.

2.3 Microbes Used in Microbial fuel cells

A wide variety of bacterial communities are found to have the ability to oxidize organic compounds and transfer electrons to the anode. Both the mixed cultures and pure bacterial cultures are utilized in the MFC. Rabaey & Verstraete, 2005reported that the mixed cultures have high resistance for process disturbances, substrate consumption and higher power output. Aerobic or facultative anaerobic bacteria are electrochemically active and the reaction temperature in MFCs depend on the bacterial tolerance to temperature (mesophilic/thermophilic) (Logan,2008;Verstraete, 2005).The most intensely studied organism in MFCs and most promising in terms of power density is *Geobacter sulfurreducens*. Beside iron-reducing bacteria (*Shewanella spp* and *Geobacter)* other bacteria such as (*Klebsiella pneumonia, Rhodopseudomonas palustris and dessulfobulbus propionicus*) isolated from the wastewater showed great potential to in MFCs.

2.4 Electron Transfer Mechanism

2.4.1 Direct electron transfer

Several microorganisms such as *Shewanella putrefaciens, Geobacter sulferreducens, Geobacter metallireducens*(Bond & Lovley, 2003) and *Rhodoferax ferrireducens* are all bioelectrically active and can form biofilm on the anode as they have high Coulombic efficiency and transfer electrons directly by conductance through the membrane and highly conductive pili(Lovley & Nevin, 2011).When these organisms are used, the anode acts as the final electron acceptor in the dissimilatory respiratory chain of the microbes in the biofilm (Du *et al*, 2007).DET can be

achieved through two naturally occurring mechanisms; Membrane-bound c-type and electronically conducting nanowires.(Mao & Verwoerd, 2013)

2.4.2. Electron transfer by own mediator /artificial mediators:

In this mechanism, electrons from microbial carriers are transported onto the electrode surface either by a microorganism's(*Shewanella oneidensis, Geothrix ferementans) o*wn mediator which in turn facilitate extracellular electron transfer or by added mediators such as neutral red, thionine, methylene blue, anthraquinone-2, 6-disulfonate, phenazines and iron chelates (Du *et al*., 2007). These MFCs use mediators as electron shuttles. Mediators provide a platform for the microorganisms to generate electrochemically active reduced products. The reduced form of the mediator is cell permeable, accept electrons from the electron carrier and transfer them onto the electrode surface (Lovely, 2006). Mediators are required in MFCs that use *Proteus vulgaris ,Escherichia coli, Streptococcus lactis,* and *Pseudomonas* species as these bacteria cannot transfer electrons outside the cell (Sengodon & Hays, 2012).Mediated electron transfer takes place by two ways; Indirect transfer systems that involve freely diffusing mediator molecules (i.e. diffusive MET) and Indirect transfer systems in which the mediator is integrated into the electrode or the cell membrane (i.e. non-diffusive MET).(Mao & Verwoerd, 2013)

2.5 Substrates

Substrate in addition to being the sources of energy influences the economic viability and performance such as columbic efficiency and power. Electricity has been generated in MFCs from various organic compounds including Carbohydrates, Proteins and fatty acids as reported by Heilmann & Logan 2006;Rega, 2006. Spectrum ranges from simple to complex mixture of organic matter present in sanitary wastes, food processing wastewater, swine wastewater and corn stover. Simple substrates are considered to be good for immediate output product although substrate rich in organic content helps in growth of diverse active microbes .Acetate is mostly preferred due to their its inertness towards microbial conversion which leads to high columbic efficiency and output ,as reported by Pant *et al* , 2010;Zhao*et al*2009whereas lignocellulosic biomass from agriculture residue as hydrolysis products are also considered as good substrate. Another promising and unusual substrate used is brewery wastewater. Chitin and Cellulose from industrial and municipal wastewater, synthetic or chemical wastewater are some unconventional substrates used in MFCs.(S.Das & Mangwani, 2010;Sengodon & Hays, 2012)

Table 2.1: List of different substrates used in MFC

Substrates type	Concentration	Current density(mA/cm)
Acetate	1gm/L	0.8
Lactate	18mM	0.005
Glucose	6.7mM	0.7
Glucoronic acid	6.7 mM	1.18
Starch	10 gm/L	1.3
Cellulosic particles	4 gm/L	0.02
Xylose	6.7 mM	0.74
Domestic wastewater	600mg/L	0.06
Brewery Wastewater	2240mg/L	0.2

(Pant *et al*., 2010)

2.6 Types of MFC

System architecture is important for the performance of MFCs. It has been recognized that improvement of the MFC design could significantly contribute to overcome some of the present limitations. With more than ten years intensive research, many different configurations of MFCs ranging from 1.5 µL to several liters have been developed. Generally, according to the numbers of reactor chamber, MFCs could be clarified as two-chamber, single-chamber and multi-chamber i.e. stack systems.

2.6.1Single chamber MFC system

This design has only one compartment that contains both the anode and the cathode. The anode is either placed away or close to the cathode separated by PEM. (Min *et al*, 2005)reported that if the anode is closer to the cathode, it reduces internal ohmic resistance by avoiding the use of catholyte as a result of combining two chambers and thus increases the power density. Compared to the two chambered MFC, it offers simple, cost effective design and produces power in a more efficient way (Du *et al*, 2007). However, in the membrane-less configuration, microbial contamination and back diffusion of oxygen from cathode to anode without PEM are the major drawbacks (Kim, 2008).

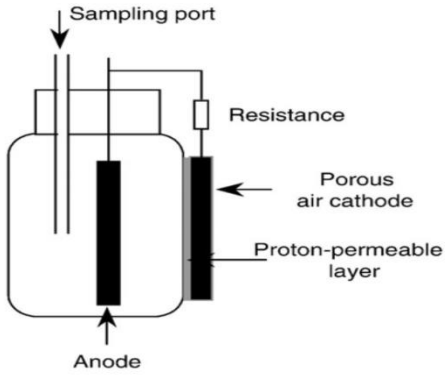


Figure 2.2: Single Chamber MFC

2.6.2 Two-chamber MFC systems

Two-chamber MFCs are commonly used for laboratory test especially in earlier studies. "Two-chamber" consists of the anode and cathode chambers separated by a separator (e.g., membrane), which allows protons to transfer across to the cathode while prevent the diffusion of cathodic electrolyte to the anode. Two-chamber MFCs can be further classified according to the different shapes of the two chambers (Du *et al*, 2007). The typical systems are salt bridge MFC, cylindrical MFC, rectangular and flat MFC, miniature MFC and up-flow tubular MFC.

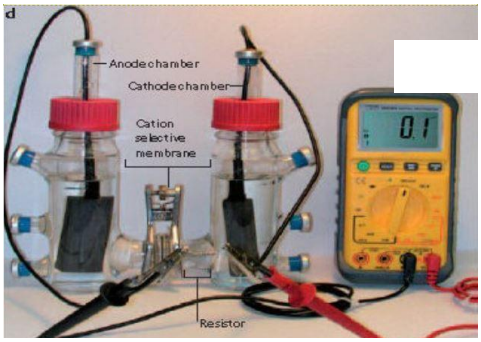


Figure 2.3: Double chamber MFC

2.6.3 Salt bridge

It is an inexpensive material made of glass tube that is heated and bent into a U-shape and filled with agar and salt, which could serve the same function as a proton exchange membrane. The salt bridge MFC is typically constructed by linking two glass bottles with salt bridge.(Song *et*

al, 2015). Min *et al*, 2005 constructed a salt bridge MFC by joining two media bottles with a U-shaped glass tube salt bridge filled with PBS. Though the salt bridge is much cheaper than a proton exchange membrane, little power can be produced from this type of MFC due to its very high resistance, which limits its applications.

2.6.4"H" shape MFC

It is a widely used two-chamber MFC design, consisting usually of two glass bottles or cylindrical chambers connected by a membrane, which is usually CEM such as Nafion etc .H-type MFCs are operated in batch mode and suitable for basic parameter research such as examining power generation from specific substrate or testing the performance of new materials, or exploring the microbial communities enriched from different substrates, but power generation is relatively low in such system. The power generation of these systems is limited by high internal resistance, small electrode surface area and membrane selectivity (Rega, 2006).The H-type or the dual chamber MFC would facilitates more accurate inputs in the anode and cathode chambers due to the fixed volume of the chambers. This set-up enables the use of other inoculum instead of just air in the cathode chamber

2.6.5 Rectangular or flat MFCs

These have been developed to reduce the electrode spacing and enlarge electrode surface. Rabaey*et al*, 2003 developed a rectangular MFC where the chambers are separated by a membrane. In such configuration design, four cells are connected into one block, thus giving rise to four cathodes and anodes. A combined electrode/proton exchange membrane system was used in a flat plate MFC to further shorten the electrode distance (Min and Logan, 2004). The flat plate MFC was designed to operate as a plug flow reactor. The reactor consisted of two chambers which were separated by the electrode/membrane assembly . The special design of flat plate or rectangular MFC is benefit to shorten the electrode spacing and increase electrode surface, but large area of membrane is also required which may increase the construction cost.

2.6.6 Miniature MFC

These have received increasing interests as they can provide unique platforms for fundamental studies of microbes such as screening environmental strains and as a portable power source for small electronic element (Qian*et al*, 2011) . A 1.2 mL MFC that demonstrates high output power (24 and 10 mW/m) based on the true surface areas of reticulated vitreous carbon (RVC) and graphite felt) has been developed by Ringeisen *et al* , 2006. A 650 µL MFC array contains 24-well was used to isolate an electrochemically active microbe that produces 2.3-fold higher

power than the wild-type *Shewanella oneidensis*16 MR-1(Hou *et al*., 2009). A MFC equipped with a nano electrode provide a new approach to investigate extracellular electron transfer at the single-cell level (Jiang *et al*., 2010). Qian *et al*, 2011 demonstrated an easily fabricated, PDMS-based, sub-5 µL MFC that generates an enhanced power density of 62.5W/m^3. Miniature MFCs are also facing some limitations such as low power densities and high fabrication costs (Qian *et al*, 2011).

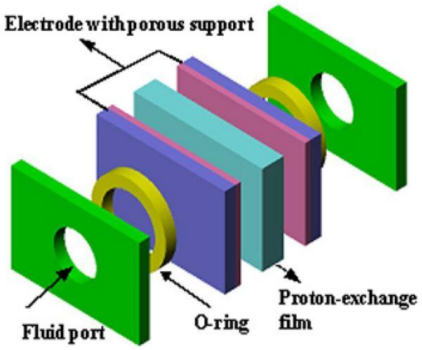


Figure 2.4: Miniature MFC

2.6.7 Up-flow mode MFCs

These are easy to scale up and thus more suitable for wastewater treatment, compared to previous two-chamber reactors. He *et al*, 2005 designed an up flow MFC which was fed with artificial wastewater and operated in continuous mode. The internal resistance of this system is 84 Ω which limits the power output. In order to further reduce the internal resistance, the same researchers developed an up flow MFC system with a U-shaped cathode inside the anode chamber, which has a small internal resistance of 4 Ω. A maximum volumetric power of 29.2 W/m^3 was produced at a loading rate of 3.40 kg COD/m^3/day and an operating temperature of 35 °C while feeding sucrose continuously (He *et al*, 2006). Normally, fluid recirculation is required in the up-flow mode MFCs, which may increase the operation costs and minimize the benefit of microbial electricity production. (Du *et al*., 2007)

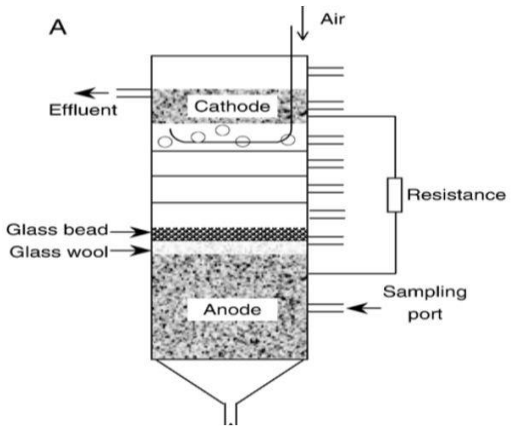


Figure 2.5:Up-Flow MFC

2.6.8Sediment MFC

Electricity can be harvested by embedding an anode into the sediment, and connecting it through an electronic circuit to a cathode in the overlying oxygenated water (Bond *et al*., 2002; Paper V; paper VI). Though there is no real separator for separating the anode and cathode, the solid and water phases can be a naturally anode chamber and cathode chamber, respectively. This system can be employed for sediment bioremediation or as power sources for fresh water or marine studies (Donovan *et al*, 2008; He *et al*., 2009a; Holmes *et al.*, 2004). The main limitations of sediment-type MFC are their low power and output voltage. Several attempts to increase the power output have been made, including modifications of the electrode material, alterations of electrode design, adding mediators or particulate substrate to the anode (Hasvold *et al* 1997; He *et al*., 2007; Hong *et al*2009; Rezaei *et al*2007; Paper V).

2.6.9 Multi-chamber/stacked MFC systems

To increase the overall system voltage or current, MFCs can be connected in series or parallel as a stacked system. Connecting several MFCs in parallel adds the current, while one common voltage applied to each cell. In case several MFCs are connected in series, the voltage is added. Therefore, any desired current or voltage could be obtained by parallel and series stacking the appropriate number of MFCs. Several reactor designs have been developed for this purpose. The first MFC stack is developed by Aelterman (Aelterman *et al,* 2006).Their MFC stack consists of six individual units with granular graphite anode. The MFC stack produced a voltage of 2.02 V at 228 W/m^3 while current of 255 mA are produced at 248 W/m^3 in parallel connection. Both open circuit voltage and short circuit current were approximately a factor of 6 higher than that of the individual MFCs. However, voltage reversal occurred in some cells at high power density during series connection, which resulted in a rapidly decrease of power and voltage output. The authors owed it to the lacing of microbial activity. In order to disclose the cause of voltage reversal in stacked MFCs, Oh and Logan, 2007 constructed a two-cell air-cathode MFC stack which produced a working voltage of 0.9V at 500 Ω and an open circuit voltage (OCV) of 1.3V when operated in fed batch mode with sufficient substrate supply. The authors found that the voltage reversal is due to loss of bacterial activity at the condition of fuel starvation. A novel configuration of stacked MFCs bridged internally through an extra CEM was assembled from two single MFCs (Liu *et al*, 2008). To minimize the limitations that non-uniform potential

distribution on the electrode surface and lower output voltage due to the potential drop, a bipolar plate stacked MFC consisting of five single cells connected in series was developed.

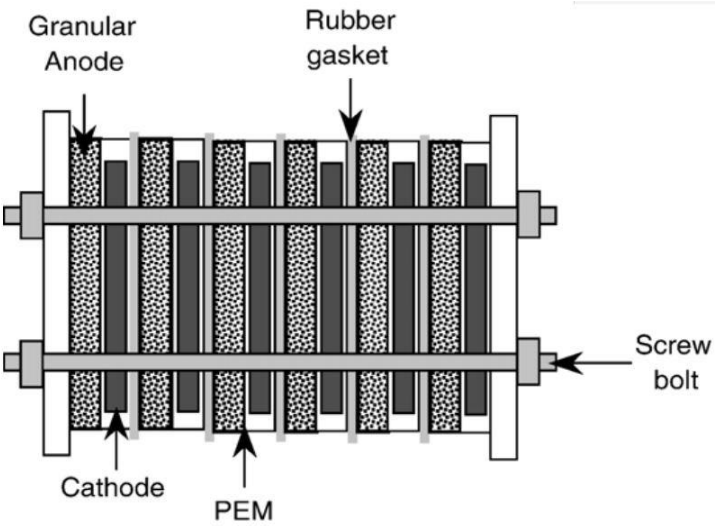


Figure2.6: Stacked MFC

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Materials

(All the reagents were purchased from Himedia, India Pvt.LTD unless stated)

3.1.2 Chemicals

1. Nutrient Broth
2. Lurea Brotani Broth
3. Potassium Dichromate (Merck Pvt. Ltd)
4. DNS (3,5-Dinitrosalicyclic acid)
5. Nesslers' Reagent(StainBiopvt.ltd)
6. Silver Sulphate (provided by Central Department of Environment)
7. Mercuric sulphate (provided by Central Department of Environment)
8. Glucose
9. Boric acid solution
10. Sulfanilamide (STAINBiopvt.ltd)
11. N- (1- naphthyl)- ethylenediaminedihydrochloride
12. Antimony potassium tartrate solution
13. Ammonium molybdate solution
14. Ascorbic acid
15. Neutral Red (Qualigens)
16. Thionyl chloride
17. Chloroform (Fisher Scientific)
18. Agarose

3.1.3 Glass wares

1. Glass Petri plates
2. Reagent bottles
3. Conical flask
4. Resistor (provided by physics department)

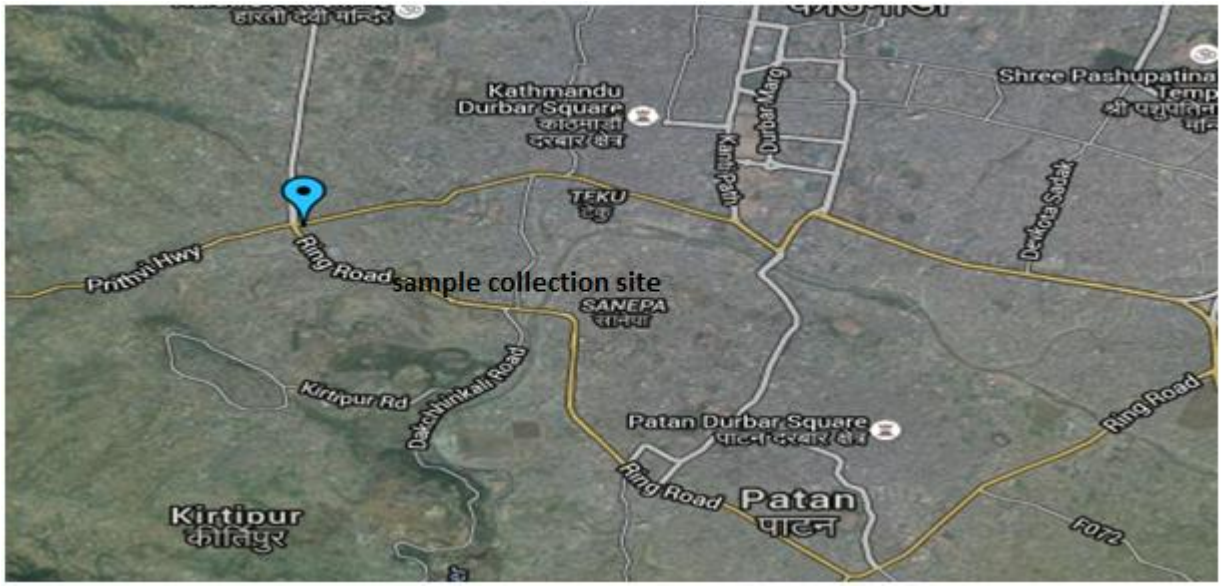
3.1.4 Equipments

1. Microscope
2. Magnetic stirrer and magnetic bar
3. Autoclave
4. Vortex
5. Laminar Flow
6. Centrifuge
7. Incubator
8. Hot air oven
9. PH meter
10. UV spectrophotometer
11. Gel Electrophoresis Chamber
12. PCR machine
13. Electrodes (Graphite,Platinum wire)
14. Multimeter (Fluke)
15. Verde flex Peristaltic Pump (major science)

3.2 Methodology

3.2.1Collection of Sample

The effluent water sample was collected from the Bagmati river near Kalanki, Kathmandu in a sterile bottle and was stored at 4°c.



3.2.2 Environmental analysis of water

3.2.2.1Determinationof Ammonia Nitrogen (Standard method for examination of water)

Standard curve of Ammonia-Nitrogen

A series of concentration ranging from 0.1mg/L, 0.2mg/L, 0.4mg/L, 0.5mg/L, 0.8mg/L, 1mg/L, 1.6mg/L, 2.0mg/Lof ammonia-nitrogen working solution was prepared by pipetting suitable volumes of the ammonia-nitrogen stock solution. Then 2 ml of Nesslers' reagent was added to each of the flasks and mix thoroughly. The solutions were allowed to sit for 20 minutes for color development. Background correction was performed with a blank solution and the absorbance of the solutions was taken at 425 nm using a glass cuvette.

Determination of Ammonia-Nitrogen

First of all 400 ml of distilled water was added to a 500-ml Kjeldahl flask with previously treated boiling chips to avoid bumping. The distillate was steamed until it showed positive reaction with Nessler reagent. Then 500 ml of sample (wastewater) was added in Kjeldahl flask and 25 ml of borate buffer was added. 150 ml of the sample was added into 25 ml boric acid solution in 250-mL Erlenmeyer flask. The distillate was made up to 250 ml with distilled water. To 50 ml of the diluted distillate, 2 ml Nessler reagent was added and was mixed well. The solutions were allowed to incubate for 20 minutes for color development. Background correction was performed with blank solution followed by sample analyses.

3.2.2.2Determination of Chemical Oxygen Demand(Standard method for examination of water)

Standard curve of Chemical Oxygen Demand

A series of concentration ranging from 20mg/L,50mg/L,100mg/L,200mg/L,400mg/L,600mg/L, and 900mg/L of phthalate working solution was prepared, by pipetting suitable volumes of the phthalate stock solution in 50-mL volumetric flasks. Then 10 ml of the reference solutions were added to the culture tubes. After then 6 ml of digestion solution was added to each of the references and mix thoroughly. To the tubes, 14 ml of catalyst solution was added to each of the references, down the side of the culture tube. It was capped tightly and was shake to mix the layers .The culture tubes were placed in an oven at 150°C for 2 hours. The tubes were cooled and any precipitate if was allowed to settle. Background correction was performed with

the blank solution (without sample) and the absorbance of the solutions was taken at 600 nm using the cuvette.

Determination of Chemical Oxygen Demand

Finally 10 ml of the sample was taken in to a culture tube and the same procedure was followed as in the standard curve preparation and COD was determined.

3.2.2.3Determination Of Nitrite-Nitrogen (Standard method for examination of water)

Standard Curve of Nitrite Nitrogen

A series of reference solutions ranging from 0.01mg/L, 0.02mg/L, 0.03mg/L, 0.04mg/L, 0.06mg/L, 0.08mg/L,0.10mg/L,0.20mg/L of nitrite solution was prepared by pipetting suitable volumes of the nitrite working solution. After that2.0 ml of buffer–color reagent to each of the references was added and mixed thoroughly and it was ensured the pH is between 1.5 and 2. The solutions were allowed to sit for 15 minutes for color development. Background correction was performed with a blank solution and the absorbance of the solutions was taken at 540 nm.

Determination of Nitrite-Nitrogen

Along with standard50 ml of the sample was transferred into a 100-mL Erlenmeyer flask. The pH of the solution was checked for alkalinity and Nitrite Nitrogen was determined as above.

3.2.2.4Determinationof Phosphorus (Standard method for examination of water)

Standard curve of Phosphorus

A series of reference solution ranging from 0.01 mg/L,0.03mg/L,0.05mg/L,0.1mg/L,0.2mg/L, 0.3mg/L,0.4mg/L,0.5mg/L of phosphorus working solution was prepared by pipetting suitable volumes of the phosphorus stock solution. The reference solutions were added to 100-mL Erlenmeyer flasks. Then 8 ml of combined reagent was added to each of the flasks and mix thoroughly. The solutions were allowed to sit for 10 to 15 minutes for color development. Background correction was performed with blank solution and the absorbance was measured at 880 nm using 10mm glass cuvette.

Determination of Phosphorus

Firstly, 50 ml of sample was transferred to 125-mL Erlenmeyer flask.Then1 ml of sulfuric acid solution was added and mixed. After that 0.4 gm of ammonium per sulfate was added. The solution was boiled gently on a preheated hot plate for approximately 30-40 min until a final volume of about 10 ml was reached. It was then cooled and diluted the sample to about 30 ml and the pH of the sample was adjusted to 7.0 ±0.2 with 1 mol/L NaOH using a pH meter, the sample was diluted to 50 ml. Then 8 ml of combined reagent was added to the sample and mixed thoroughly. Background correction was performed with blank solution and followed sample absorbance measurements.

3.2.2.5 Determination of Reducing Sugar Using DNS method (Miller *et al* 1959)

Standard curve of reducing sugar

Firstly stock solution of Glucose was prepared. 25µl of stock was taken and then was added in order to make the solution ranging from concentration 0.05mg/L, 0.10mg/L ,0.2mg/L ,0.4mg/L ,0.6mg/L ,0.8mg/L ,1.00mg/L. Then 500 µl of sample was taken into which 500ul of citrate buffer and 3ml DNS reagent was added. After the addition of DNS it was kept in boiling water. It was allowed to cool and then absorbance was taken at 540nm.

Determination of Reducing Sugar

500µl of sample was taken and the same procedure was followed along with the preparation of standard curve.

3 .2.3Isolation and identification of Bacteria

3.2.3.1 Isolation of microorganism

1 ml of sewage sample was diluted upto 10^{-5} dilution using serial dilution. 0.1ml from each dilution was spread plate on the agar plate. The plates was sealed with parafilm, labelled and kept in the 30°C kept in incubator for 24hrs.1 ml sample taken from municipal sludge was cultured in LB broth. Isolation of these microorganisms until single colony obtained had been done by using serial dilution method and spread plate method. Plates of agar plate were prepared aseptically. After agar plate was ready, was the fast growing bacteria plate was taken. The different colonies of bacteria on the plate were chosen. By using aseptic technique, bacteria were streaked on the agar plate. The streak agar plate was sealed and kept in 30°C

incubator for growing. Besides that, from the petriplate, gram staining was done. Samples were observed under microscope and characterized (Lozano *et al*., 2009).

3.2.3.2. Biochemical Identification of microorganism

Biochemical test were performed by standard procedure based on Bergey's manual (1994). For the identification of these microorganisms from municipal sludge, several biochemical identification methods such as Gram stain, spore forming, strict anaerobes, starch hydrolysis, Voges-Proskauer had been used.

3.2.3.3 Maintenance of microorganisms

All the organisms were maintained at Nutrient Agar plate. The organisms were sub-cultured at 15 days interval and were preserved using 15%Glycerol stock.

3.2.4 Construction of MFC

3.2.4.1. Treatment of PEM (Mann *et al*, 2007)

Nafion 115 membranes were first boiled in successive bathes of 0.5M H_2SO_4 at 80°c to 100°c for 2 hours, then was boiled in H_2O at 80°c to 100°c for 2 hours after that was boiled in 3% H_2O_2 at 80°c to 100°c for 2 hours and finally was boiled in H_2O at 80°c to 100°c for 2 hours. This process was repeated after the Nafion was used for 4-5 times.

3.2.4.2Neutral Red immobilization of Graphite Felt (Jeon& Park, 2010)

Firstly the graphite felt was stitched with Teflon wire. It was then soaked in methanol to absorb methanol. Excess methanol was drained from Graphite. After that it was dipped in 1% polyvinylchloride solution for about 3-4 hours and dried in oven at 80°c. Then it was kept in a beaker containing 0.01% Neutral red in chloroform and 10% Thionyl chloride. It was left for 2-3 hours for soaking .It was then kept in a thick layer of newspaper for soaking and was left for whole night to dry or exhaust. After drying it was washed with water for 2-3 times until it showed red color. It was then oven dried at 50°-60°c for whole night. Finally a hole was made and sealed with a rubber. This process was repeated after the graphite was used for 4-5 times.

3.2.4.3Treatment of Cellulose acetate

Cellulose acetate was soaked in autoclaved water and was left overnight.

3.2.4.4. MFC Set up

A MFC was constructed by joining two plastic bottles of 500ml capacity connected via a glass tube with the help of a rubber gasket. Graphite felt was used as anode and the platinum coated wire was used as cathode in the MFC. Anode compartment was filled with 400 ml of sample whereas the cathode compartment was filled with 0.1M Phosphate buffer. Open circuit voltage was observed by using multimeter (FLUKA). External resistance of 500Ω was applied to the system to generate current voltage and power.

1. 400ml of wastewater sample was taken in the anode compartment and 400ml of phosphate buffer was taken in the cathode compartment. Graphite felt was used as anode and the platinum coated wire was used as cathode in the MFC. 4ml of sewage sample as the source of microorganism was added into the anode compartment .Cellulose acetate membrane was used as the Proton exchange membrane. External resistance of 500Ω was applied to the system to generate current voltage and power. The experiment was performed in triplicates and the current and voltage generated was observed using an ammeter and voltmeter respectively.
2. 400ml of wastewater sample was taken in the anode compartment and 400ml of phosphate buffer was taken in the cathode compartment. Graphite felt was used as anode and the platinum coated wire was used as cathode in the MFC. 4ml of sewage sample as the source of microorganism was added into the anode compartment .Hydrogen peroxide at the rate of 1ml/min was added continuously into the cathode compartment using a peristaltic pump. Nafion (117) membrane was used as the Proton exchange membrane .External resistance of 500Ω was applied to the system to generate current voltage and power. The experiment was performed in triplicates and the current and voltage generated was observed using an ammeter and voltmeter respectively.
3. 400ml of autoclaved wastewater sample was taken in the anode compartment and 400ml of phosphate buffer was taken in the cathode compartment. Graphite felt was used as anode and the platinum coated wire was used as cathode in the MFC. 4ml of sewage sample as the source of microorganism was added into the anode compartment. Hydrogen peroxide at the rate of 1ml/min was added continuously into the cathode compartment using a peristaltic pump. Cellulose acetate membrane was used as the Proton exchange membrane. External resistance of 500Ω was applied to the system to generate current voltage and power. The experiment was performed in triplicates and the current and voltage generated was observed using an ammeter and voltmeter respectively.

4. 400ml of cheese whey sample was taken in the anode compartment and 400ml of phosphate buffer was taken in the cathode compartment. Graphite felt was used as anode and the platinum coated wire was used as cathode in the MFC. 4ml of yeast culture as the source of microorganism was added into the anode compartment. External resistance of 500Ω was applied to the system to generate current voltage and power. The experiment was performed in triplicates and the current and voltage generated was observed using an ammeter and voltmeter respectively.

Figure 3.1: Microbial Fuel Cell with the addition of Hydrogen Peroxide using a peristaltic pump.

3.2.4.5. MFC for Wastewater Treatment

The MFC was operated for five days and the absorbance of solution was noted daily. After that a graph of absorbance versus concentration was plotted from which the concentration of COD was estimated. Finally graph of COD versus days was plotted as shown in fig 4.6.The process was performed in triplicates and the results were calculated on the basis of average values of those three experiments. The same process was repeated for observing the reducing Sugar removal efficiency.

CHAPTER FOUR

RESULTS

4.1 Determination of Environmental parameters of Waste water:

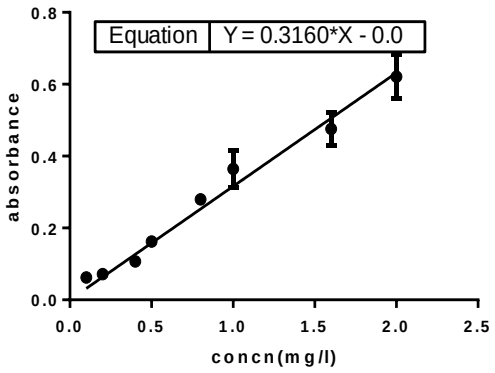


Figure 4.1: Standard curve of Ammonical Nitrogen

Calibration linearity curve was generated using seven different levels of calibration standards in the range from 0.1 mg/L to 2.0 mg/L NH_3-N including the blank as first level. Results showed linearity with correlation coefficient of 0.9854.

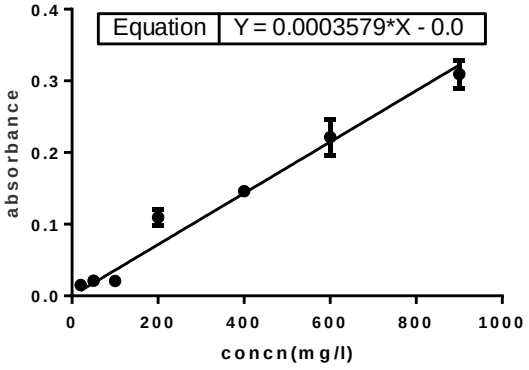


Figure 4.2: Standard curve of Chemical Oxygen Demand

Calibration linearity curve has been generated using eight different levels of calibration standards in the range from 20 mg/L to 900 mg/L including blank as first level. Results showed linearity with a correlation co-efficient of 0.9788.

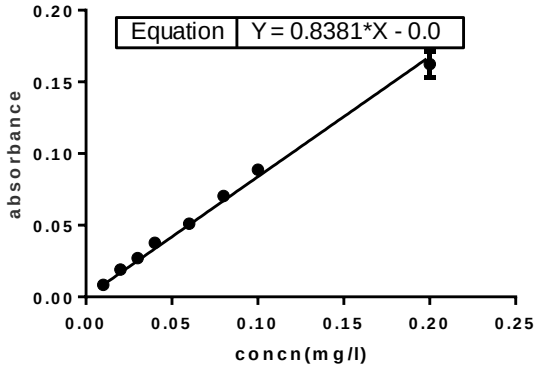


Figure 4.3: Standard Curve of Nitrite Nitrogen

Calibration linearity curve has been generated using nine different levels of calibration standards in the range from 0.01 mg/L to 1.0 mg/L including a blank as first level. Results showed linearity with correlation coefficient of 0.9973.

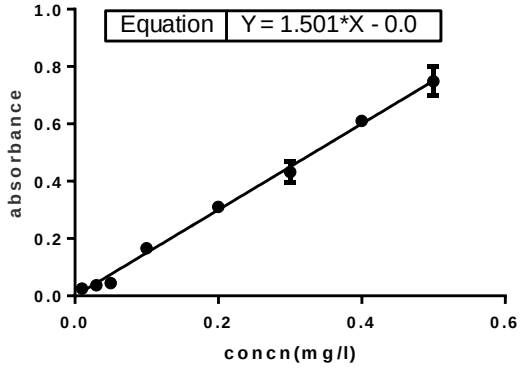


Figure 4.4: Standard Curve of Phosphorus

Calibration linearity curve was generated using nine different levels of calibration standards in the range from 0.01 mg/L to 0.5 mg/L including a blank as first level. Results showed linearity with correlation co-efficient of 0.9966.

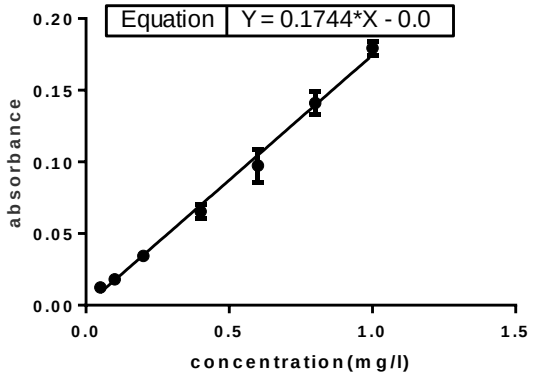


Figure 4.5: Standard curve of reducing sugar

Calibration linearity curve was generated using nine different levels of calibration standards in the range from 0.05 mg/L to 1.0 mg/L including a blank as first level. Results showed linearity with correlation co-efficient of 0.9955.

Table 4.1: Environmental Analysis of Wastewater:

Analytical parameters of water	**Concentration(mg/L)**
Ammonia- Nitrogen	1.457±0.392
Chemical Oxygen Demand	33.363±0.760
Total phosphorus	0.537±0.023
Reducing sugar	0.105±0.033
Nitrite Nitrogen	0.139±0.107

Table4.1 above shows the contaminants i.e Ammonia Nitrogen, Chemical Oxygen Demand, Total Phosphorus, Reducing Sugar and Nitrite Nitrogen present in wastewater.

4.2 Isolation and identification of bacteria

From the waste water sample, 6 different bacteria were isolated based on their morphology using 10^{-6} and 10^{-5} dilution. The isolates were named as BT1, BT2, BT3, BT4, BT5 and BT6.Table 4.2 shows the biochemical analysis for BT1, BT2, BT3, BT4, BT5 and BT6. Based on the staining technique and the biochemical test, the bacterial isolates were identified.

Table 4.2 : Morphological and biochemical characteristics of the isolates

Character	BT1	BT2	BT3	BT4	BT5	BT6
Morphology	Gram negative, motile	Gram positive, non-motile	Gram negative, motile	Gram negative, motile	Gram negative,non-motile	Gram negative,motile
Catalase test	+ve	+ve	+ve	+ve	+ve	+ve
Methyl red test	_ve	+ve	_ve	+ve	_ve	+ve
Voges-Proskaur	+ve	+ve	_ve	_ve	+ve	_ve
Indole test	+ve	_ve	+ve	+ve	_ve	_ve
Citrate test	_ve	_ve	+ve	_ve	+ve	+ve
Nitrate test	+ve	+ve	+ve	+ve	_ve	+ve
Urease test	_ve	+ve	_ve	_ve	+ve	_ve

Microorganism isolated were subjected to biochemical tests such as catalase, Methyl red, voges-Prosakaur, Indole, citrate, Nitrate and urease test and the results were reported as in the table 4.2.Gram staining was also performed and observed under the microscope.

Table 4.3: Biochemical Identification of bacterial isolates

Strain	**Identified bacterial isolates**
BT1	*Enterobacterspp*
BT2	*Staphylococcus spp*
BT3	*Pseudomonas spp*
BT4	*Escherichia coli*
BT5	*Klebsiellaspp*
BT6	*Salmonella spp*

After the biochemical tests and gram staining, *Staphylococcus* spp, *Pseudomonas*, *E.coli*, *Klebsiella spp* and *Salmonella spp* were found to be present in the wastewater.

4.3 MFC for wastewater treatment:

Ammonia Nitrogen, COD, Phosphorus, Nitrite Nitrogen and Reducing sugar were estimated before and after the operation of MFC and removal efficiency was noted. However significant results were not obtained for the removal of Ammonia Nitrogen, Nitrite Nitrogen and Phosphorus, therefore the results of COD and reducing sugar are only shown.

4.3.1 Removal of COD

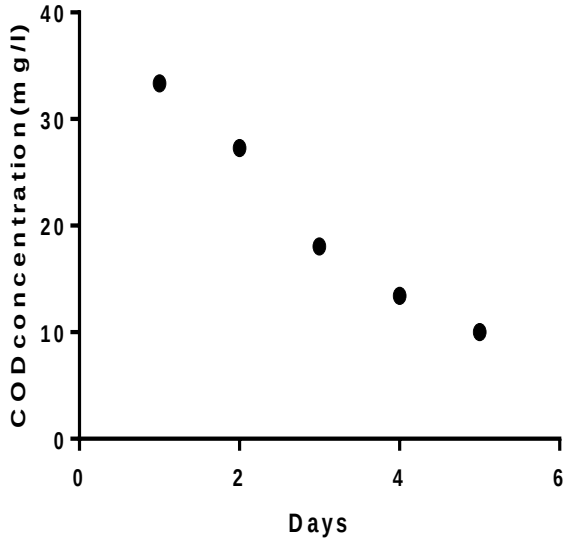


Figure 4.6: Reduction of COD versus number of days

The MFC was operated for five days and the absorbance of solution was noted daily. The COD of wastewater was found to be reduced from 33.363mg/L to10.019 mg/L on the fifth day which was a decrease by 69.96%.

4.3.2Removal of reducing sugar

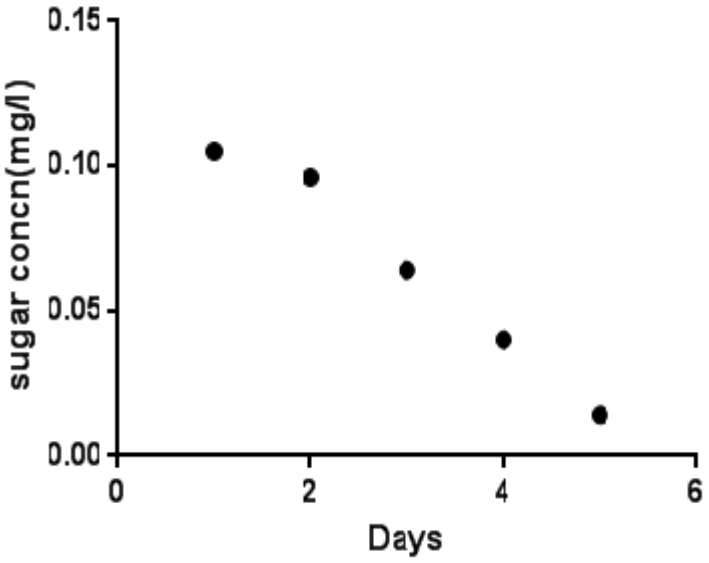


Figure 4.7: Reduction of reducing sugar versus number of days

The MFC was operated for five days and the absorbance of solution was noted daily. The concentration of reducing sugar was found to gradually decrease from 0.104mg/L to 0.010mg/L on the fifth the day and remained approximately 0.00 on the sixth day. The concentration of reducing sugar was lowered by 90.38%.

4.4 Power, voltage and current Generated by MFC in different conditions

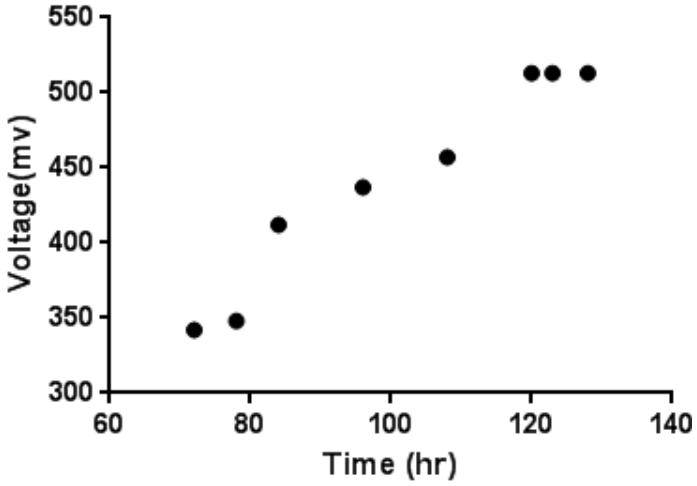


Figure 4.8: Open circuit voltage in MFC using Cellulose acetate membrane

MFC showed an open circuit voltage 513mV on the fifth day. The voltage increased gradually up to the fifth day and remained constant on the following day.

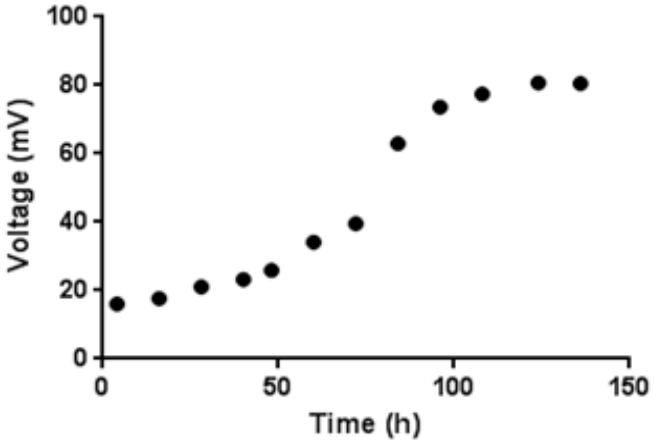


Figure 4.9: Voltage generated in MFC using cellulose acetate with external resistance of 500Ω

MFC was run for about 7 days, and the voltage was recorded daily at an interval of 1 hour. In this experiment, cellulose acetate membrane was used as Proton Exchange Membrane. The voltage was 19mV on the first day which kept on increasing smoothly and reached 80.74mVon the fifth day. After that the voltage generation decreased dramatically and remained almost zero.

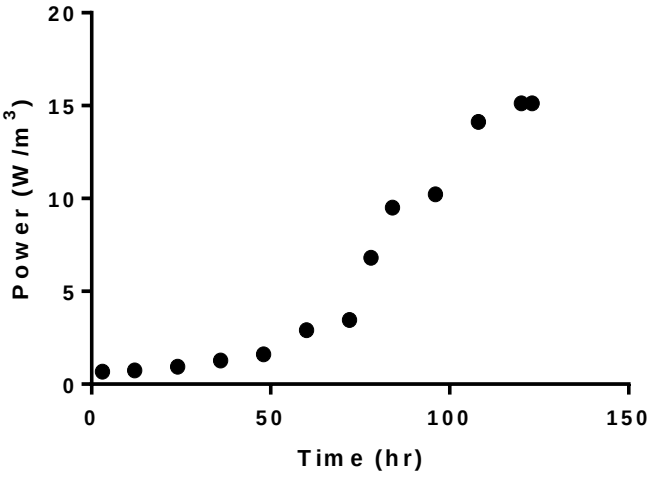


Figure 4.10:Power generation in MFC using cellulose acetate membrane with external resistance of 500Ω

MFC was run for about 7 days, and the power was calculated by taking into account the voltage and current produced. In this experiment, cellulose acetate membrane was used as Proton Exchange Membrane. The power was 1w/m^3 on the first day which kept on increasing smoothly and reached 15.13 W/m^3 on the fifth day. The power production decreased in the similar pattern of voltage decrement after the sixth day.

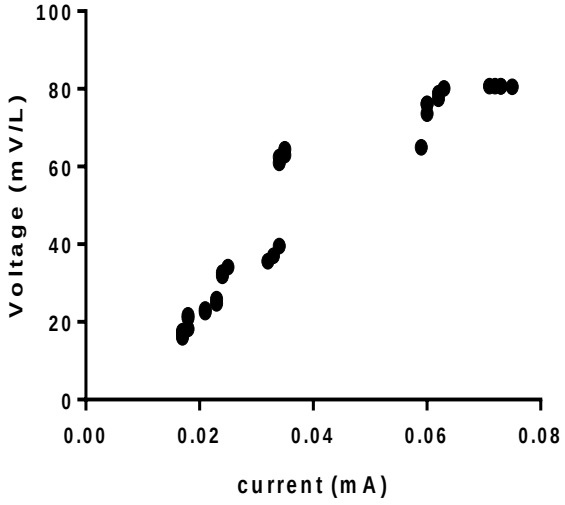


Figure 4.11:Variation of voltage with current in MFC while using acetate membrane with external resistance of 500Ωs

As the current flow increased, voltage also increased in the same proportion. Both the current and voltage were found to increase with the no of days initially and later on decreased.

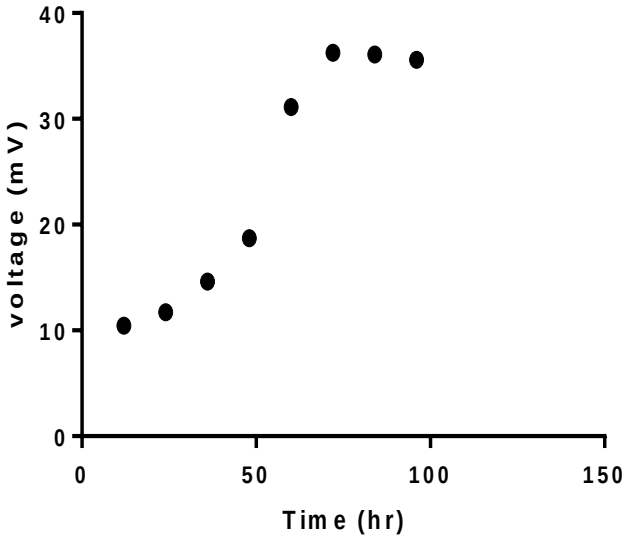


Figure 4.12: Voltage generated in MFC while using Nafion membrane with external resistance of 500Ω

MFC was run for about 5 days, and the voltage was recorded daily at an interval of 1 hour. In this experiment, Nafion membrane was used as Proton Exchange Membrane. The voltage was 10mV on the first day which kept on increasing smoothly and reached 36.26mV on the third day. After that the voltage generation decreased dramatically and remained almost zero.

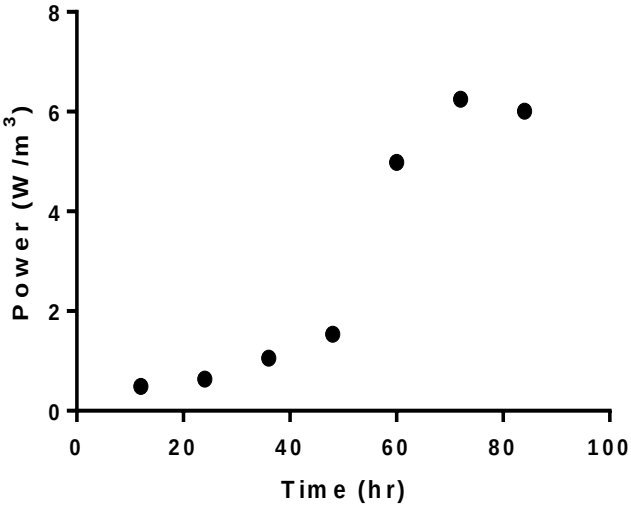


Figure 4.13: Power Generation in MFC using Nafion Membrane with external resistance of 500Ω

MFC was run for about 5 days and the power was calculated by taking into account the voltage and current produced. In this experiment, Nafion membrane was used as Proton Exchange Membrane. The power was 0.8 W/m^3 on the first day which kept on increasing smoothly and reached 6.25W/m^3 on the third day. The power production decreased in the similar pattern of voltage decrement after the fourth day.

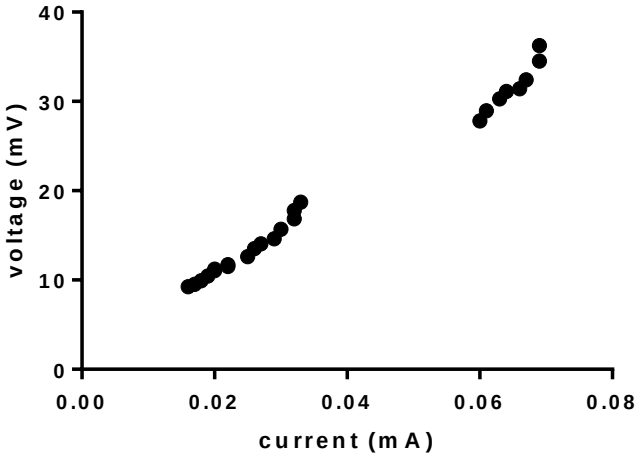


Figure 4.14:Variation of voltage with current using Nafion Membrane with external resistance of 500Ω

As the current flow increased, voltage also increased in the same proportion. Both the current and voltage were found to increase with the no of days initially and later on decreased.

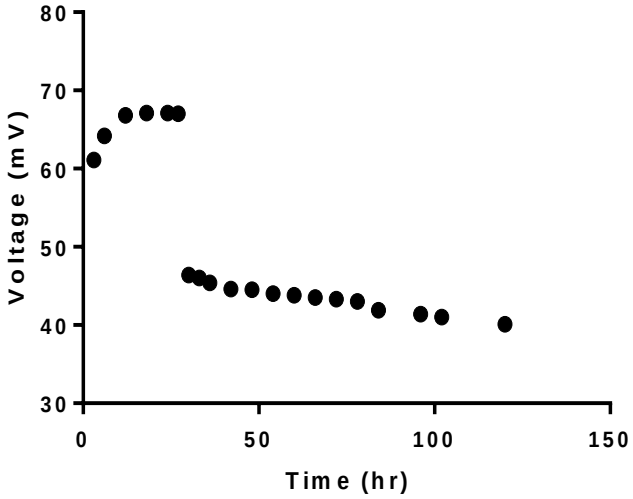


Figure4.15:Voltage generated in MFC using cellulose acetate membrane with 1% H_2O_2 at the flow rate of 1ml/min at cathode with external resistance of 500Ω

MFC was run for about 7 days, and the voltage was recorded daily at an interval of 1 hour. In this experiment, Cellulose acetate membrane was used as Proton Exchange Membrane When Hydrogen peroxide was used as a source of oxygen; the voltage production was maximum on the first day i. eabout4 times larger than in normal cases. However the voltage generation started to decrease drastically from the second day and remained almost constant after that day.

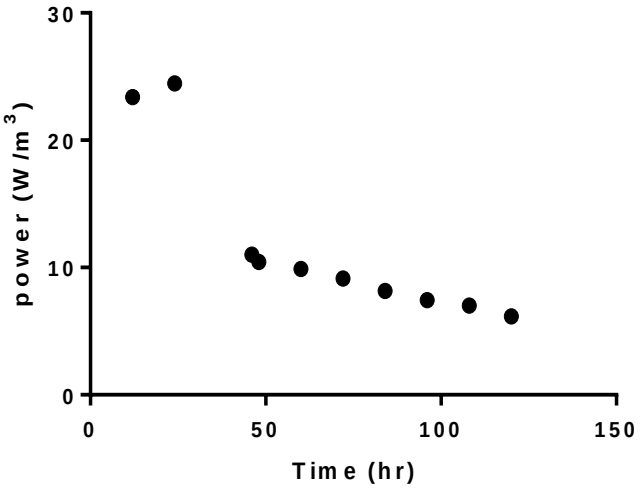


Figure4.16: Power Generation in MFC while using cellulose acetate membrane with 1% H_2O_2 at the flow rate of 1ml/min at cathode with external resistance of 500Ω

MFC was run for about 5 days and the power was calculated by taking into account the voltage and current produced. In this experiment cellulose acetate membrane was used as Proton Exchange Membrane. When Hydrogen peroxide was used as a source of oxygen, the power production was maximum on the first day i.e. about 4 times larger than in normal cases. However the power generation started to decrease drastically from the second day and remained almost constant after that day.

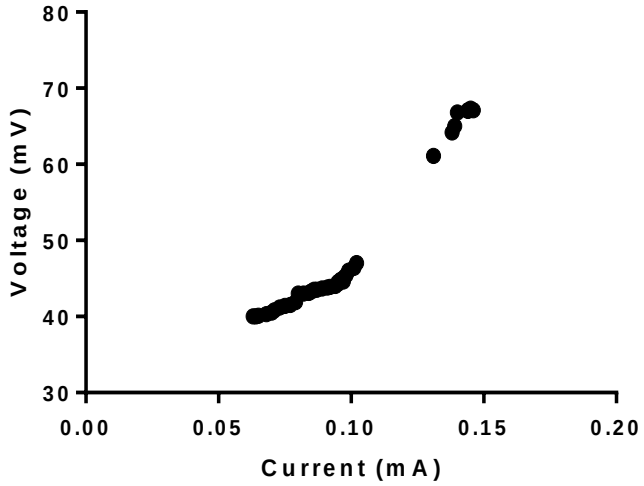


Figure 4.17: Variation of voltage with current using cellulose acetate membrane with 1% H_2O_2 at the flow rate of 1ml/min at cathode with external resistance of 500Ω

As the current flow increased, voltage also increased in the same proportion. Both the current and voltage were found to decrease after the second day subsequently.

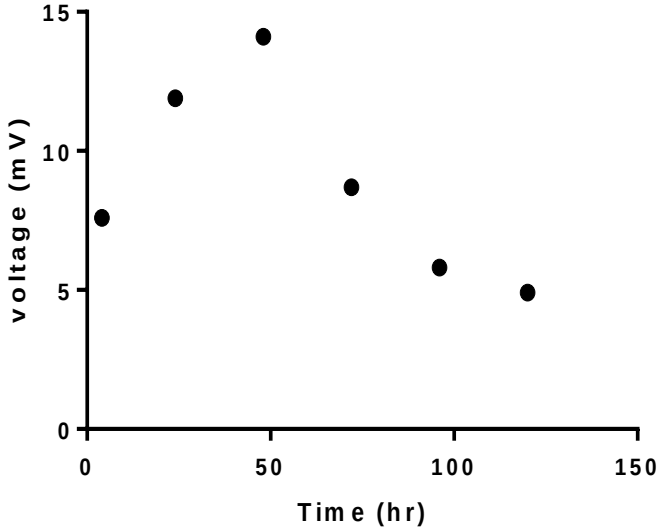


Figure 4.18: Voltage generated in MFC using cellulose acetate membrane with autoclaved waste water as substrate with external resistance of 500Ω

MFC was run for about 5days, and the voltage was recorded daily at an interval of 1 hour. In this experiment Cellulose acetate membrane was used as Proton Exchange Membrane When autoclaved wastewater was used, the voltage production was maximum on the second day

however was very less in comparison to that of raw wastewater. The voltage generation started to decrease drastically from the third day and remained almost constant after that day.

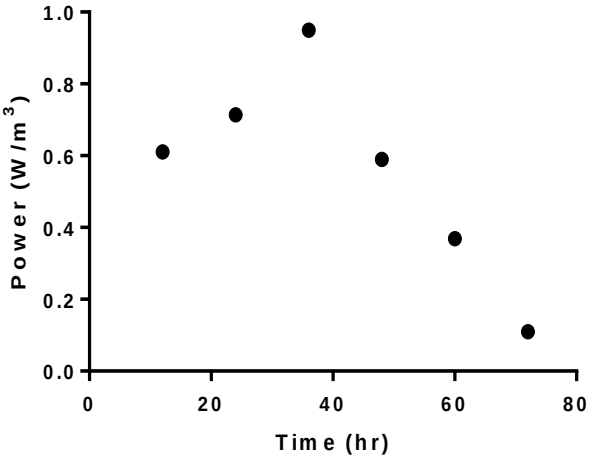


Figure 4.19: Power generated in MFC using with autoclaved waste water as substrate with external resistance of 500Ω

MFC was run for about 5 days and the power was calculated by taking into account the voltage and current produced. In this experiment cellulose acetate membrane was used as Proton Exchange Membrane. When autoclaved wastewater was used, the power production was maximum on the Second day. The power generation started to decrease drastically from the second day and remained almost constant after that day.

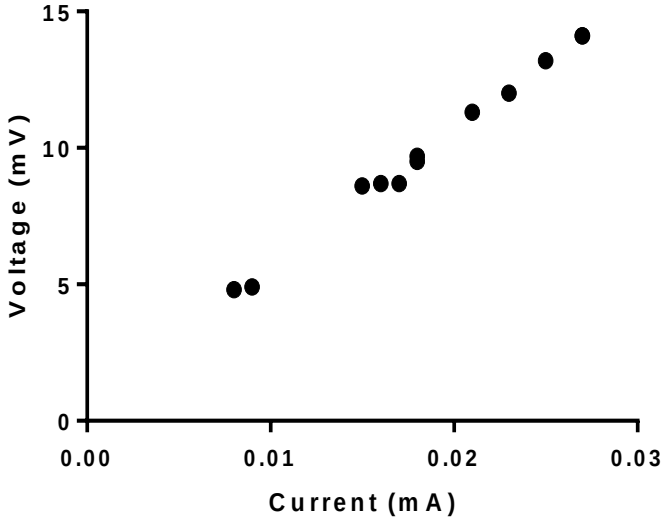


Figure 4.20: Variation of voltage with current using cellulose acetate membrane with autoclaved water with external resistance of 500Ω

As the current flow increased, voltage also increased in the same proportion. Both the current and voltage were found to increase with the no of days initially and later on decreased.

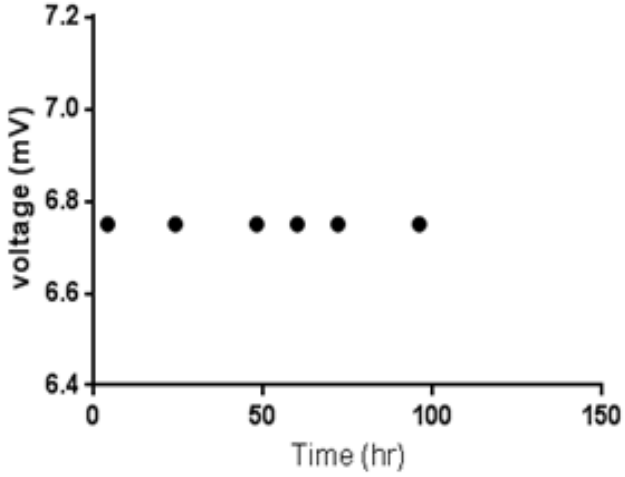


Figure 4.21: Voltage Generated in MFC using Cheese whey and *Saccharomyces Cerevisiae* with cellulose acetate membrane with external resistance of 500Ω

MFC was run for about 5days, and the voltage was recorded daily at an interval of 1 hour. In this experiment, Cellulose acetate membrane was used as Proton Exchange Membrane When cheese whey was used as sample the voltage production was constant on each day.

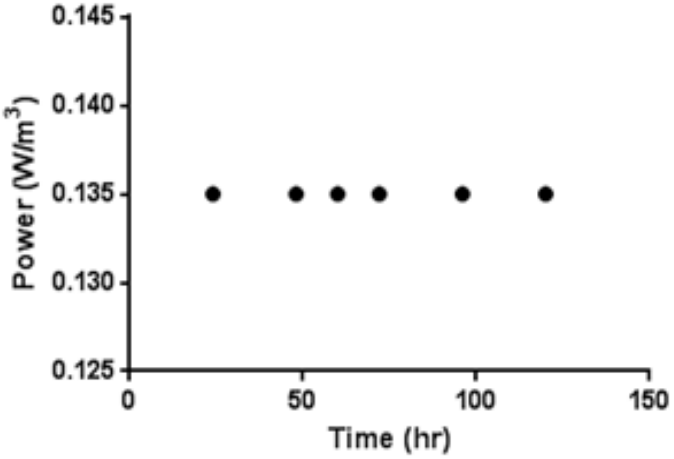


Figure 4.22: Power Generated in MFC using Cheese whey and *Saccharomyces Cerevisiae* with cellulose acetate membrane with external resistance of 500Ω

MFC was run for about 5 days and the power was calculated by taking into account the voltage and current produced. In this experiment cellulose acetate membrane was used as Proton

Exchange Membrane. When cheese whey was used as sample and *Saccharamyces cerevisiae* was used as inoculums, power production was constant on each day.

Table 4.4: Relative power generated by Biofuel cell at different conditions

Membrane	substrate	Organism	Maximum current(mA)	Maximum voltage(mV)	Maximum power (W/m^3)
Cellulose acetate	Wastewater	Mixed culture	0.075 (5^{th} day)	80.74 (5^{th} day)	15.13(5^{th} day)
Nafion membrane	Wastewater	Mixed culture	0.069 (3^{rd} day)	36.26(3^{rd} day)	6.25(3^{rd} day)
Cellulose acetate on addition of 1%H_2O_2	Wastewater	Mixed Culture	0.146 (1^{st} day)	67.0(1^{st} day)	24.45(1^{st} ay)
Cellulose acetate(autoclaved water)	Wastewater	Mixed culture	0.027(2^{nd} day)	14.1(2^{nd} day)	0.95(2^{nd} day)
Cellulose acetate	Cheese Whey	*Saccharomyces cerevisiae*	0.008	6.75	0.135

The table shows the relative voltage, current and power produced by Microbial Fuel cell in different conditions. For each case the experiment was repeated three times and the average values were taken into consideration. Maximum voltage and power was observed in the addition of Hydrogen Peroxide at the rate of 1ml/min continuously.

CHAPTER FIVE

DISCUSSION

5.1 Environmental Analysis Of waste water

Our observation showed Phosphorus to be 0.537mg/L which was found to be as similar to (Green *et al*., 2003) according to whom, total Phosphorus was found to range from 0.3mg/L to 1.7mg/L. Whereas Nitrite Nitrogen was found to be 0.139mg/L in our experiment which was in contrast to Green *et al*., 2003, that showed 0.6mg/L to 10mg/L Nitrite Nitrogen in one of the polluted river of Nepal. This may be due to the presence of less Nitrogenous substances in our waste. There are no studies till date done that determined the reducing sugar in wastewater. The wastewater showed COD of 33.36mg/L which indicated the sample collected was not that polluted as described in Water Quality Parameter of the Bagmati River at Sundarighat which was found to be up to 255 mg/L COD (ENPHO, 2003).This may be because the water is flowing from less polluted area. However the COD obtained was higher than that of Bhattarai *et al*,2008 i.e 13mg/L, which may be due to high amount of domestic and agricultural wastes in the study sites from nearby settlement areas.

5.2 Isolation and Identification of organism

Enterobacter spp, *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia* coli, *Klebsiella* spp and *Salmonella* spp were the species recorded in our wastewater sample. As similar to our result ,M. P. Das, 2013 suggested the presence of *Enterobacter* spp, *Staphylococcus spp* and *Pseudomonas* spp. Park *et al*, 2014 also reported the predominancy of Pseudomonas species in waste water. Similarly as per our result,(Vasileva-tonkova*et al* 2008, reported the presence of *Pseudomonas spp, Staphylococcus spp and Escherichia spp.* Also H.A *et al* 2002 reported the presence of *klebsiella spp, Staphylococcus spp, Pseudomonas spp* and *E.coli* in the wastewater.

5.3 MFC for wastewater treatment and power generation

Chemical oxygen Demand determines the amount of organic pollutants found in surface water (e.g. lakes and rivers) or wastewater, making COD a useful measure of water quality. COD is considered to be a indirect measure for the pollutant in the wastewater. Since MFC was able to show the removal of Chemical Oxygen Demand by 69.96%,it can taken as a new approach for wastewater treatment which is in accordance with the research by Ghangrekar & Shinde, 2006 who claimed MFC are capable of reducing the COD from 50% to 90%.

The amount of reducing sugar lowered from 0.105mg/L to 0.014 mg/L on the fifth day. The result indicates the utilization of sugar present in the wastewater. Although the concentration of reducing sugar was very low in the sample it was able to produce considerable power showing that other organic matter in the wastewater has equal contribution in the production of electricity. Youngho *et al*, 2010 reported that total Nitrogen was not effectively removed in continuous mode MFCs when the reactors were operated under high organic loading rates of 6.0 –182 g COD/L-d. While total nitrogen removals were high in elongated fed batch mode.

According to Rabaey *et al.,* 2005, *Pseudomonas* spp, *Escherichia* coli are good microbes for electricity generation in microbial fuel cell. Using complex mixed cultures (anodic microcosm) allows much wider substrate utilization. It means that the MFCs have much wider substrate specificity when mixed than do pure cultures. Based on these studies mixed culture of organism obtained from anerobic sludge was used.

Open circuit voltage of 513mV was obtained on the fifth day when mixed culture of organism was used. According to Du *et al*., 2007, the mixed culture is found to be efficient. The obtained value was slightly lower than that of brewery wastewater which was found to be 534mV according to Rohan *et al*., 2013 which was obtained on the first day however we obtained the maximum voltage on the fifth day. This might be because the brewery wastewater is excessively rich in organic contents. Similarly in a double chamber MFC voltage up to 680mV as reported by Rahimnejad *et al,* 2015 but the organism and substrate used was different than ours. We recorded the open circuit voltage to be 513mV by using wastewater while using mixed culture whereas Klebsilla *pneumonae* showed an open voltage of 453mV when Paneer whey was used as the sample as stated by Dalvi *et al* , 2011,this is because our wastewater contained very less organic content.

A maximum voltage of 0.08V was obtained when mixed culture of organism and a 500Ω resistor was used in our experiment however Pandey *et al* 2011 reported that Microbial fuel cell produced 0.5 V when Proteus vulgaris was used. Any significant voltage production was not shown in our research when cheese whey was used and inoculum provided was *saccharomyces cerevisiae* but when glucose was used as substrate *saccharomyces cerevisiae* produced a power output of 110mW/m^2 and a open circuit voltage of 479mV when Methylene Blue was used as a electron mediator (Rossi*et al*, 2015).It is because *Saccharomyces cerevisiae* did not utilize the whey. In addition, cheese whey we used might have not contained high amount of reducing sugar. Neutral Red used as electronophore might have influenced the result to some extent as the mediators used are different with distinct mechanisms.

Autoclaved wastewater was also used to examine power generation in the MFC. The maximum power with the autoclaved wastewater was 0.95W/m^3 which was very much lower than that of fresh wastewater 15.13W/m^3.The result was different from the research by Min *et al*., 2005 where the CE with the autoclaved wastewater was slightly lower (17%), but not drastically different from that with the fresh wastewater (20%). Autoclaved wastewater reduced the concentration of bacteria in wastewater which led to generation of low voltage and current. This could have been affected by other factors also and the condition in these two different studies.

When 30g/l of glucose which was highly concentrated than our wastewater was used, the maximum obtained voltage, current, and power density for Nafion 117 were 668 mV, 60.28 mA/m^2 and 9.95 mW/m^2 as reported by Rahimnejad *et al*., 2015, respectively which differed with our experiment when we used Nafion 117 , it is because the volume was twice than ours and the organism was also different than others. Other parameters such as system architecture (of different surface area) and the calculation in a different way i.e w/m^2 in their study which is different from ours must have influenced the result.

Despite all these possibilities of MFC a technique for wastewater treatment, we had problems in scaling up and enhanching the power generated by the system. We operated similar kind of Microbial Fuel cell of 2 litres at same conditions not varying the perimeters, but we could not obtain expected results. The power density is found to decrease as the system size increases and further improvements are needed to construct highly efficient reactors with reduced internal resistance and electrode over-potential to maximize power in large scale systems (Cheng & Logan, 2011) .It was concluded the internal resistance of the system played a vital role in the power generation . The surface area of electrodes could not be reduced as the distance between two compartments which might have been responsible for high resistance of the system. According to Oh *et al*., 2010 the electricity produced by MFCs will never be a cost effective source of energy in its own right but it's contribution will be in reducing the energy used in wastewater treatment.

Deep understanding of the acclimatization of the communities in MFCs and their response to environmental perturbations would reduce the perceived risks and accelerate the adoption of MFCs. However, even if this deep understanding of the biology at work in an MFC takes many years to achieve it is clear that, even in the short term, MFCs will have role in sustainable wastewater treatments.

CHAPTER SIX

CONCLUSION

The MFC showed the COD and reducing sugar removal efficiency of about 70% and 90% respectively indicating it to be suitable for wastewater treatment. Simultaneously showed a maximum voltage of 513mVin an open circuit on the fifth day and remained constant thereafter. The MFC with cellulose acetate generated power of 15.13 w/m^3whereas Nafion membrane generated 6.25 W/m^3 only hence cellulose acetate membrane as PEM was found to be better in microbial fuel cell for power generation than Nafion membrane .Nafion membrane is not suitable for neutral pH and in the presence of cation species such as Na^+, K^+, and NH^{4+} (when 10^5 times higher than H^+ concentrations). These species have more potential to transfer through the membrane rather than protons. This process causes pH increase in the cathode chamber. The high cost of Nafion along with the physical instability at the temperatures higher than 100^{o}C is the other challenge in using Nafion as membrane material. When 1 ml/min 1% H_2O_2 was supplied in cathode, power generation drastically increased, and reached to 24.45 W/m^3during 24 hours and gradually decreased. The maximum power generated was very high comparing to the power reported in normal cases which may be due to good redox activity of H_2O_2.H_2O_2 oxidizes one pollutant over another, or even to favor different oxidation products from the same pollutant. However, on the following day the decrease in power may be due to the leakage ofH_2O_2into anode which inhibited the growth of microorganism. This study revealed to show presence of oxygen enhanced power generation. It is because, in the presence of oxygen at cathode compartment, the reaction ½ O_2+2e^- $\Longrightarrow$ H_2O, E = +0.816 V which is highly feasible takes place due to which fast consumption of $e^-$and H^+ occurs and ultimately anodic reaction becomes faster. It was concluded various organic rich content substrates can be used for the generation of electricity, though not significantly high, if the organism selected is an effective one.

If certain parameters such as substrate, microorganism, external resistance and electrode materials are varied and efficiency is improved, the application of MFC can go beyond laboratory, MFC can be a emerging wastewater treatment technology along with some power production.

Summary

MFCs are a promising technology for the production of electricity from organic material and wastes. Wastewater as fuel source while achieving wastewater treatment has aided numerous startups to focus on the commercial application of MFC technology.

Currently limited applications are possible because of low MFC power output. An understanding of the microbiology of the current producing process is required before further advances in power output are possible. Three major problems that need to be addressed is proton accumulation within the biofilm, over potential at the cathode and high internal resistance of the system. Of interest are some current applications of MFCs where current production is not the major advantage, but wastewater treatment or bioremediation using a cathode or anode maybe much more promising then the electrical production of the MFC itself.

MFC technology can be a practical approach for wastewater treatment as it is sustainable, environmental friendly and effective than other conventional techniques.

Recommendation

- Molecular characterization of the organism isolated from wastewater.
- Operation of MFC with different substrates such as Glucose, lactose and other carbohydrates
- Use of any other electrode material and observing the changes in voltage produced
- Use of different organism individually and check the efficiency of MFC.
- Scaling up the volume
- Operation of MFC by varying the external resistance

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Appendices

A. Preparation of media

1. YEPD MEDIA

Ingredients	Gm/litre
Yeast extract	10
Peptone	20
Dextrose	20

2. Nutrient Agar

Ingredients	Gm/litre
Beef extract	3.0
Peptone	5.0
Agar	15.0

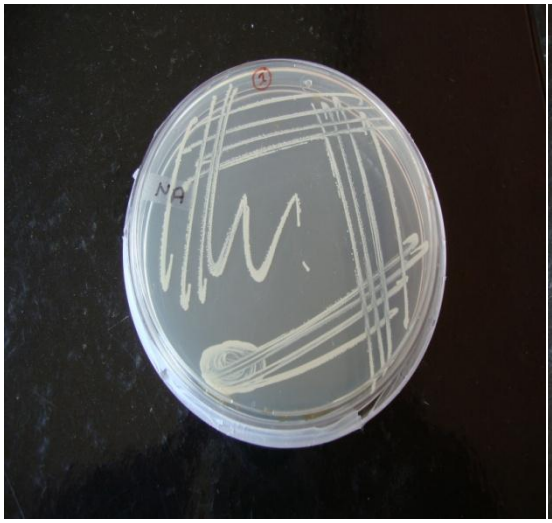
3. Lurea Bertani Broth

Ingredients	Gm/litre
Tryptone	10
Yeast extract	5.0
Sodium chloride	10

B. Preparation of reagent

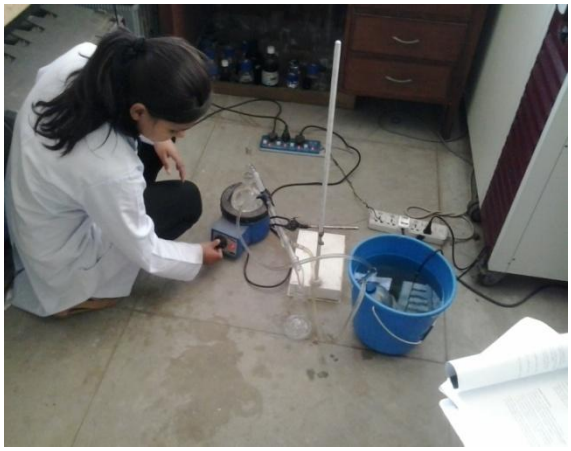
1.Nessler reagent	Dissolve 100 g mercuric iodide and 70 g of potassium iodide in small amount of water. Add this mixture to a cooled solution of 160 g NaOH in 500 mL of distilled water and dilute up to the mark with distilled water in a 1000-mL volumetric flask
2. Sodium Tetraborate solution 0.025 mol/L.	Dissolve 5 g of anhydrous sodium tetraborate, Na2B4O7 (or Na2B4O7.10H2O) in distilled water and dilute up to the mark with distilled water in 1000-mL volumetric flask.
3. Digestion solution:	Add 10.2 g of potassium dichromate K2Cr2O7, 167 mL of conc.sulfuric acid, H2SO4, and 33.3 g of mercury sulfate HgSO4 to 500 mL of distilled water, cool and dilute to 1000 mL with distilled water in 1000-mL volumetric flask
4. Catalyst solution:	Add 22 g of silver sulfate, AgSO4 to a 4.09 kg bottle of concentrated sulfuric acid, H2SO4 and let stand for 2 days until dissolved
5. Buffer Color reagent	Add 105 mL of concentrated hydrochloric acid (HCl), 5.0 g sulfanilamide and 0.5 g N- (1-naphthyl)- ethylenediamine dihydrochloride to 250 mL of distilled water, stir until dissolved and add 136 g of sodium acetate and stir again until it dissolves.Dilute the solution to 500 mL with distilled water and store in dark
6.Potassium Phosphate buffer	61.5ml of 1M K_2HPO_4 and 38.5ml of 1M KH_2PO_4 is mixed and PH is adjusted to 7.

Bacterial plates



Photos

Sample collection



Distillation for estimation of ammonia

Presentation

International Young Scientist Award For Best Oral Presentation on 5th International Science Congress (ISC-2015) 8th-9th December, Tribhuvan University, Kathmandu ,Nepal

Oral Presentation on '**Microbial fuel cell : A promising technology for Wastewater Treatment and generation of Electricity'on** 1st International conference On Bioscience And Biotechnology February 4-6 Kathmandu Nepal

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