Enhancement in treatment of dairy industrial waste water and electricity production using graphite electrode

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List of Abbreviation and Acronym

BOD- Biological Oxygen Demand
CNT- Carbon nanotube
COD- Chemical Oxygen Demand
CV- Cyclic Voltammetry
DDC- Dairy Development Co-operation
DNS- Di-nitro Salicylic Acid
DO- Dissolved oxygen
EB- Emeraldine Base
ES- Emeraldine Salt
Gm- gram
LB- Luria-Burtani
mA- Milli Ampere
MA- Mac Conkey Agar
MFC- Microbial Fuel Cell
µL- Microlitre
mg/L- Milligrams per litre
mg- Milligrams
mL- Milliliter
mV- Millivolts
NA- Nutrient Agar
NAD- Nicotinamide adenine dinucleotide
NB- Nutrient Broth
Nm- Nanometer
O/F- Oxidative/Fermentative
OCV- Open circuit voltage
PEM- Proton exchange membrane
Ppm- Parts per million
SIM- Sulphide, Indole and Motility
UV- Ultraviolet
VP- Voges Proskauer
W- Watt

Abstract

Microbial fuel cell (MFC) can be used for dairy waste treatment and bioelectricity production. MFC can act as ecofriendly and low cost management of energy production.

In this study, microbial fuel cell uses the fuel source from the dairy waste (Paneer whey) biomass. The MFC was designed as two chambered that included an anaerobic anode and aerobic cathode compartment, and was separated from each other by nafion membrane. The anode and cathode electrodes were made from graphite felt hung with platinum wires. The bacterial mixed culture used was obtained from sewage of DDC enriched in LB broth and lactobacillus strains isolated from the fermented pickle. MFC showed a maximum open circuit voltage of 633mV and rendered maximum power of 25.869W/m^3on the fifth day using the electrode coated with carbon nanotube (CNT) under 1000Ω external load. The dairy waste contained 1.8432 mg/L of reducing sugar, 0.23450 mg/L of phosphorous, 0.0359 mg/L of Ammonical-Nitrogen and 689.163 mg/L of COD before treatment. The treatment in microbial fuel cell resulted in reduction of reducing sugar by 95.06%, reduction of phosphorous by 65.67%, reduction of Ammonical- Nitrogen by 67.13% and reduction of COD by 58.27%. The anode chamber was inoculated with single strain of bacteria like *Klebsiella*, *Lactobacillus* and yeast Y2. Open circuit voltage in single strain of these bacteria and yeast were less than the mixed culture of bacterial strains (*Klebsiella* sps, *Salmonella* sps, *E.coli*, *Pseudomonas* sps and *Lactobacillus* sps). The power production using phosphate buffer in the mixed culture and lactobacillus strain was more than the power production using sodium acetate buffer. The MFC showed maximum power production of 22.621W/m^3using the phosphate buffer mixed with potassium ferricyanide as a chemical oxidizer than the sodium acetate mixed with potassium ferricyanide. The cyclic voltammetry resulted that the MFC is reasonably stable and can be used for the energy production. The result showed that coating the graphite electrode with CNT has a huge potential regarding enhancement of production for bioelectricity and dairy waste water treatment.

Keywords: Microbial fuel cell, Mixed culture, Carbon Nanotube, Cyclic voltammetry, Dairy waste treatment, Bioelectricity

CHAPTER ONE

INTRODUCTION

Wastes are any substance or object which the holder discards or intends or is required to discard. They are substances or objects which are required to be disposed of by the provisions of national law. 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 (Bontoux et al., 2007).

Toxic waste materials can contaminate surface water, groundwater, soil, and air which causes more problems for humans, other species, and ecosystems. Using waste as fuel can offer important environmental benefits. It can provide a safe and cost-effective option for wastes that would normally have to be dealt with through disposal. It can help reduce carbon dioxide emissions by diverting energy use from fossil fuels, while also generating energy and using waste as fuel can reduce the methane emissions generated in landfills by averting waste from landfills Waste treatment and disposal produces significant green house gas (GHG) emissions, notably methane, which are contributing significantly to global warming.

Environmental issues related to energy and waste, are the two biggest problems which cannot be overlooked. There is mismanagement of waste which is causing irreversible damage to the environment. For energy, there is still very much dependency on non-renewable sources. The demand of energy is such that, there is a need to promote energy production from renewable sources. These two environmental concerns can be handled in one go by Microbial Fuel Cell (MFC).

MFCs are fuel cells that are capable of converting chemical energy available in organic substrates into electrical energy using bacteria as a biocatalyst to oxidize the biodegradable substrates (www.microbialfuelcell.org). The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production (Schwartz, 2007).

In an MFC, bacterial community present in the anode compartment uses organic substrates as fuels to produce electrons and protons through biological processes (Rabaey and 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 (Logan and Regan, 2006; Salgado, 2009). Bacteria then transfer these electrons to anode from where 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 anode compartment is typically maintained under anaerobic conditions as oxygen inhibits electricity generation whereas the cathode is exposed to oxygen (Logan, 2008; Rahimnejad Mostafa, 2009). Du et al., 2007 reported that 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.

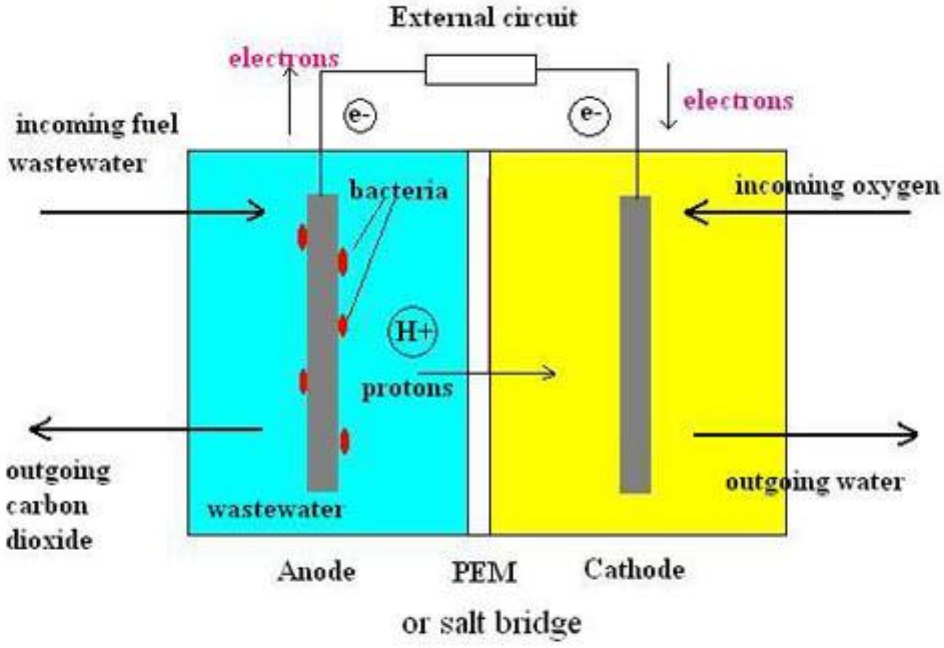

Fig1.1: Schematic representation of Microbial fuel cell (illumin, 2016)

Commercial application of MFCs for wastewater treatment is difficult, because most of the mediators are expensive and toxic in nature. Hence, today emphasize is being given on development of mediator less MFC, enhancing its power production, and reduction of its operational cost, to increase its acceptance as wastewater treatment process (Ghangrekar & Shinde, 2006)

Most of the research these days are focused on the biomass based energy. Biomass is a term used for organic material produced by living organisms where the chemical energy found in the bonds between the atoms in the organic matter is used up .From crops to the industrial waste, or house hold and domestic waste there is plenty of biomass as possible fuel sources. Microbial fuel cell is applicable in neutralizing the pollutants from the contaminated sites .Direct disposal of these waste may cause serious environmental problems because of the presence of high organic content in the waste .These waste are responsible for higher Chemical Oxygen demand phosphorous and nitrogen content ,which major factor for water pollution. So microbial

fuel cell can be further exploited for treatment of these wastes and converting the chemical energy to electrical energy by oxidation of biodegradable organic substrates.

The recent energy crisis has reinvigorated interests in MFCs among academic researchers as a way to generate electric power or hydrogen from biomass without a net carbon emission into the ecosystem. MFCs can also be used in wastewater treatment facilities to break down organic matters (Zhuwei et al., 2007)

Microbial production of electricity may become an important form of bioenergy in future because MFCs offer the possibility of extracting electric current from a wide range of soluble or dissolved complex organic wastes and renewable biomass. A large number of substrates have been explored as feed. The major substrates that have been tried include various kinds of artificial and real wastewaters and lignocellulosic biomass. Though the current and power yields are relatively low at present, it is expected that with improvements in technology and knowledge about these unique systems, the amount of electric current (and electric power) which can be extracted from these systems will increase tremendously providing a sustainable way of directly converting lignocellulosic biomass or wastewaters to useful energy (Pant et al.,2010).

Statement of problem

More than 7 billion people in our planet are directly or indirectly exploiting the energy stored in fossil fuels. This has supported the global industrialization and economic growth during the past hundred years. This is not a sustainable method for energy utilization. Fossil fuel will not actually run out for the next 100 years but it’s for sure there is increasing demand of fossil fuel for the upcoming years.

The use of the fossil fuels tends to increase in the concentration of carbon dioxide in the atmosphere. This has led to global warming and melting of glaciers resulting in rising of sea levels. Global warming can be slowed, and perhaps reversed, only when society replaces fossil fuels with renewable, carbon-neutral alternatives.

The best option is bioenergy: the energy is captured in biomass and converted to energy forms useful to modern society. To make a dent in global warming, bioenergy must be generated at a very high rate, since the world today uses ∼10 TW of fossil-fuel energy. And, it must do so without inflicting serious damage on the environment or disrupting our food supply. For bioenergy several microorganism-based options have the potential to produce large amounts of renewable energy without disruptions. The bioenergy is stored in the form of electrical energy by oxidation of biodegradable organic substrates.

This microorganism based option is symbolically, the Microbial fuel cell reactor. In one approach, microbial communities convert the energy value of various biomass residuals to socially useful energy. Biomass residuals come from agricultural, animal, and a variety of industrial operations, as well as from human wastes. Microorganisms can convert almost all of the energy in these wastes to methane, hydrogen, and electricity.

Another problem microbial fuel cell can address is the treatment of the organic waste. Waste water treatment has become a worldwide problem as conventional sewage wastewater treatment requires a lot of energy for aerating the sludge. In recent years researches are being done to generate anaerobic method which minimizes the cost and energy. Researchers are opting to find an innovative method which can solve these two problems simultaneously i .e treatment and generation of electricity through so called waste.

Rationale

Clean fuels, significantly fuel cells and biofuels, as new sources of energy without any pollution are suitable replacements of traditional fossil fuels. Although in is its infancy MFC is a promising renewable and sustainable technology that is considered to be the most efficient for the production of electricity with minimal or zero use of hydrocarbon from the substrate which would otherwise considered as waste. MFCs are one of the newest technologies to produce energy from different sources of substrates. Because of the promise of sustainable energy generation from different substrates such as organic wastes, research has been intensified in this field in the last few years. The generated power in MFC is still too low and researchers are working to improve it for commercial application.

Sustained innovations and continuous development efforts have established the usefulness of MFCs towards many specialized and value-added applications beyond electricity generation. The MFCs have the potential to remove organic carbon matter by treating almost all kind of organic rich wastewater ranging from human faces , manure sludge, cassava mill water, confectionery wastewater ,fermented vegetable waste, domestic wastewater, paper industry wastewater (Mathuriya & Yakhmi, 2014).Sediment.

The dairy wastes from the industries tends to pollute the water sources more due to high amount of lactose content ,carbohydrate and chemical oxygen demand.In this study, we aim to develop a MFC which is capable of enhancing the treatment of industrial dairy waste (Paneer whey) by decreasing the reducing sugar, COD, Phosphorous and Nitrogen content. Additionally, we aim to produce electricity by using substrate from the dairy waste. This can be significant to mitigate fuel and electricity crisis in long run.

HYPOTHESIS

Due to unmanagement of waste water produced from household and industries, environmental pollution is a major concern. Chemical treatment of these pollutants includes sophisticated technology, to remove contaminants or the specific pollutants, but the process is very expensive. This can also lead to risk of various chemicals, on the water sources, affecting the ecology.

Thus, MFC may possess the capability in neutralizing the pollutants generally from the industrial dairy waste. The fuel cell can be enhanced and optimized using various bacterial strains for reducing the contaminants and efficient electricity production.

OBJECTIVES

General Objectives

- To prepare a MFC in laboratory and to determine its efficiency.
- To produce and study the generation of electricity and dairy waste treatment using paneer whey as substrate by using cultures from DDC sewage and lactose utilizing strain.

Specific Objectives

- To isolate, characterize and identify the microorganisms present in the sewage waste water.
- To use the microorganisms to remove or neutralize the pollutants from the dairy waste and analyze the changes on the COD, Phosphorous level, sugar concentration and Nitrogen.
- To measure the voltage of the electricity produced with and without external resistance (load) over the fixed time period in different concentrations of substrate.
- To study the variations in electricity production using different enhancement method to treat the dairy waste and produce electricity.

CHAPTER TWO
LITERATURE REVIEW

2.1 Industrial Dairy Waste

The dairy industry, like many others, is challenged with rising costs for wastewater treatment and disposal. Tighter environmental restrictions coupled with rising energy and process costs limit production and expansion in many facilities. Existing water treatment systems are often loaded beyond design flows, causing inefficient operation. Wastewater from dairy processing can contain variable and high levels of nutrients, TSS, BOD, and floatable materials. The wastewater from a dairy can amount to 20–30 million liters, which requires large areas for sedimentation basins for settleable solids. The average characteristics of the wastewater from a milk processing includes a biochemical oxygen demand (BOD) of about 1,000 mg/L, a chemical oxygen demand (COD) of about 1,900 mg/L, a total solids content of 1,600 mg/L, and a suspended solids content of 300 mg/L (Jonsson et al.,2006).

Whey is the liquid remaining after milk fat and casein have been separated from whole milk. It can be used directly as a substrate for the growth of different microorganisms (Paneesar & Kennedy, 2011). Paneer whey is rich in lactose. Presence of lactose in whey is responsible for more than 90% biochemical oxygen demand (BOD) (Patel & Murthy, 2011).

2.2 Role of MFC in waste water treatment

There has been great interest in using MFCs for wastewater treatment and power generation has been shown using a variety of wastewaters including both domestic and industrial wastewaters (Aelterman *et al.*, 2006).One of the first tests exploring industrial wastewater treatment using an air–cathode MFC with graphite rods demonstrated 26 mW/m2 (Liu et al., 2004), with a total COD removal of about 50% and current recovery of 4%. A higher power density of 464 mW/m2 (15.5 W/ m3), with total COD removal of 40–50% and coulombic efficiencies of 20%, was achieved by flowing the wastewater through the carbon cloth anode (Cheng et al., 2006).

2.3 Working Principle of MFC

The first MFC concept was demonstrated by MC Potter in 1910. Using platinum electrodes electricity was produced from living cultures of Escherichia coli and Saccharomyces. In the 1980's it was discovered that current density and power output could be greatly enhanced by the addition of electron transfer mediators, or electron shuttles which can carry electrons from inside the cell to exogenous electrodes(Du, Li, & Gu, 2007).

Microbial fuel cells (MFCs) are devices that use bacteria as the catalysts to oxidize organic and inorganic matter and generate current. In an MFC, bacterial community present in the anode compartment uses organic substrates as fuels to produce electrons and protons through biological processes (Rabaey and Verstraete, 2005). Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material containing a resistor, or operated under a load (i.e., producing electricity that runs a device). By convention, positive current flows from the positive to the negative terminal, a direction opposite to that of electron flow. Electrons can be transferred to the anode by electron mediators or shuttles by direct membrane associated electron transfer or by so-called nanowires produced by the bacteria, or perhaps by other as yet undiscovered means (Logan et al.,2006). The protons generated are diffused through the PEM to the cathode and subsequently combine with the electrons and oxygen to form water.

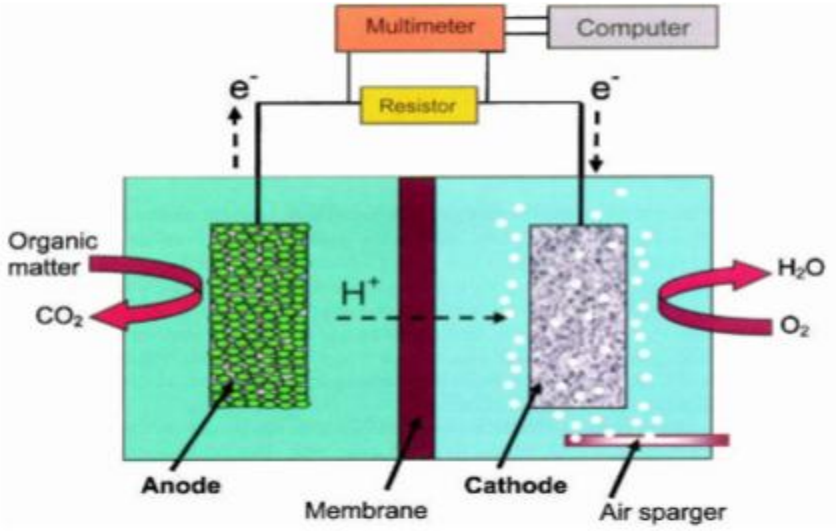

Fig2.1: Graphical representation of microbial fuel cells (Logan, B.E. 2008.Microbial fuel cells)

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

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

2.4 Components of MFCS (Juan, 2013)

The microbial fuel cell consists of simple yet vital components to effectively harness the energy are as follows:

1. Electrodes- Both in cathode and anode chambers
2. Proton Exchange Membranes- Widely used as naffion membrane as least resistive membrane.
3. Substrate - Any organic matter used as source for microorganisms.eg: waste water
4. Bacteria - Exoelectrogens most suited for MFC.

Table 2.1: Basic components of MFC (Juan, 2013)

Items	**Materials**
Anode	Graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black, reticulated vitreous carbon (RVC)
Cathode	Graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black, RVC
Anodic Chamber	Glass, polycarbonate, Plexiglass
Cathodic Chamber	Glass, polycarbonate, Plexiglass
Proton Exchange Membrane (PEM)	Nafion, Ultrex, polyethylene.poly (styrene-co-divinylbenzene); salt bridge, porcelain septum or solely electrolyte
Electrode Catalyst	Pt, Pt black, MnO2, Fe^{3+}, polyaniline, electron mediator immobilized on anode

2.4.1 Substrates used in MFC

Substrate provides not only energy for the bacterial cells to grow in the MFCs but also influences the economic viability and overall performance such as power density and coulombic efficiency of MFCs. The composition, concentration and type of the substrate also affect the microbial community and power production (Cheng and Logan, 2011; Pant D, 2010). Many organic substrates including carbohydrates, proteins, volatile acids, cellulose and wastewater have been used as feed in MFC studies. It can range from simple, pure, low molecular sugars to complex organic matter containing waste water to generate electricity.

Table 2.2: List of substrates in MFC (Pant D, 2010)

Type of Substrate	Concentration	Current Density(mA/cm^2)
Acetate	1g/L	0.8
Lactate	18Mm	0.005
Glucose	6.7Mm	0.7
Glucoronic acid	6.7Mm	1.18
Starch	10gm/L	1.3
Cellulosic Particles	4gm/L	002
Xylose	6.7mM	0.74
Domestic Wasterwater	600mg/L	0.06
Brewery Wastewater	2240mg/L	0.2

2.4.2 Electrode Materials

The efficiency of MFC is dependent on the material of electrode used. The electrodes have a certain resistance hence the most effective ones are the least resistive. The anodic resistance contributes to the overall cell resistance in the MFC operation. However, the use of highly efficient electrode materials (i.e Platinum) is not economically feasible for the large scale applications, thus investments on more cost-effective alternatives is priority in MFC research. The material characteristics which are critical for an effective electron transfer are high conductivity and mechanical strength. There is no requirement for bacteria adhesion. The scalability and cost-effectiveness are also taken into consideration (Juan, 2014).

For anode, carbon cloth, carbon felt, graphite felt, carbon mesh and graphite fiber brush are frequently used due to their stability, high electric conductivity and large surface area (Logan, 2010; Logan and Regan, 2006). For cathodes, platinum (Pt), platinum black, activated carbon (AC), graphitebased cathodes and biocathodes are used (Chen et al., 2008; Du et al., 2007). Though platinum coated electrodes are more efficient and superior in power production due to higher catalytic activity with oxygen than other electrodes, they are not cost effective (Logan, 2010; Oh et al., 2004). Alternate catalysts for platinum include ferric iron, manganese oxides, iron and cobalt based compounds. Ferricyanide (K_3 $(Fe(CN)_6)$ is frequently used as an electron acceptor in the MFCs due to its good performance and low overpotential3 (Logan and Regan, 2006). Biocathodes increases the power by decreasing the overpotential (Huang et al., 2011). Alternately, the cathode can contain oxygen and is preferred because it simplifies the operation of the cell and is the most commonly used electron acceptor in MFC (Sengodon 2012)

2.4.3 Microbes in MFCs

A wide variety of bacterial communities are found to have the ability to oxidize organic compounds and transfer electrons to the anode. MFCs make use of both the mixed cultures and pure bacterial cultures (Cheng et al., 2005). Rabaey et al (2005) reported that the mixed cultures have high resistance for process disturbances, substrate consumption and higher power output. The electrochemically active bacteria in MFCs may be aerobes or facultative anaerobes and the reaction temperature in MFCs depend on the bacterial tolerance to temperature (mesophilic/thermophilic) (Logan, 2008; Rabaey and Verstraete, 2005). Not only the electrochemically active, iron-reducing bacteria (Shewanella and Geobacter) but also other group of bacteria (Klebsiella pneumonia, Rhodopseudomonas palustris, Dessulfobulbus propionicus) that are isolated from the wastewater showed great potential to be used in MFCs (Sharma and Kundu, 2010). A number of recent reports reviewed screening, identification of microbes, their ability to generate electric current and power densities in detail (Logan, 2009; Logan et al., 2005). Extra attention will be paid to certain bacteria which have the ability to generate electricity and to transfer electron effectively in the anode. This type of bacteria is called Exoelectrogens "exo-"for exocellular and "electrogens" based on the ability to directly transfer electrons to a chemical or material that is not the immediate electron acceptor. These exoelectrogens can be sourced in a number of places, according to Du et al, they are found in soil, marine sediment, waste water, fresh water sediment and activated sludge, which are rich with these microorganisms.

2.4.4 Proton Exchange Membrane

The proton exchange membrane is a core component that greatly influences electrochemical performance in MFCs. The PEM has a structure which enables only hydrogen ions or protons to pass through. "Hydrogen with proton exchange membrane fuel cells (PEMFCs) is currently considered as a potential next generation alternative energy technology because of the high energy density and high abundance of hydrogen in nature."The most widely used polyelectrolyte for proton exchange membrane is the Nafion ionomer which increases the three- dimensional zone of catalytic activity. As the hydrogen ions passes through the membrane, it forms water and carbon dioxide with the electrons in the reduction process and completing the circuit.

2.5 Electron Transfer in MFC

2.5.1 Mediated MFC

Most microbial cells are electrochemically inactive. Electron transfer from microbial cells to the electrode is facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid and neutral red. Most available mediators are expensive and toxic (Logan etal.,2006)

2.5.2 Mediator less MFC

If no exogenous mediators are added to the system, the MFC is classified as a “mediator-less” MFC even though the mechanism of electron transfer may not be known. Mediator-free microbial fuel cells use electrochemically active bacteria to transfer electrons to the electrode (electrons are carried directly from the bacterial respiratory enzyme to the electrode). Among the electrochemically active bacteria are *Shewanella putrefaciens*, *Aeromonas hydrophila* and others. Some bacteria are able to transfer their electron production via the pili on their external membrane. Mediator-free MFCs are less well-characterized, such as the strain of bacteria used in the system, type of ion-exchange membrane and system conditions (temperature, pH, etc.) (Juan, 2013).

2.6 MFC Designs

2.6.1 Single chambered MFCs

The one-compartment MFC consists of an anode in a rectangular anode chamber coupled with air-cathode. Protons are transferred from the anolyte solution to the porous air –cathode. The cube is usually made of perspex plastic. A typical one-compartment MFC eliminates the need for the cathodic chamber by exposing the cathode directly to the air. The most common is the cube reactor design which is shown in Figure. Compared to the two chambers 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).

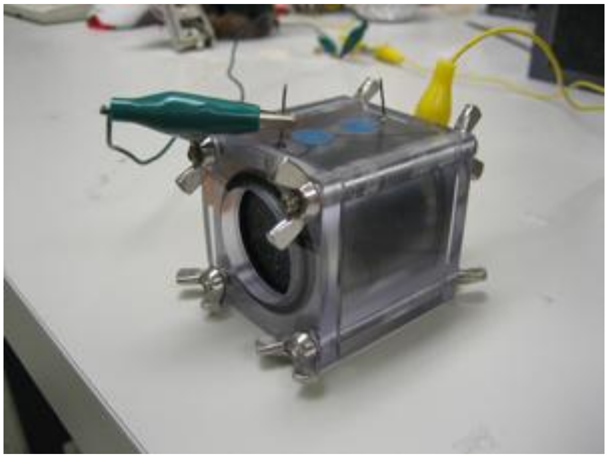

Fig2.2: A single-chamber MFC (courtesy of Penn State)

2.6.2 Double Chambered MFCs

This is the most widely used design consisting of two chambers with the anode and cathode compartments separated by an ion exchange membrane. The anode chamber is kept oxygen free for anaerobic breakdown process to occur, which is usually purged with nitrogen. Although the H-type or dual-chambered microbial fuel cells is the most common in laboratory use, it is the most challenging to scale up due to the impractical configuration, high internal resistance and electrode based losses (Du et al., 2007; Logan and Regan, 2006; Nwogu, 2007).

Fig 2.3: A double chambered MFC (Courtesy of Penn State)

2.6.3 Tubular MFCs

The single-chambered, tubular, continuous microbial fuel cell uses granular graphite matrix as the node which generates high power outputs. This type of MFC have proven to be most effective in continuous flow operation, however, there is about 57% changes in the chemical oxygen demand concentration across the reactor and a significant decrease in current density. This set up does not require a strictly controlled anaerobic environment and adapted the form of a helix which allows the fuel to flow through at a certain flow rate. This MFC configuration is most applicable in commercial use as it yields high power densities with minimum cost in terms of the materials used. The coiled helix tubular MFC concept might be the next step to realizing practical applications (Scott et al., 2012)

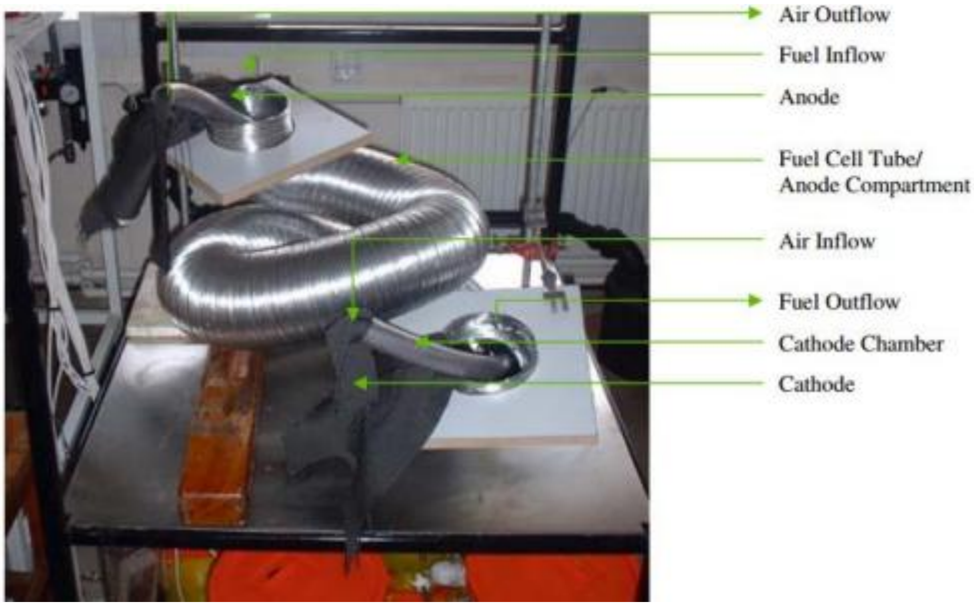

Fig 2.4: A tubular MFC (Juan 2013)

2.6.4 Stacked MFC

In this design, several single cell MFCs are connected together in series or in parallel to achieve high current output (Du et al., 2007). Due to higher electrochemical reaction rate, a parallel connection can generate more energy than a series connection when operated at the same volumetric flow but is prone to higher short circuiting compared to a series connection (Aelterman et al., 2006; Schwartz, 2007).

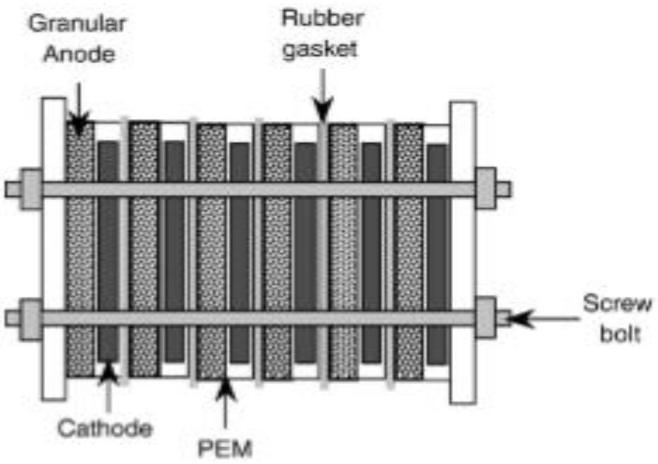

Fig2.5: A Stacked MFC (Schwartz, 2007)

2.6.5 Up-flow MFC

The cylinder shaped MFC consists of the anode (bottom) and the cathode (top) partitioned by glass wool and glass beads layers. The feed is supplied from the bottom of the anode passes upward of the cathode and exits at the top. The diffusion barrier among the electrodes provides a gradient for proper operation of the MFCs (Du et al., 2007; Kim 2008; Schwartz, 2007). This design has no physical separation and so there are no proton transfer associated problems and is attractive for wastewater treatment (Kim 2008).

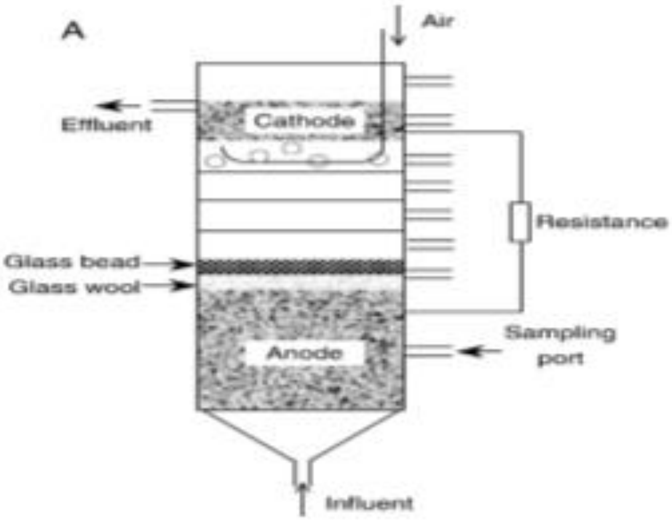

Fig 2.6: An up-flow MFC (Zhuwei et al., 2007)

2.7 Application of MFC

2.7.1 Waste water treatment

Micro-organisms can perform the dual duty of degrading effluents and generating power. MFCs are presently under serious consideration as devices to produce electrical power in the course of treatment of industrial, agricultural, and municipal wastewater. When micro-organisms oxidize organic compounds present in waste water, electrons are released yielding a steady source of electrical current. If power generation in these systems can be increased, MFCs may provide a new method to offset operating costs of waste water treatment plants, making advanced waste water treatment more affordable in both developing and industrialized nations. In addition, MFCs are also known to generate less excess sludge as compared to the aerobic treatment process (Logan et al., 2006).

2.7.2 BOD sensing

Another potential application of the MFC technology is to use it as a sensor for pollutant analysis and *in situ* process monitoring and control. Biological Oxygen Demand (BOD) is the amount of dissolved oxygen required to meet the metabolic needs of aerobic organisms in water rich in organic matter, such as sewage. The proportional correlation between the coulombic yield of MFCs and the concentration of assimilable organic contaminants in wastewater make MFCs possible usable as BOD sensors. An MFC-type BOD sensor can be kept operational for over 5 years without extra maintenance, far longer in service life span than other types of BOD sensors (oji et al., 2012)

2.7.3 *Power supply to remote sensors*

With the development of micro-electronics and related disciplines the power requirement for electronic devices has drastically reduced. Typically, batteries are used to power chemical sensors and telemetry systems, but in some applications replacing batteries on a regular basis can be costly, time-consuming, and impractical. A possible solution to this problem is to use self-renewable power supplies, such as MFCs, which can operate for a long time using local resources. Extensive research toward developing reliable MFCs to this effect, is focused mostly on selecting suitable organic and inorganic substances that could be used as sources of energy (Yang et al., 2015).

2.7.4 Hydrogen production

Hydrogen production by modified MFCs operating on organic waste may be an interesting alternative. In such devices, anaerobic conditions are maintained in the cathode chamber and additional voltage of around 0.25 V is applied to the cathode. Under such conditions, protons are reduced to hydrogen on the cathode. Such modified MFCs are termed bio-electrochemically assisted microbial reactors (Regan et al., 2008).

2.7.5 Implanted Medical Devices

A major application of the MFC is in the powering of implanted medical devices using glucose in human body and oxygen from blood as anode and cathode respectively. This application of MFC supplements power requirements for biomedical devices implanted in the human body reducing the need for routine surgery for the replacement of batteries. eg pacemakers (Opara et al., 2011)

Other applications of Microbial fuel cell are in brewery industries, desalination of plants and pollution remediation.

2.8 Use of organic electronic in MFC

Organic electronics provides a technology that allows the production of flexible, conformal, rollable or large area electronic devices; thus introducing features that cannot be done or struggle to do the conventional electronics, and therefore increasingly creating new markets.

One of the most important advantages of organic electronics is the variety of device fabrication methods that can be used, covering from the expensive and more conventional technology based on vacuum deposition, to the less expensive solution-based processes, such as printing, doctor blading, or spin/spray coating technologies. While the former is based on production steps that require high temperature and high vacuum, the latter are generally characterized by room temperature and atmospheric pressure condition, leading to cheaper devices.

In recent years, organic electronics have attracted great attention due to their multiple advantages such as light weight, flexibility, large area fabrication and cost-effective production processes. The recent progress in fabricating organic electronic devices has been achieved with the development of new materials which provide competing functionalities to the electronics devices. One of the material with great potential in the organic electronics field is carbon nanotubes (CNTs) (Qiao et al., 2007).

2.8.1 CNT based material

CNTs, allotropes of carbon, have been shown to be promising alternative material for MFC electrode because of their electrical conductivity, chemical stability, biocompatibility, high specific area and also catalytic properties. It is also reported that CNTs have strong cell adhesion, cell attachment and growth properties (Mustakeem, 2015).

With the incorporation of CNT, the nanofibrillar composite reaches conductivities 20 times higher than that of the pure polymer. In the recent years, a variety of approaches have been proposed to solubilize them and thus enhance their processability, including the covalent functionalization by treatment with strongly oxidizing acids , extensive high-power sonication followed by non-covalent modification of the nanotubes with polymers and surfactants (Qiao et al.,2007)

2.8.2 Composite material

The combination of CNT with polymers could introduce also new electronic properties based on the new morphological disposition or based on the electronic interaction between the two components which leading to new functionalities and applications (Cabezas, 2013).It is reported that CNTs incorporated with a conductive polymer can lead to a synergistic effect (Qiao et al., 2010) showed that a CNT-polyaniline composite enhanced the electrocatalytic property and adhesion with the bacterial cell. They assumed that it was because of the protective effect of polyaniline and large increase in the surface area. Other reports showed that CNTs deposition over carbon paper increases the power density by six times as compared to a graphite electrode. Polypyrrole coated CNTs were tested in a dual chambered MFC and characterized by cyclic voltametry. The result showed high peak current compared to the separation between the peak voltages, indicating that the polypyrrole - CNT composite increases the electrochemical active area (Mustakeem, 2015).

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. DNS (3, 5-Dinitrosalicyclic acid)
4. Sodium Hydroxide
5. Sodium Tetra Borate
6. Nesslers’ Reagent
7. Glucose
8. Ammonium Chloride
9. Potassium Hydrogen Phthalate
10. Silver Sulphate
11. Mercuric sulphate
12. Potassium Dichromate
13. Sulfuric Acid
14. Ammonium Per sulfate
15. Potassium Dihydrogen Phosphate
16. Antimony potassium tartarate
17. Ammonium Molybdate

18. Ascorbic Acid

19. Sodium Acetate

20. Potassium Ferricyanide (Merck Pv.Ltd)

21. Agarose (Merck Pv.Ltd)

22. Methanol

23. Acetone

24. Hydrogen peroxide

25. Sodium potassium tartarate

3.1.3 Glass wares

1. Glass Pipette

2. Glass Beaker

3. Conical flask

4. Reagent bottles

5. Petriplates

3.1.4 Instruments and Equipments

1. Microbial fuel cell setup

2. Incubator

3. Laminar flow

4. Hot air oven

5. Water Bath

6. PH meter

7. Centrifugation machine

8. UV Transilluminator

10. UV Spectrophotometer
11. Autoclave Cooker
12. Microscope
13. Magnetic stirrer
14. Gel electrophoresis tank
15. PCR machine
16. Electodes (Graphite, Platinum wire)
17. Resistor
18. Verde Flex Peristaltic Pump (Major Science)
19. Nafion membrane (117)
20. Battery (9V, 1.5V)
21. Multimeter (FLUKA)

3.2 Methodology

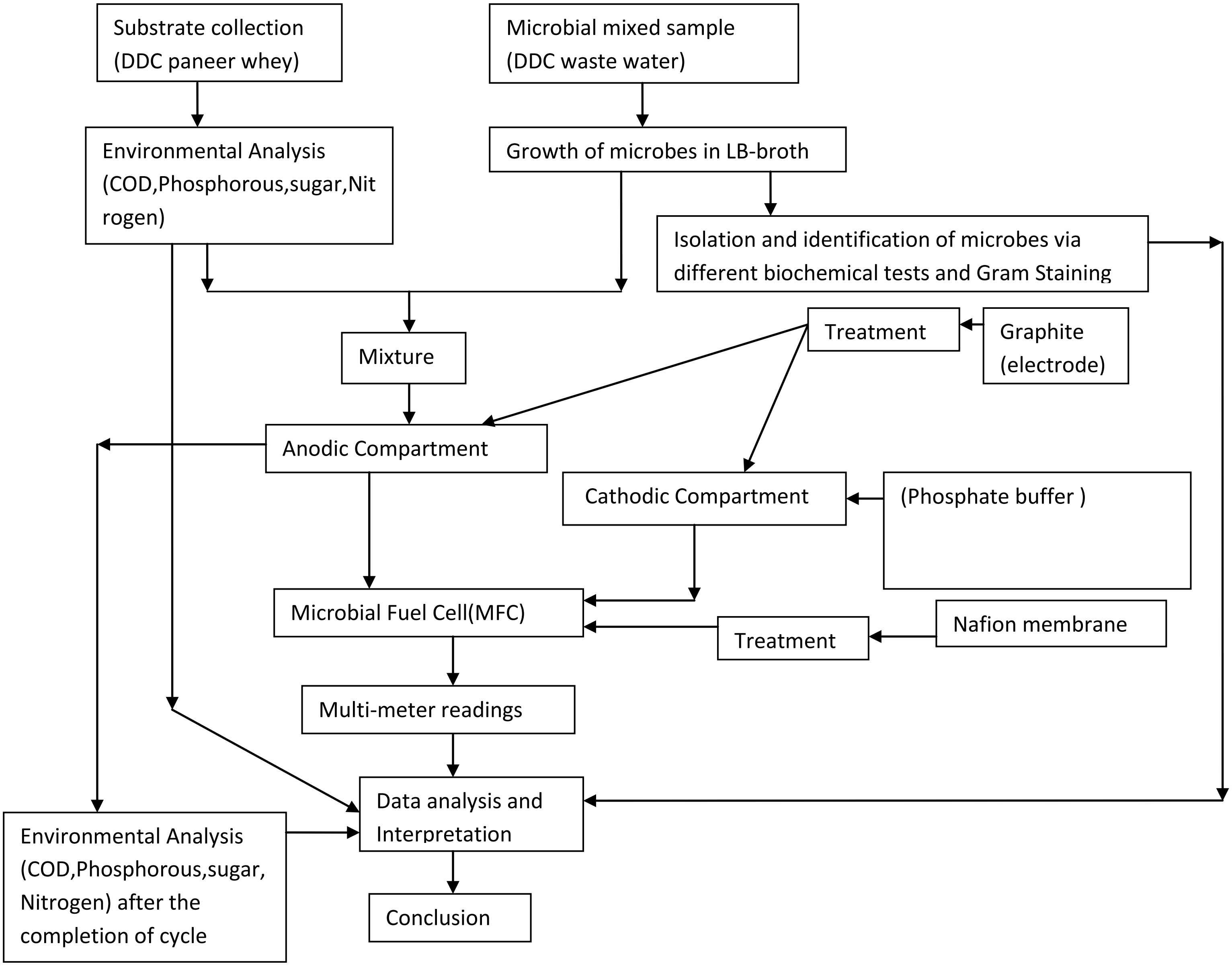

Fig3.1: Diagrammatic sketch of methods and methodology

3.2.1 Sample Collection

The freshly prepared paneer whey sample was collected from Dairy Development Corporation (DDC), lainchaur. The samples were collected in a sterile bottle and stored at 4°C.

The sewage sample containing waste water was collected from the drainage of DDC, Lainchaur and store at 4°C.This sewage sample containing bacterial strains was used as a culture in MFC.

3.2.2 Environmental analysis of Paneer whey

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

Standard curve of Reducing sugar

Firstly stock solution of Glucose was prepared.200mg of glucose was mixed with 100ml of water.500µl of sample was taken into which 500µl of citrate buffer and 3ml of DNS reagent was added. Standard glucose stock was prepared ranging from 100mg/L, 200mg/L, 300mg/L, 400mg/L, 500mg/L, 600mg/L, 700mg/L, 800mg/l, 900mg/L, and 1000mg/L. After the addition of DNS it was kept in boiling water for about 15 minutes. It was cooled at room temperature and distilled water was added to make the final volume up to 10ml. The mixture was thoroughly homogenized and absorbance reading was done at 575nm.

Determination of Reducing Sugar

100ul of sample was taken and the same procedure was followed along with the preparation of standard curve.

3.2.2.2 Determination 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 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.

3.2.2.3 Determination of 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 was prepared by pipetting suitable volumes of the phosphorus working 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, 5 ml of sample was transferred to 125-mL Erlenmeyer flask. Then 0.1ml of sulfuric acid solution was added and mixed. After that 0.04 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 1 ml was reached. It was then cooled and diluted the sample to about 3ml 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 5ml. Then 8ml of combined reagent was added to the sample and mixed thoroughly. Background correction was performed with blank solution and followed sample absorbance measurements.

3.2.2.4 Determination of 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/L was prepared by pipetting suitable volumes of the ammonia-nitrogen working 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.

3.2.3 Isolation and identification of Bacteria

3 .2.3.1 Isolation of Microorganisms

1ml of the sewage sample from the drainage of DDC was taken in a tube, where 9ml of distilled water was added. Serial dilution was done up to 10^{-5}, and the sample was spread plated in NA plate. Incubation was done at 37°C for 24 hours. The different bacterial colonies were selected according to the colour and morphology .They were thus subcultured in NA plate to get a pure culture of bacterial strains, and further tested for biochemical test (Lozano *et al*.2009)

Similarly, about 2ml of the sewage sample was taken and cultured in LB broth. The culture was incubated at 37°C for 3-4 days and store at 4°C.This culture is primarily used in anaerobic growth of bacteria in MFC.

3.2.3.2 Biochemical identification of microorganisms

Biochemical test are the quickest and the easiest test for identifying bacteria because they use deductive principles to reduce the number of possible species present very quickly. Additionally, because bacteria are microorganisms visual identification is not always possible and is never easy. Genetic testing is possible for some well known bacteria, but it is expensive and time consuming. So biochemical test are the widely used method so far (Goshen, 2012).

The bacteria was identified by the Bergey's Manual of systematic bacteriology (1994).Several test were performed such as gram staining, IMVIC, Catalase, Oxidase, Urease etc were done (Holt *et al*.1994)

3.2.3.3 Storage of Microorganisms

The isolated colonies were obtained in NA plate sealed with the parafilm tape and stored at 4°C for the long term storage. The organisms were sub cultured at 15day interval and preserved using 50% glycerol stock.

3.2.3.4 Culture of Yeast

The yeast sample from the bark of plant was cultured in YEPD media at 28°C for about 2-3 days. The isolated yeast was further cultured in YEPL media to check its lactose fermenting capacity, present in paneer whey. The durham tube was kept in test tube to observe the gas formation, indicating the fermentation of lactose.

3.2.4 Extraction of Genomic DNA

From Bacteria (Nishiguchi et al., 2010)

For liquid culture 1-3 ml of culture was kept in an ependroff tube and centrifuged at 13000rpm for about 2 minutes and the supernatant was removed.567µl of TE buffer was added to the pelleted cells. The pellet was resuspended by repeating pipetting or by gently vortexing so that the cells becomes resuspended.30µl of 10% SDS and 3µl of 20mg/ml solution of proteinase k was added , mixed and incubated for 1 hour at 37°C.After incubation 100µl of 5M Nacl was added and mixed. Afterwards, 80µl of CTAB/Nacl solution (0.7M Nacl, 10% CTAB) was added. This solution was incubated at 65°C for 10 minutes. After incubation, equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed. Centrifugation was done for 5 minutes and the aqueous solution was transferred to the new tube. Again centrifugation was done at 14000 rpm for 5 minutes and transferred the supernatant to the new tube. Again 0.6µl of isopropanol was added and mixed gently until the DNA precipitates. Centrifugation was done to remove the isopropanol and 1ml of 70% ethanol was added to wash the salt away from DNA. Centrifugation was done to discard ethanol, drying on the benchtop and the pellet was resuspended in 50µl of TE buffer and kept at 4°C.The obtained g-DNA was undergone through gel electrophoresis and visualized under UV- transilluminator.

From Yeast

Genomic DNA of yeast was isolated by using the Wizard genomic DNA purification kit, gel electrophoresis was done and visualized under UV- transilluminator.

3.2.5 Polymerase Chain Reaction

After the genomic DNA was extracted, the PCR was performed .There are different PCR conditions for different species (bacteria, yeast).It is generally used for the amplification of certain segment of DNA.

PCR Reaction Mixture

Reagents	Amount (µl)
Master Mix (2X)	10
Mgcl$_2$ (2X)	0.6
Forward primer (10pmol)	1
Reverse Primer (10pmol)	1
DNA template (100ng/µl)	1
Nuclease free water	6.4
Total	20

3.2.5.1 Thermocyclic condition of PCR for bacteria (Klindworth *et al*.2012)

Stage	Cycle	Step		Temperature (in °C)	Time
1	1	1	Initial denaturation	95	5 min
2	25	1	Denaturation	95	40 sec
		2	Annealing	55	2 min
		3	Extension	72	1 min
3	1	1	Final extension	72	7 min
		2	Hold	4	Hold

3.2.5.2 Thermocyclic condition of PCR for Yeast

Stage	Cycle	Step		Temperature (in °C)	Time
1	1	1	Initial denaturation	95	5 min
2	25	1	Denaturation	95	40 sec
		2	Annealing	55	2 min
		3	Extension	72	1 min
3	1	1	Final extension	72	7 min
		2	Hold	4	Hold

3.2.6 Pretreatment of Carbon nanotubes (Cabezas, 2013)

First 10 mg of CNT was mixed in 35ml of 2:5 v/v of HNO_3 / H_2SO_4 .The mixture was ultrasonicated for 6 hours and centrifuged at 4000rpm for 15 minutes. If not settled properly the rpm was slightly increased. The CNT was washed with deionized water several times until the PH reached close to 7. Finally the CNT was washed with 100% ethanol. The solid black powder was dried in vaccum at 80°C for about 8-12 hours.

3.2.7 In situ oxidative polymerization of aniline (Cabezas, 2013)

The reaction was done in an ice bath. The reaction vessel containing 1M Hcl (9.55ml) and MWCNT was kept at 4ºC with constant stirring. First aniline monomer was added to nanotube solution and then a solution of 1M Hcl containing APS (aniline:APS molar ratio) was added dropwise to the reaction vessel. Finally the mixture was further stirred for 2 hours at 4°C to ensure a good polymerization. Thus, the composite was formed in ES form.

3.2.8 Composite washing and dedoping process (Sainz et al., 2005; Cabezas, 2013)

Composite was collected as solid with a Buchher funnel and resulting cake was carefully washed, first with distilled water, until the final filtrate was neutral and then with methanol. To the mixture of CNT and PANI 50mg of NMP was put per 100ml of methanol. In order to transform the emeraldine salt into emeraldine base (EB), the composite was dedoped by stirring for 2 hours with 3wt % ammonium hydroxide (NH_4OH), filtered and washed with water, ethanol, water and this was followed by vaccum drying at room temperature for 24 hours. The dedoped composite was dissolved in 50mg of N-Methyl -2-Pyrrolidone (NMP) per 100ml of methanol. Sonication was done in an ice bath for 15 minutes. The graphite electrode was dipped in the NMP solution. Further vaccum dry was done at 70°C for 24 hours. Finally, oven dry was done at 55ºC for 8-12 hours.

3.2.9 Anode material coated with Carbon nanotube composite (Wang *et al*.2009)

The treated CNT was mixed with absolute ethanol and vortex with the help of magnetic stirrer for about 15 minutes. The treated graphite electrodes was dipped in the mixture and sonicated for about 15 minutes. Then vaccum dry was done for about 12 hours and dried in an oven for 12 hours at 60°C.

3.2.10 Treatment of PEM (Najafpour *et al.*2010)

The PEM was used as Nafion 117 membrane. Before its use the membrane were treated properly. Nafion proton exchange membrane was subjected to a course of pretreatment to take off any impurities that was boiling the film for 1h in 3% H_2O_2, washed with deionized water, 0.5 M H_2SO_4 and then washed with deionized water. The anode and cathode compartments were filled by deionized water when the biological fuel cell was not in use to maintain membrane for good conductivity.

3.2.11 Treatment of Graphite electrodes

The graphite electrodes were firstly treated with 70% methanol and ultrasonicated for 15 minutes at a temperature of 25°C.This was followed by treatment with distilled water and ultrasonication for 15 minutes. Finally the graphite electrodes were treated with 70% acetone and ultrasonicated for 15 minutes followed by treatment with distilled water and ultrasonication for minutes. The electrodes were then oven dried at 60°C for 1 day. Before the use, these electrodes were treated in UV for about 15 minutes.

3.2.12 Cyclic voltammetry (Molina et al., 2011)

Hokuto-DenkoHA151 potentiostat was used in combination with the work station. The measurements were performed in a three electrode arrangement. The counter electrode employed was made of platinum. The pretreatment consisted of washing with distilled water. The reference electrode was used as calomel (mercury chloride). The measurements were done between -1v to +1v. The characterization by means of CV was done at scan rate of 0.1v/sec. The data were collected at the interval of 10mV to obtain the stable current values. The cyclic voltametry was done up to 10 cycles.

3.2.13 Construction of MFC

The MFC set up was done by joining the two plastic bottles each capacity of 1000ml via a glass tube with the help of a rubber gasket. Nafion117 was used as a PEM. The graphite felt was used as anode and cathode respectively were a platinum wire was used to hung the electrodes to the wire. In the cathode compartment 700ml of 0.1M, PH 7.6 phosphate buffer was kept whereas in the anode compartment 5% of bacterial culture was kept along with 665ml of Paneer whey. The electrodes were dipped properly and in equal ratio in the anode and the cathode compartment. The wires arising from the anode and the cathode compartment were connected to the multimeter (FLUKA) and open circuit voltage was observed. The closed circuit was obtained by connecting the external resistance of 1000Ω in series. Thus, power, voltage and current was obtained.

1. In the cathode compartment 700ml of 0.1M Phosphate buffer was kept, whereas in the anode compartment 665ml of filtered paneer whey was taken containing 35ml of bacterial culture obtained from the sewage of DDC. The graphite felt was taken as an electrode. The Nafion117 was used as PEM and an external resistance of 1000Ω was used to form a closed circuit. Thus voltage, current and power was obtained with the help of multimeter.

2. In the cathode compartment 700ml of 0.1M Sodium Acetate buffer was kept, whereas in the anode compartment 665ml of filtered paneer whey was taken containing 35ml of bacterial culture obtained from the sewage of DDC. The graphite felt was taken as an electrode. The Nafion117 was used as PEM and an external resistance of 1000Ω was used to form a closed circuit. Thus voltage, current and power was obtained with the help of multimeter.

3. In the cathode compartment 700ml of 0.1M Phosphate buffer along with 0.1M Potassium ferricyanide was kept, whereas in the anode compartment 665ml of filtered paneer whey was taken containing 35ml of bacterial culture obtained from the sewage of DDC. The graphite felt was taken as an electrode. The Nafion117 was used as PEM and an external resistance of 1000Ω was used to form a closed circuit. Thus voltage, current and power was obtained with the help of multimeter.

4. In the cathode compartment 700ml of 0.1M Phosphate buffer along with 0.1M Potassium ferricyanide was kept, whereas in the anode compartment 665ml of filtered paneer whey was taken containing 35ml of bacterial culture obtained from the sewage of DDC. The graphite felt coated with CNT was taken as an electrode. The Nafion117 was used as PEM and an external resistance of 1000Ω was used to form a closed circuit. Thus voltage, current and power was obtained with the help of multimeter.

3.2.14 Operational procedures in MFC

The MFC was constructed and operated for about 5-8 days. Each day the anodic sample was taken for the determination of removal efficiency of reducing sugar. The initial and final COD, Phosphorous and Nitrogen was determined and compared for the percentage reduction. The open circuit reading was taken for the optimization of MFC. Afterwards, power, voltage and current were obtained from the closed circuit possessing 1000Ω resistance.

Similarly, different buffers and graphite electrode coated with CNT was used in MFC to study its efficiency. All the procedures in MFC and spectrophotometric reading were done in triplicates to get the accuracy of data.

Fig3.2 Microbial fuel cell with the drop wise addition of Potassium ferricyanide and Phosphate buffer using the peristaltic pump.

CHAPTER FOUR

RESULTS

4.1. Determination of Environmental parameters of waste water.

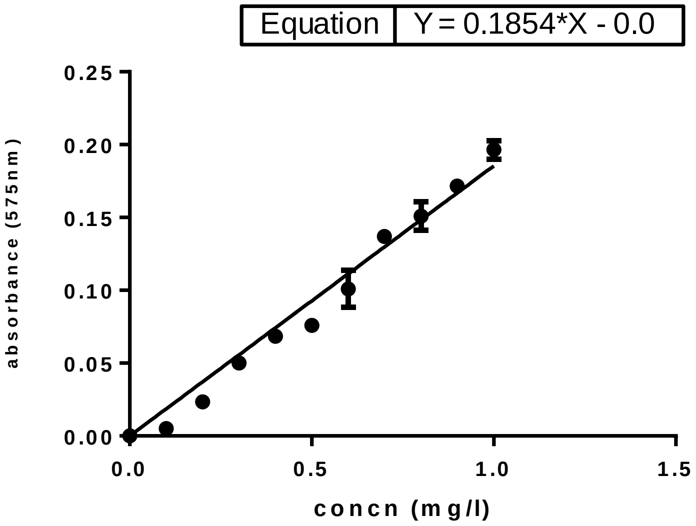

Fig4.1: Standard curve of reducing sugar

Calibration linearity curve was obtained using eleven different levels of calibration standards in the range from 0.1mg/L to 1mg/L including the blank .Our result showed the linearity with correlation coefficient of 0.9962.

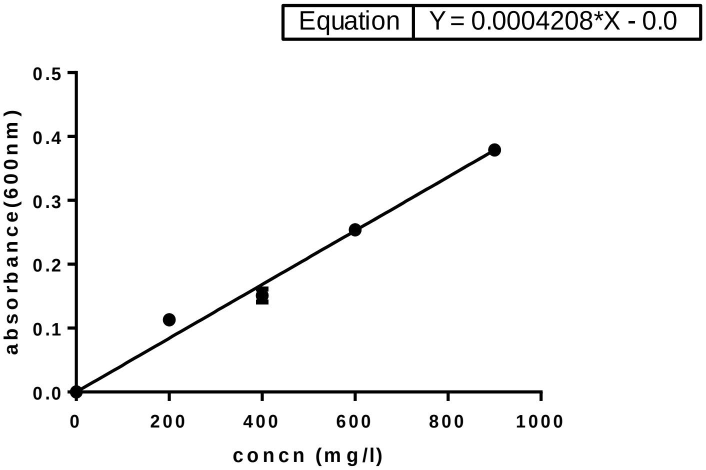

Fig4.2: Standard curve of Chemical oxygen Demand

Calibration linearity curve was obtained using eight different levels of calibration standards in the range from 20mg/ltr to 900mg/ltr including the blank .Our result showed the linearity with correlation coefficient of 0.9843.

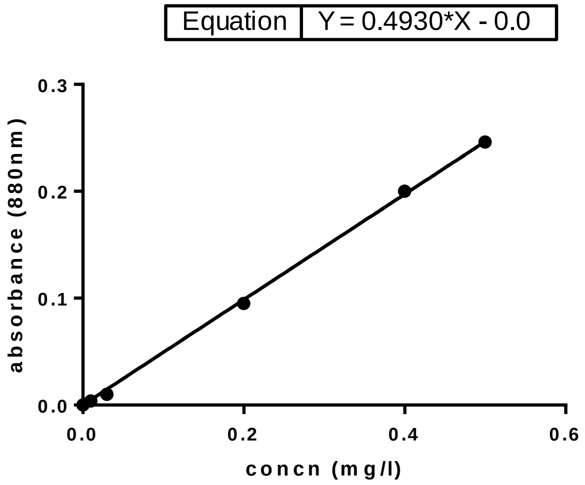

Fig4.3: Standard curve of Phosphorous

Calibration linearity curve was obtained using nine different levels of calibration standards in the range from 0.01mg/L to 0.50mg/L including the blank .Our result showed the linearity with correlation coefficient of 0.9998.

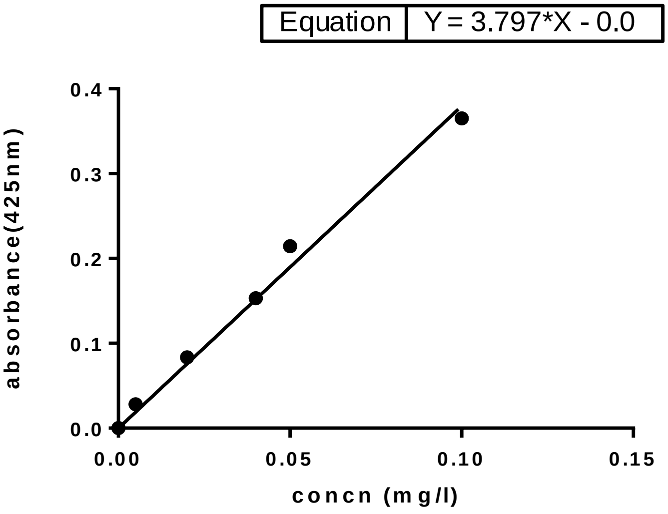

Fig4.4: Standard curve of Ammonical Nitrogen

Calibration linearity curve was obtained using nine different levels of calibration standards in the range from 0.005mg/L to 0.100mg/L including the blank .Our result showed the linearity with correlation coefficient of 0.9962.

Table 4.1 Environmental analysis of paneer whey

Analytical parameters of paneer whey	**Concentration(mg/L)**
Reducing sugar	1.8432
Chemical Oxygen Demand	689.163
Total phosphorus	0.2345
Ammonical Nitrogen	0.0359

Table 4.1 represents the presence of contaminants i.e. Reducing sugar, Chemical oxygen Demand, Total Phosphorus and Ammonical Nitrogen in Paneer whey sample.**(PH- 5.37, Temperature- 75°C)**

4.2 Characterization of Bacteria from the sewage sample of DDC

After the serial dilution of 10^{-5}, spread plate was done to get the bacterial isolates. Different bacteria isolated were obtained based on their morphology. The isolates were named as 1,2,3,4. Based on the staining technique and biochemical test, the different bacteria were identified. Gram staining was done to identify the gram positive and negative bacterial, whereas biochemical test were performed according to the bergey’s manual.

Table 4.2 Morphology and biochemical characteristics of the isolates

Character	**1**	**2**	**3**	**4**
Morphology	Gram negative, non-motile	Gram negative, motile	Gram negative, motile	Gram negative, Motile
Catalase test	+ve	+ve	+ve	+ve
Methyl red test	_ve	+ve	_ve	+ve
Voges-Proskaur	+ve	_ve	_ve	_ve
Indole test	_ve	_ve	+ve	+ve
Citrate test	+ve	+ve	+ve	_ve
Nitrate test	_ve	+ve	+ve	+ve
Urease test	+ve	_ve	_ve	_ve

Table 4.3 Biochemical Identification of Bacteria

Strain	**Identified bacterial isolates**
1	*Klebsiella spp*
2	*Salmonella spp*
3	*Pseudomonas spp*
4	*Escherichia coli*

The biochemical test and gram staining resulted to the presence of *Klebsiella, Salmonella, Pseudomonas* and *Escherichia coli* in sewage sample of DDC.

4.3 Isolation of Lactose fermenting yeast

After the yeast was isolated from YEPD media, it was further cultured in YEPL media for 2-3 days at 28°C. The gas formation in durham tubes were observed indicating fermentation of lactose in the presence of yeast. Three strains of yeast Y2, Y3, Y4 were observed to produce gas in durham tubes indicating as lactose fermenting yeast. Out of which Y2 posessed the maximum gas production. Sample Y2 was found to be *Rhodotorula mucilaginosa.*

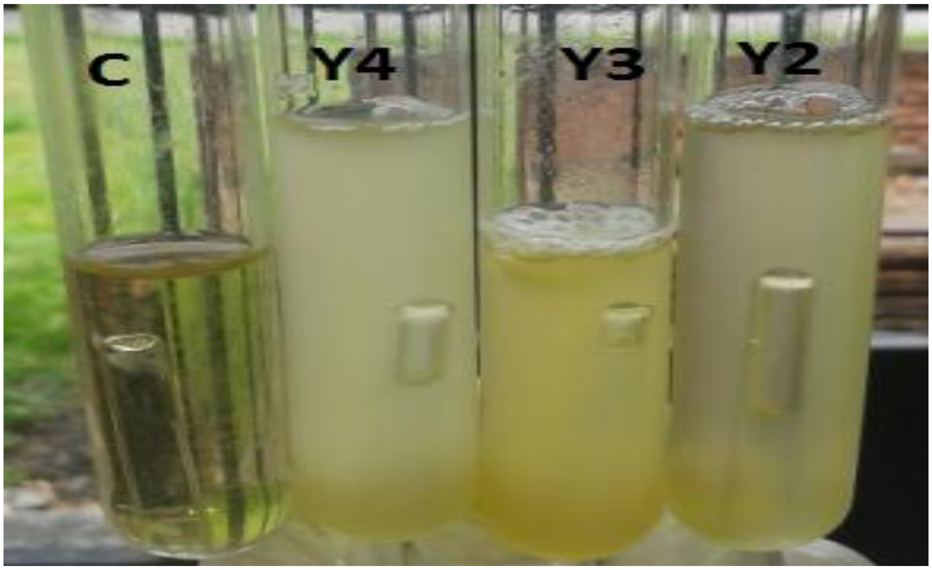

Fig4.5 Identification of Lactose fermenting yeast in YEPL media

4.4 Analysis of various parameters in MFC

After the MFC was run the initial and final COD, reducing sugar, Phosphorous and Ammonical Nitrogen were estimated. This gave the percentage removal efficiency of different parameters. Also the open circuit voltage was obtained and closed circuit current, voltage and power was calculated. The MFC was run in different bacterial samples, single culture, mixed culture, change in buffers, addition of chemical oxidizers and modification in the anode coated with CNT composite.

4.4.1 Removal of reducing sugar (100% substrate i.e paneer whey) using mix culture of bacterial strains.

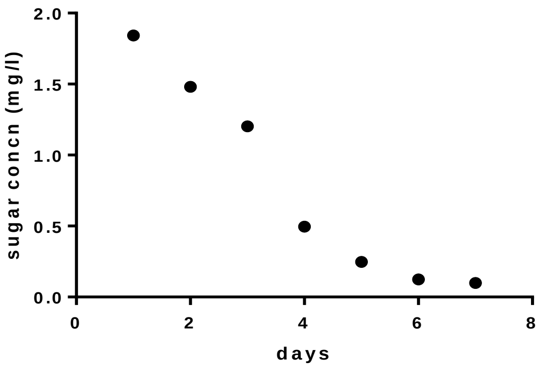

Fig 4.6 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.0993mg/L on the seventh day. The concentration of reducing sugar was lowered by 94.61%.

4.4.2 Removal of reducing sugar using lactobacillus only

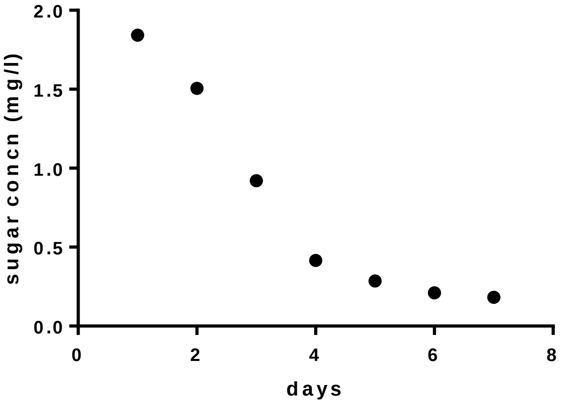

Fig 4.7 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.1833mg/L on the seventh day. The concentration of reducing sugar was lowered by 90.05%.

4.4.3 Removal of reducing sugar using yeast sample (Y_2)

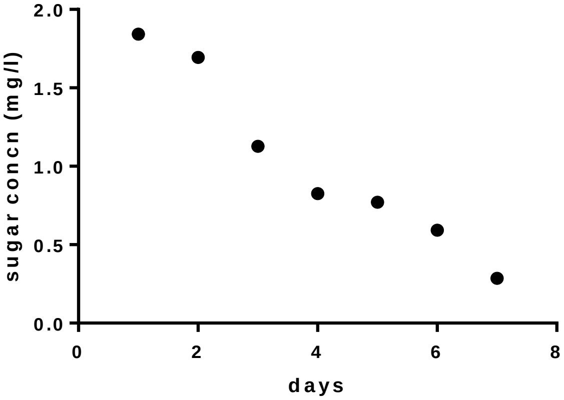

Fig 4.8 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.2858mg/L on the seventh day. The concentration of reducing sugar was lowered by 84.49%.

4.4.4 Removal of reducing sugar using Klebsiella only

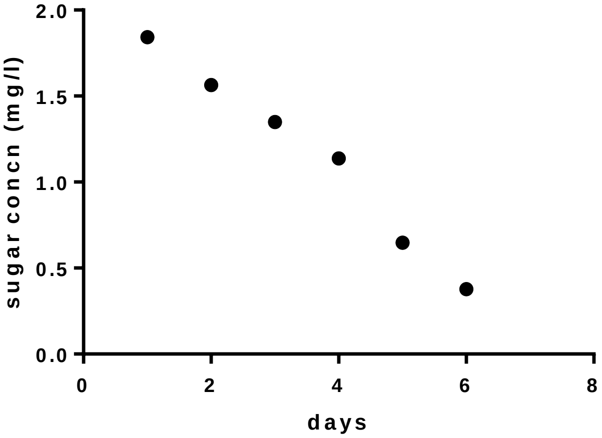

Fig 4.9 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.3775mg/L on the sixth day. The concentration of reducing sugar was lowered by 79.51%.

4.4.5 Removal of reducing sugar using mix culture of bacterial strain and lactobacillus

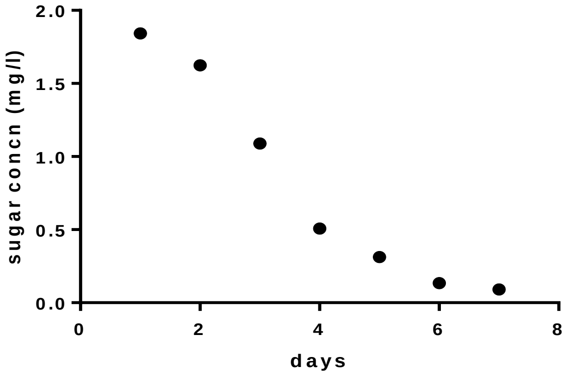

Fig 4.10 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.091mg/L on the seventh day. The concentration of reducing sugar was lowered by 95.06%.

4.4.6 Removal of reducing sugar coating electrode with CNT (Emeraldine salt form)

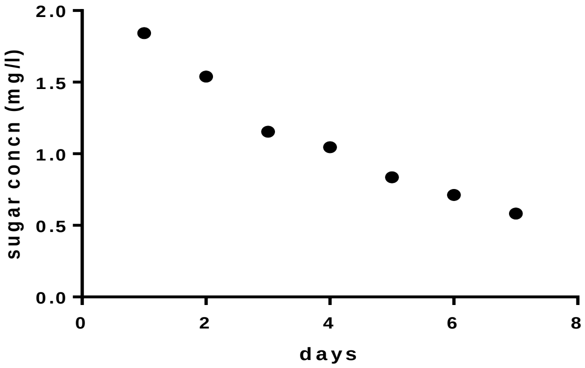

Fig 4.11 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.5825mg/L on the seventh day. The concentration of reducing sugar was lowered by 68.39%.

4.4.7 Removal of reducing sugar coating electrode with CNT (Emeraldine base form)

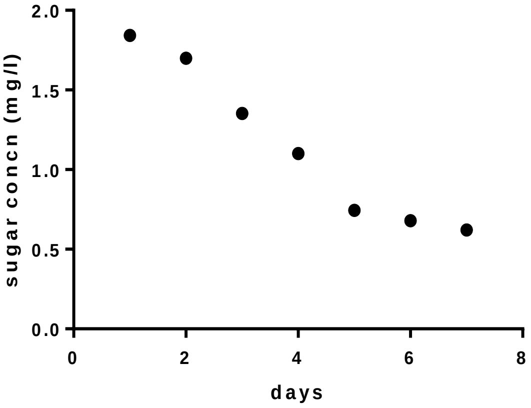

Fig 4.12 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.6202mg/L on the seventh day. The concentration of reducing sugar was lowered by 66.35%.

4.4.8 Removal of reducing sugar using 0.1M sodium acetate buffer

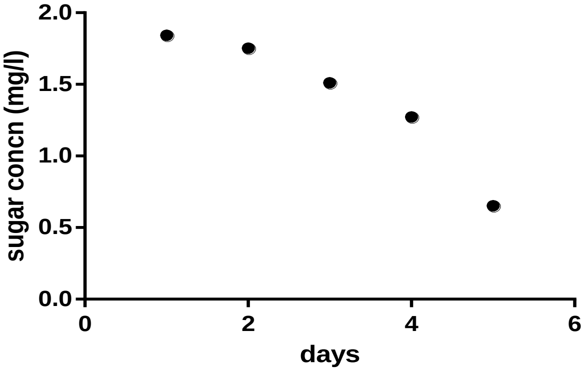

Fig 4.13 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.6526mg/L on the fifth day. The concentration of reducing sugar was lowered by 64.59%.

4.4.9 Removal of reducing sugar using 0.1M phosphate buffer and 0.1M potassium ferricyanide

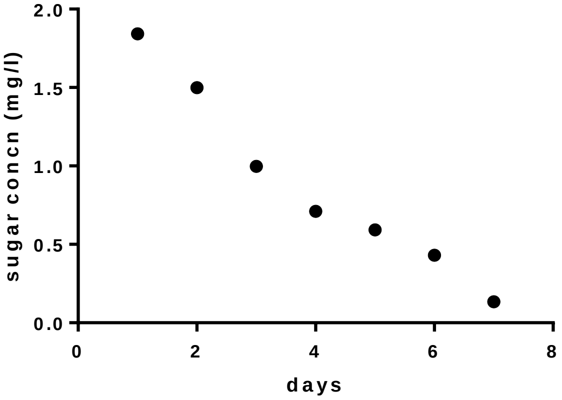

Fig 4.14 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.1348mg/L on the seventh day. The concentration of reducing sugar was lowered by 92.68%.

4.4.10 Removal of reducing sugar using 0.1M Sodium Acetate and 0.1M potassium ferricyanide

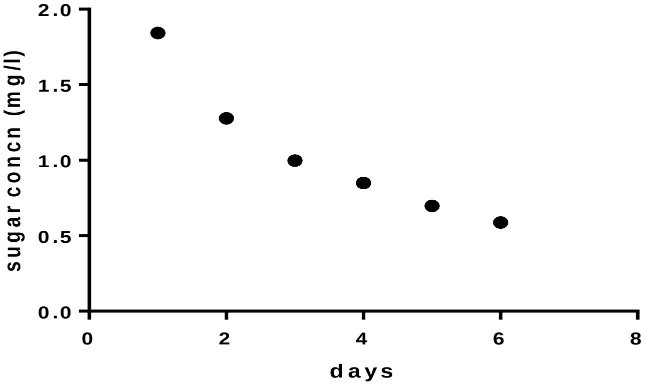

Fig 4.15 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.5879mg/L on the sixth day. The concentration of reducing sugar was lowered by 68.10%.

4.4.11 Removal of reducing sugar using electrode coated with CNT and ethanol

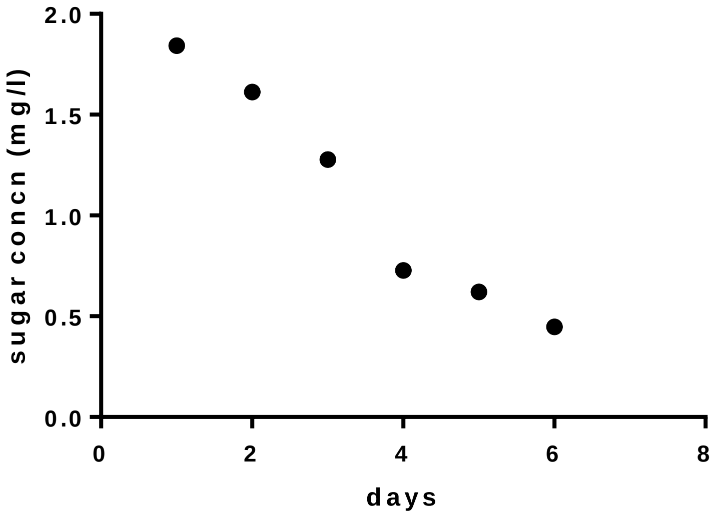

Fig 4.16 Reduction of Sugar concentration versus number of days

Reducing sugar gradually decrease from 1.8432mg/L to 0.4476mg/L on the sixth day. The concentration of reducing sugar was lowered by 75.71%.

4.5 Analysis of reduction of COD, Phosphorous and Ammonia-Nitrogen

Table4.4 Removal of COD

Samples	**Electrode coating**	**Buffer**	**Initial concentration (mg/ml)**	**Final concentration (mg/ml)**	**% Reduction**
Mixculture strains			689.163	344.58	50.00
Lactobacillus			689.163	401.615	41.72
Yeast (Y_2)			689.163	423.003	38.62
Klebsiella			689.163	270.912	60.68
Mixculture +Lactobacillus			689.163	287.547	58.27
Mixculture+lactobacillus	CNT ES		689.163	280.418	59.31
Mixculture+lactobacillus	CNT EB		689.163	297.053	56.89
Mixculture+lactobacillus	CNT+ ethanol		689.163	268.536	61.03
Mixculture+lactobacillus		Sodium acetate	689.163	237.642	65.51
Mixculture+lactobacillus		Phosphate+ Potassium ferricyanide	689.163	308.935	55.17
Mixculture+lactobacillus		Sodium acetate+ Potassium ferricyanide	689.163	318.441	53.79

Table4.5 Removal of Ammonia-Nitrogen

Samples	**Initial concentration (mg/ml)**	**Final concentration (mg/ml)**	**% Reduction**
Mixculture strains	0.0359	0.0131	63.50
Lactobacillus	0.0359	0.0118	67.13
Yeast (Y_2)	0.0359	0.0250	30.36
Klebsiella	0.0359	0.0237	33.98
Mixculture +Lactobacillus	0.0359	0.0118	67.13

Table4.6 Removal of Phosphorous

Samples	Electrode coating	Buffer	Initial concentration (mg/ml)	Final concentration (mg/ml)	% Reduction
Mixculture strains			0.2345	0.107	54.20
Lactobacillus			0.2345	0.148	36.58
Yeast (Y$_2$)			0.2345	0.136	41.87
Klebsiella			0.2345	0.117	49.88
Mixculture +Lactobacillus			0.2345	0.080	65.67
Mixculture+lactobacillus	CNT ES		0.2345	0.179	23.36
Mixculture+lactobacillus	CNT EB		0.2345	0.161	31.30
Mixculture+lactobacillus	CNT+ Ethanol		0.2345	0.119	48.91
Mixculture+lactobacillus		Sodium acetate	0.2345	0.092	60.38
Mixculture+lactobacillus		Phosphate+ potassium ferricyanide	0.2345	0.097	58.59
Mixculture+lactobacillus		Sodium acetate+ Potassium ferricyanide	0.2345	0.109	53.30

4.6 Power, voltage and current generated by MFC in different conditions

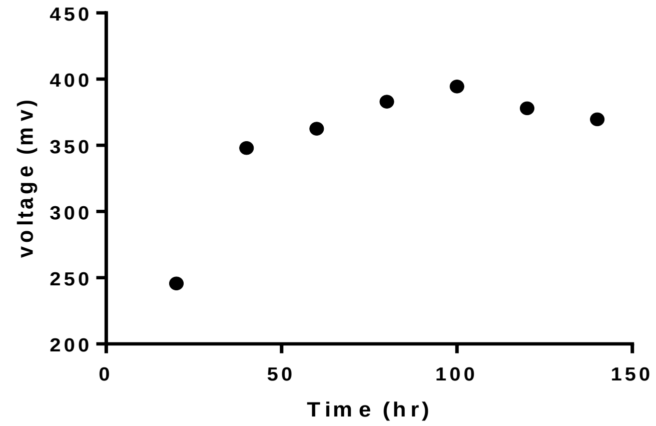

Fig4.17 Open circuit voltages obtained from MFC using mixculture strains

While using the mixed culture strain the MFC showed a maximum open circuit voltage of 394.4mV on the fifth day. Then the voltage gradually decreased on the following day.

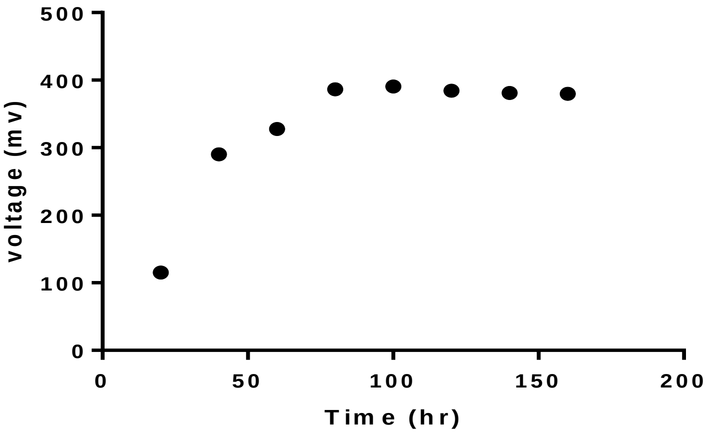

Fig4.18 Open circuit voltages obtained from MFC using Lactobacillus strain

While using the Lactobacillus strain the MFC showed a maximum circuit voltage of 390.7mV on the third day. The voltage remained constant for few hours and gradually decreased on the following day.

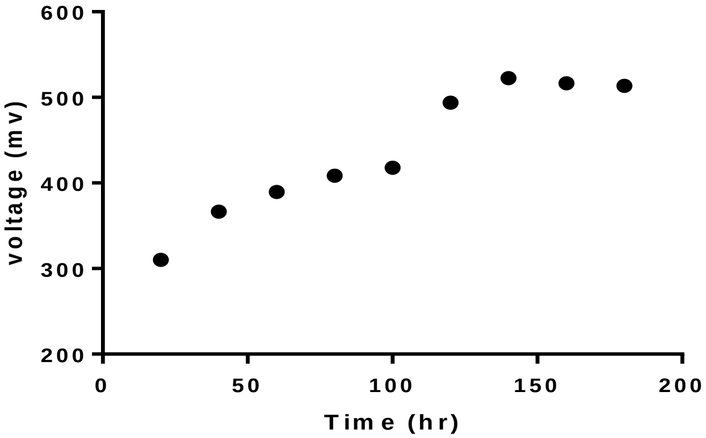

Fig4.19 Open circuit voltages obtained from MFC using Yeast (Y_2) strain

While using the Yeast (Y_2) strain the MFC showed a maximum open circuit voltage of 522.6mV on the sixth day. The voltage remained constant for few hours and gradually decreased on the following day.

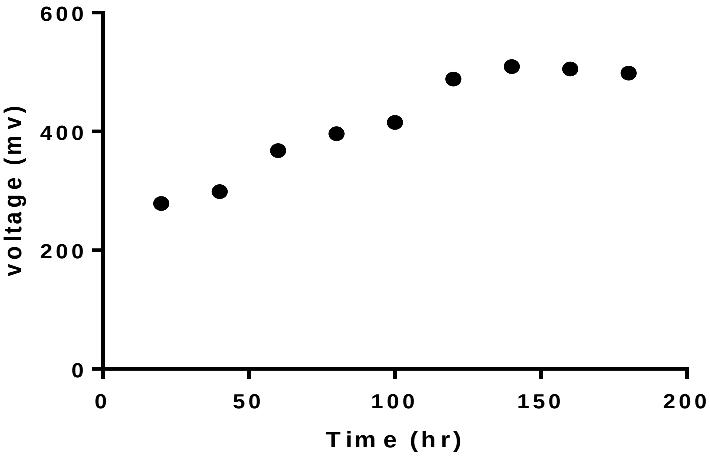

Fig4.20 Open circuit voltages obtained from MFC using Klebsiella strain

While using the Yeast (Y_2) strain the MFC showed a maximum open circuit voltage of 509.4mV on the sixth day. The voltage gradually increased up to the sixth day and remained constant for few hours .Then the voltage gradually decreased.

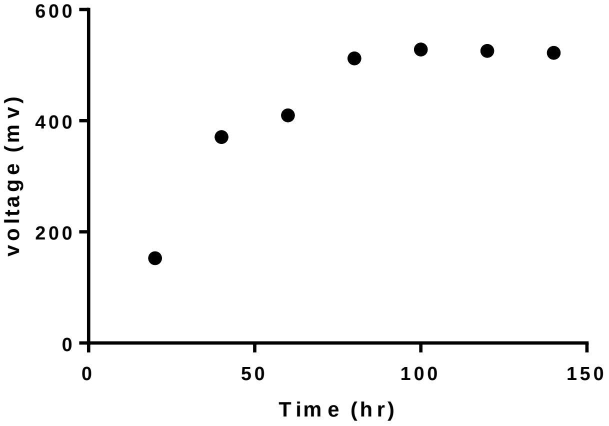

Fig4.21 Open circuit voltages obtained from MFC using Mixed culture and Lactobacillus strain

While using the Mixed culture and *Lactobacillus* strain the MFC showed a maximum open circuit voltage of 528.4mV on the fifth day. Then voltage remained constant for few hours then gradually decreased.

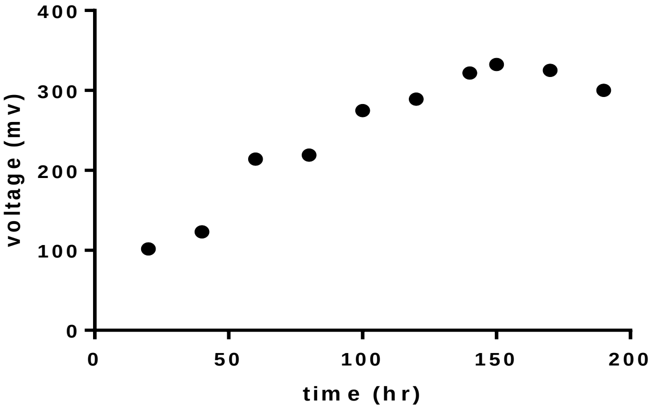

Fig4.22 Open circuit voltages obtained from MFC using an electrode coated with CNT in ES form

While using the electrode coated with CNT in Emeraldine salt (ES) form the MFC showed a maximum open circuit voltage of 333.4mV on the Fifth day. The voltage gradually increased up to the fifth day and remained constant for few hours .Then the voltage gradually decreased.

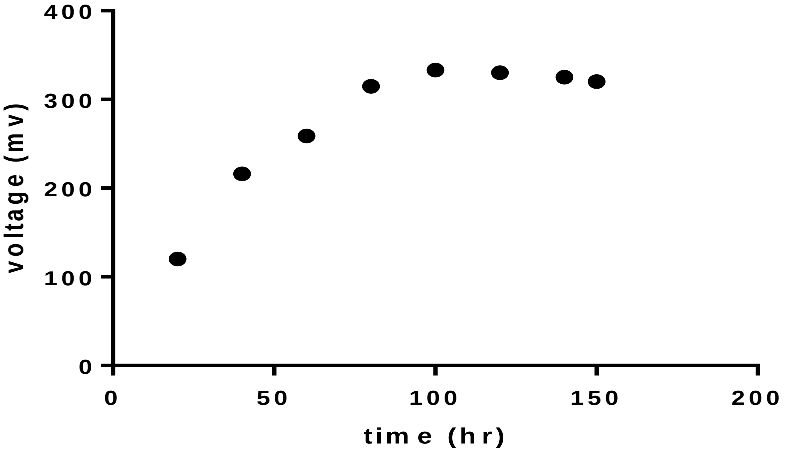

Fig4.23 Open circuit voltages obtained from MFC using an electrode coated with CNT in EB form

While using the electrode coated with CNT Emeraldine Base (EB) form the MFC showed a maximum open circuit voltage of 333.3mV on the Fifth day. The voltage gradually increased up to the fifth day and remained constant for few hours .Then the voltage gradually decreased.

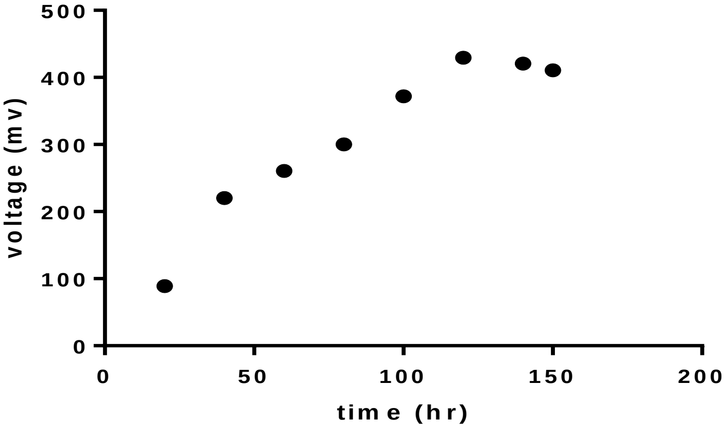

Fig4.24 Open circuit voltages obtained from MFC using Sodium acetate buffer

While using the sodium acetate buffer the MFC showed a maximum open circuit voltage of 429.4mV on the Fifth day. The voltage gradually increased up to the fifth day and remained constant for few hours .Then the voltage gradually decreased.

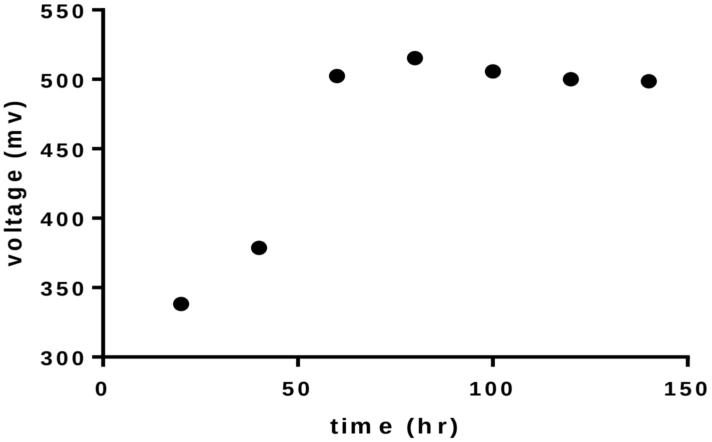

Fig4.25 Open circuit voltages obtained from MFC using mixture of sodium acetate buffer and Potassium ferricyanide

While using the mixture of sodium acetate buffer and potassium ferricyanide the MFC showed a maximum open circuit voltage of 515.4mV on the Fourth day. The voltage gradually increased sharply up to the fourth day and gradually decreased the following day.

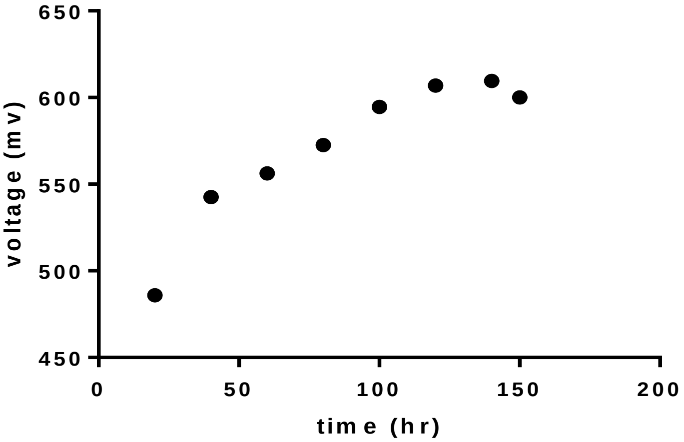

Fig4.26 Open circuit voltages obtained from MFC using mixture of Phosphate buffer and potassium ferricyanide

While using the mixture of phosphate buffer and potassium ferricyanide the MFC showed a maximum open circuit voltage of 609.5mV on the sixth day. The voltage gradually increased sharply up to the sixth day and gradually decreased the following day.

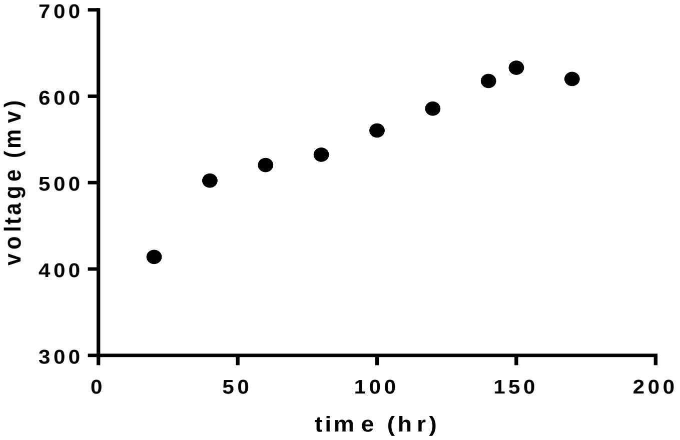

Fig4.27 Open circuit voltages obtained from MFC using electrode coated with CNT treated with ethanol

While using the electrode as CNT treated with ethanol the MFC showed a maximum open circuit voltage of 633mV on the fifth day. The voltage gradually increased sharply up to the fifth day and gradually decreased the following day.

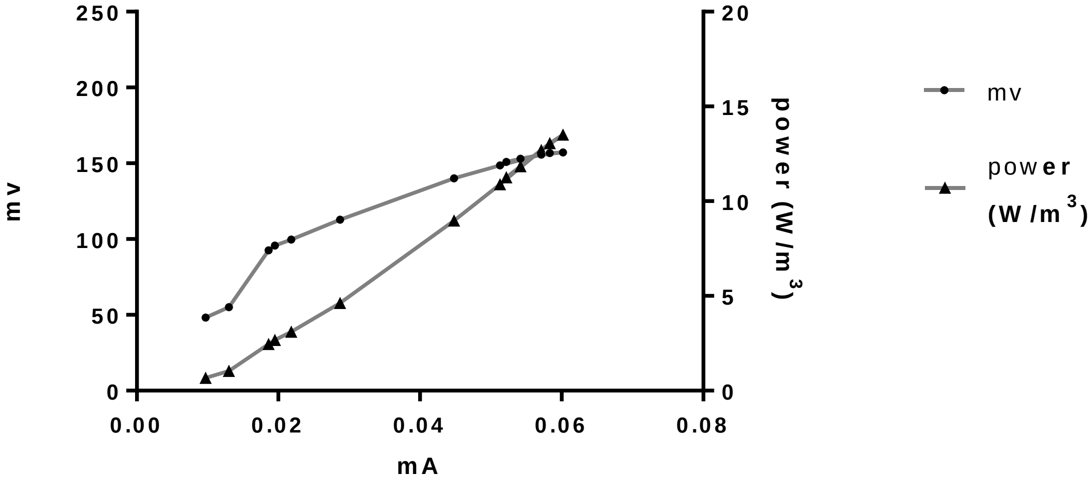

Fig4.28 Power and voltage generation in MFC using the Mixed culture and Lactobacillus in phosphate buffer with an external resistance of 1000Ω

The power was calculated by taking account of voltage and current. Initially both the voltage and power were less but as the days increased both increased and gradually decreased. The maximum voltage was obtained to be 157.1mV with the maximum power of 13.5w/m^3 when the maximum current was 0.060mA.

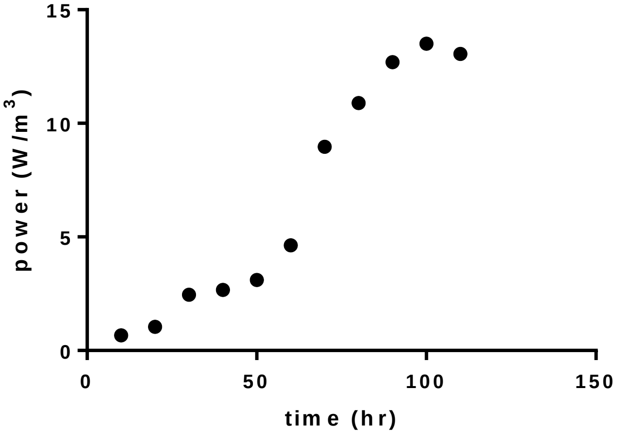

Fig4.29 Power generation in MFC using the Mixed culture and Lactobacillus in phosphate buffer with an external resistance of 1000Ω

The MFC was run for about six days. The power obtained on the first day was 0.667W/m^3 which kept on increasing and reached the maximum power of 13.510W/m^3 on the fifth day. On the sixth day the power gradually decreased.

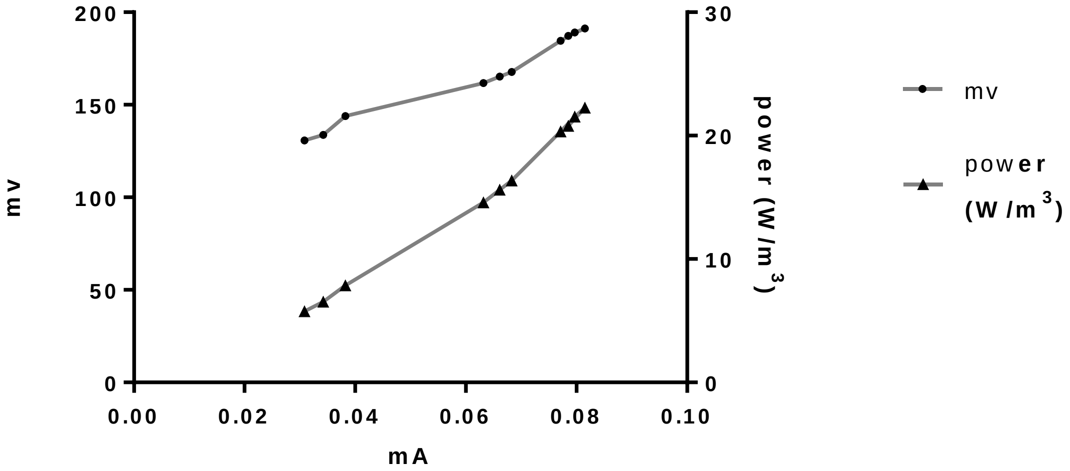

Fig4.30 Power and voltage generation in MFC using the Mixed culture and Lactobacillus in phosphate buffer and potassium ferricyanide with an external resistance of 1000Ω

The power was calculated by taking account of voltage and current. Initially both the voltage and power were less but as the days increased both increased and gradually decreased. The maximum voltage was obtained to be 191.2mV with the maximum power of 22.261w/m^3 when the maximum current was 0.0815mA.

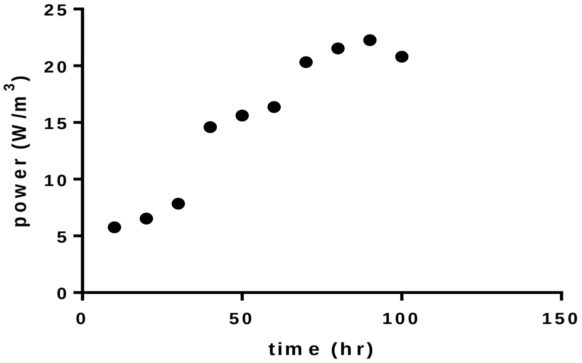

Fig4.31 Power generation in MFC using the Mixed culture and Lactobacillus in phosphate buffer mixed with potassium ferricyanide with an external resistance of 1000Ω

The MFC was run for about five days. The power obtained on the first day was 5.750W/m^3 which kept on increasing and reached the maximum power of 22.261W/m^3 on the fourth day. On the fifth day the power gradually decreased.

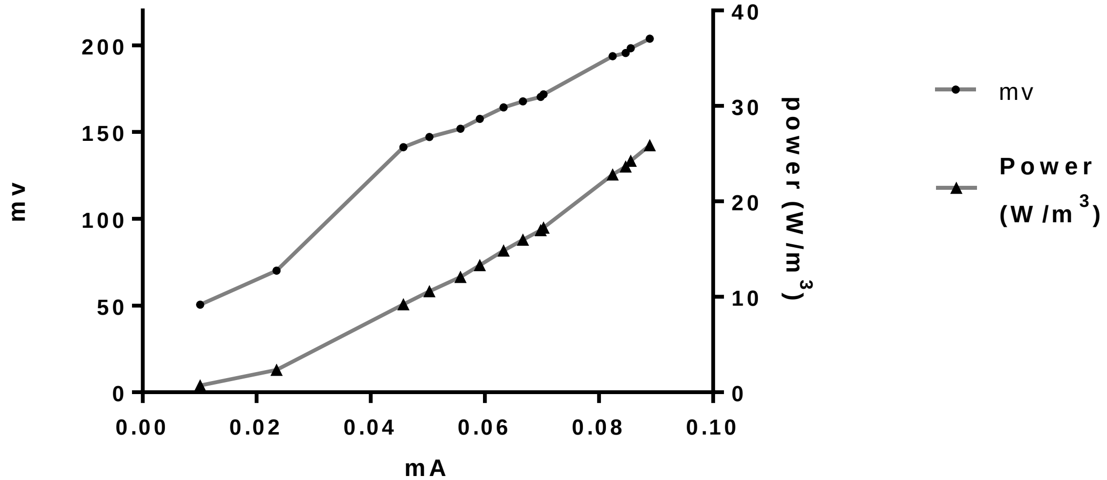

Fig4.32 Power and voltage generation in MFC using the Mixed culture and Lactobacillus in mixture of phosphate buffer and potassium ferricyanide with electrode coated with CNT treated in ethanol with an external resistance of 1000Ω

The power was calculated by taking account of voltage and current. Initially both the voltage and power were less but as the days increased both increased and gradually decreased. The maximum voltage was obtained to be 203.7mV with the maximum power of 25.869w/m^3 when the maximum current was 0.0889mA.

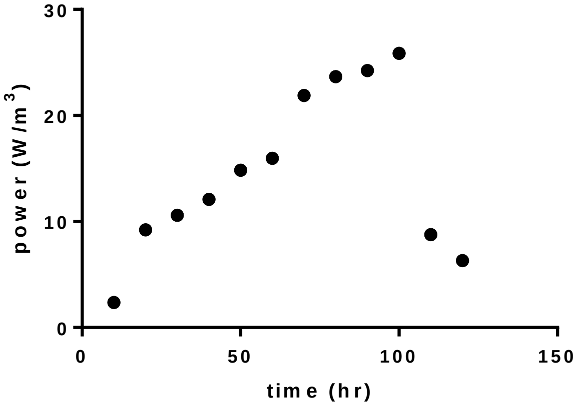

Fig4.33 Power generation in MFC using the Mixed culture and Lactobacillus in mixture of phosphate buffer and potassium ferricyanide with an electrode coated with CNT treated in ethanol with an external resistance of 1000Ω

The MFC was run for about six days. The power obtained on the first day was 1.921W/m^3 which kept on increasing and reached the maximum power of 25.869W/m^3 on the fifth day. On the sixth day the power sharply decreased.

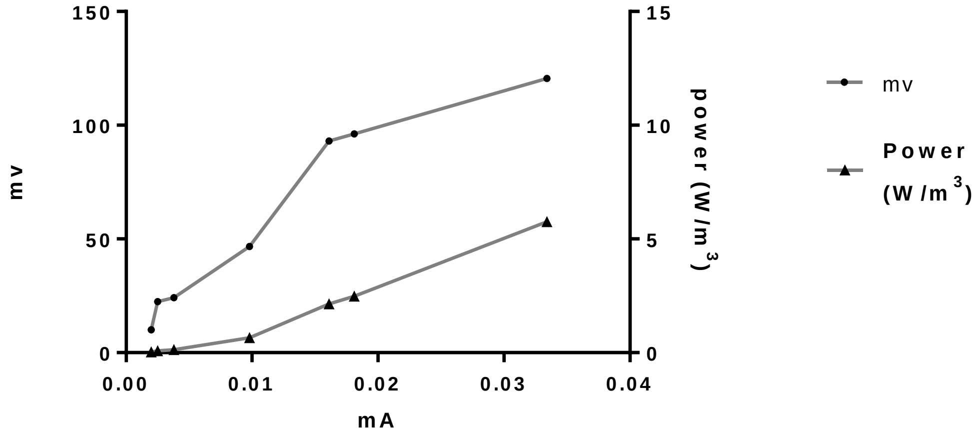

Fig4.34 Power and voltage generation in MFC using the Mixed culture and Lactobacillus in sodium acetate buffer with an external resistance of 1000Ω

The power was calculated by taking account of voltage and current. Initially both the voltage and power were less but as the days increased both increased and gradually decreased. The maximum voltage was obtained to be 120.6mV with the maximum power of 5.754w/m^3 when the maximum current was 0.0334mA.

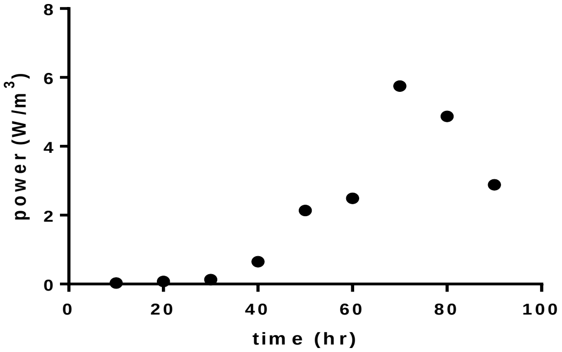

Fig4.35 Power generation in MFC using the Mixed culture and Lactobacillus in sodium acetate buffer with an external resistance of 1000Ω

The MFC was run for about five days. The power obtained on the first day was 0.131W/m^3 which kept on increasing and reached the maximum power of 5.754W/m^3 on the third day. After the fourth day the power sharply decreased.

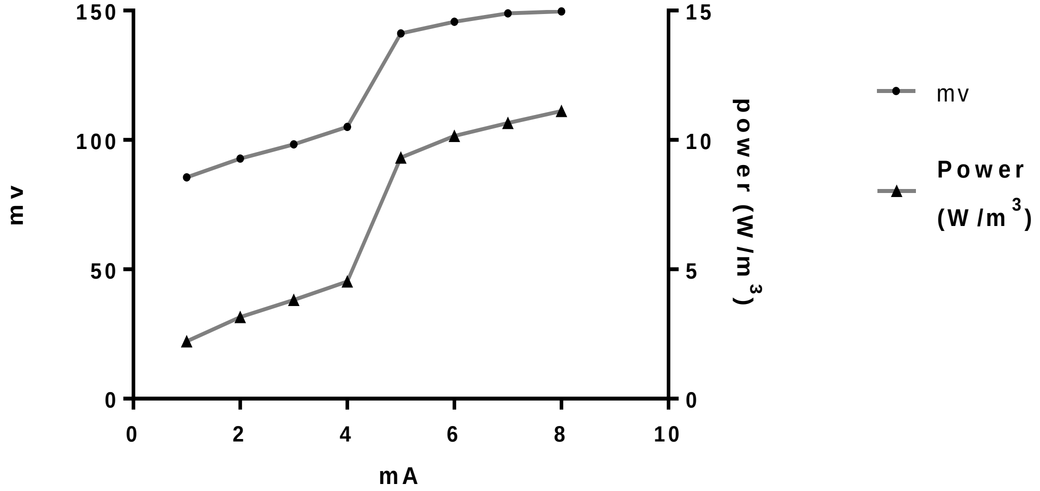

Fig4.36 Power and voltage generation in MFC using the Mixed culture and Lactobacillus in sodium acetate buffer and potassium ferricyanide with an external resistance of 1000Ω

The power was calculated by taking account of voltage and current. Initially both the voltage and power were less but as the days increased both increased and gradually decreased. The maximum voltage was obtained to be 149.7mV with the maximum power of 11.120w/m^3 when the maximum current was 0.052mA.

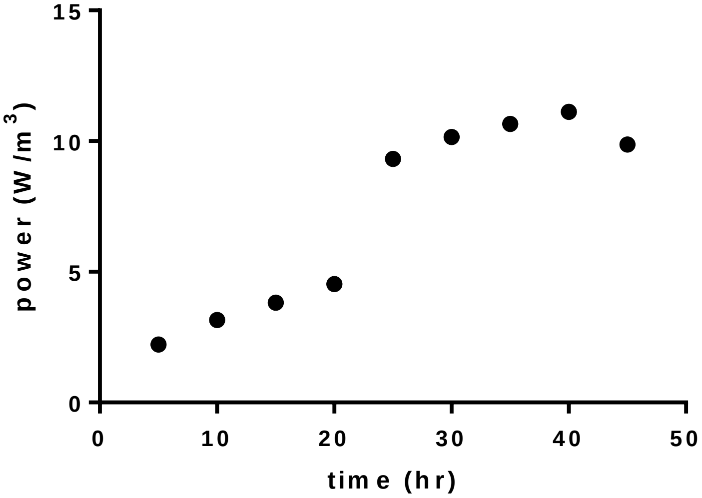

Fig4.37 Power generation in MFC using the Mixed culture and Lactobacillus in sodium acetate buffer mixed with potassium ferricyanide with an external resistance of 1000Ω

The MFC was run for about four days. The power obtained on the first day was 2.225W/m^3 which kept on increasing and reached the maximum power of 11.120W/m^3 on the second day. After the third day the power sharply decreased.

4.7 Cyclic voltammeter measurement

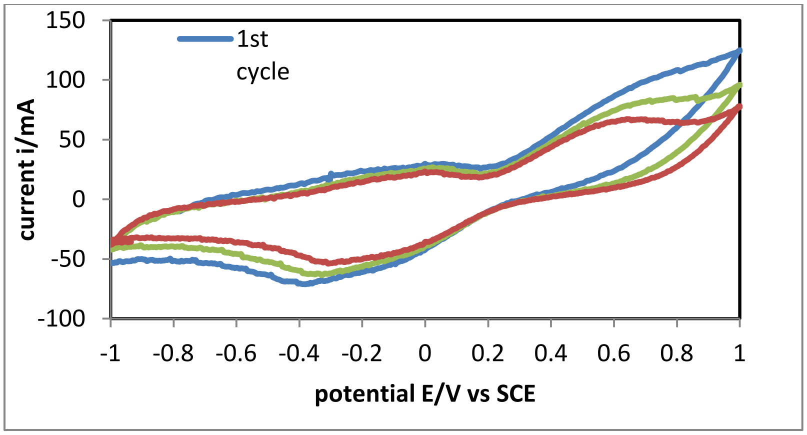

Fig4.38 Shows a typical cyclic voltammogram of graphite felt eletrode used as anode in the MFC.

4.8 Result of genomic DNA

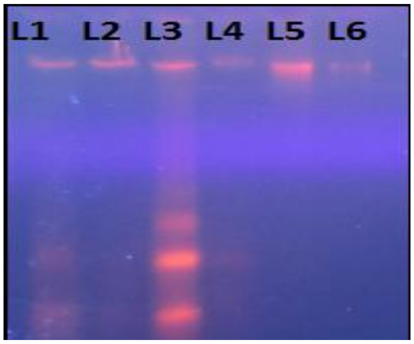

Fig4.39 Genomic DNA of bacterial and yeast sample on 0.8% agarose gel visualized under UV transilluminator

L1- genomic DNA of *Salmonella* spp (5ul DNA +1ul loading dye)

L2 - geno1mic DNA of *Klebsiella* spp (5ul DNA +1ul loading dye)

L3 - genomic DNA of *Pseudomonas* (5ul DNA +1ul loading dye)

L4- genomic DNA of *E.coli* (5ul DNA +1ul loading dye)

L5- genomic DNA of *Rhodotorula mucilaginosa* (5ul DNA +1ul loading dye)

L6- genomic DNA of yeast Y3 (5ul DNA +1ul loading dye)

4.9 Result of PCR product

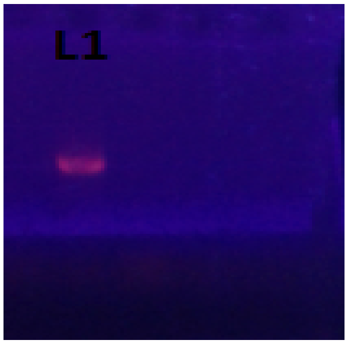

Fig4.40 PCR product on Agarose gel 1% visualized under UV transilluminator

L1 - PCR product of *Klebsiella* sps (5ul PCR product +1ul loading dye)

CHAPTER FIVE

DISCUSSION

5.1 Environmental analysis of paneer whey

From our observation, in paneer whey the reducing sugar was found to be 1.8432mg/ltr, Phosphorous was found to be 0.2345mg/L, Ammonical Nitrogen was found to be 0.0359mg/ltr and COD was found to be 689.163mg/L. Though very less research is done regarding the environmental analysis of paneer whey; our result of COD contrasted with Macwan et al., 2016, that showed the COD of 60,000 – 80,000mg/L. This might be due to the variation in chemical composition while making the paneer. According to Goyal and Gandhi, 2009; the processing conditions have greater influence in parameters like proteins and total solids that cause the change in various factors.

5.2 Identification of Microorganisms and Yeast

Various microorganisms were isolated and identified by biochemical method. They were *Klebsiella* sps, *Pseudomonas* sps, *Salmonella* sps and *E.coli*. The yeast sample Y2 was identified by sequencing and it was found to be *Rhodotorula mucilaginosa*. Generally bacterial like *E.coli*, *Klebsiella* sps and *Pseudomonas* sps are commonly found in waste water as reported by Kalia, 2015. Isolation of genomic DNA for all the bacterial isolates and yeast was successfully done. But the PCR product for only one strain of bacteria ie. *Klebsiella* sps was observed .Proper optimization of PCR must be done for the molecular characterization of bacterial and yeast isolates.

5.3 Waste water treatment and enhancement method for electricity production using MFC

Our MFC showed the removal of reducing sugar by 95.06%, removal of phosphorous by 65.67%, removal of Ammonical –Nitrogen by 67.13% and removal of COD by 58.27%. According to Hisham *et al*., 2013 the Ammonical – Nitrogen reduction using MFC was 65.28%, which shows that our data is in accordance with the Ammonical- Nitrogen removal efficiency. As reported by Bako et al., 2014; the removal of COD is 75% in two chamber MFC but the organism and substrate was different than ours. Also the research by Ghangrekar and shinde, 2006 has shown the capability of MFC to remove the COD by 50% - 90 %.This shows that our MFC is efficient in removing the COD from the paneer whey. The maximum removal of reducing sugar indicates that there is largely presence of organic nutrients, predominantly lactose in paneer whey.

The sharp increase in the voltage is observed in the graph. This is due to the gap while taking the reading. After the sharp increase the voltage is observed to be constant and later decreasing. This means that the voltage sharply increase due to the growth of the microorganisms. When the microorganisms are in exponential phase they tend to grow as much in the presence of the substrate. Later they reach the stationary phase resulting to a constant voltage and finally in the death phase resulting in sharply decrease in voltage production.

The inoculum prepared from mixed culture of bacteria obtained from the sewage of DDC and *lactobacillus* sps obtained from the fermented pickle showed the maximum open circuit voltage (OCV) of 528.4mv on the fifth day. Whereas the single strain of *Klebsiella* sps showed the maximum Open circuit voltage of 509.4mv on the sixth day and *Lactobacillus* sps showed the maximum open circuit voltage of 390.7mv on the third day. Due to the presence of lactose utilizing bacteria like *Klebsiella*, *E.coli* and *lactobacillus* in the mixed culture the OCV might be more as compared to single culture of bacteria. This shows that the MFC inoculated with mixed culture of bacteria is capable of producing the maximum OCV in comparison to the single culture of bacteria.

When the yeast sample *Rhodotorula mucilaginosa* was used the OCV of 522.6mv was obtained on the sixth day in a mediatorless MFC. This value is higher than the single strain of lactose utilizing *Klebsiella* sps and *Lactobacillus* sps. According to Gunawardena et al,.2008; the *Saccharomyces cerevisiae* based fuel cell showed improved performance when methylene blue and ferricyanide was used as electron mediators rendering a maximum power generation of 0.1467W/m^3 and maximum OCV of 383.6mv under 100KΩ resistance. Comparing with this data our yeast sample has a lot of potential for power generation when the mediators is used as it is capable of utilizing the lactose as substrate.

The maximum power of 13.510W/m^3 on the fifth day using resistor of 1000Ω was obtained in our MFC where the mixed culture of bacteria and *lactobacillus* sps were inoculated as anode and the phosphate buffer was used as cathode. According to Wang et al,.2008; the maximum power density of 12W/m^3 was obtained in single chamber MFC using the brewery waste water. In comparison to this data, our double chambered MFC is more efficient in power production than single chambered MFC. Other factors like presence of more organic nutrients in whey, presence of lactose utilizing microorganisms and use of graphite electrode might be supporting for more power generation.

In comparison between Phosphate and sodium acetate buffer, phosphate buffer was able to produce maximum power of 13.510W/m^3 on the fifth day whereas sodium acetate buffer was able to produce maximum power of 5.754W/m^3 on the third day using the resistor of 1000Ω and mixed culture of bacteria along with *Lactobacillus* species. Although the same PH of 7.6 and 0.1M concentration was used; the phosphate buffer produced the maximum power. According to Fan et al.,2007; phosphate buffer maintains suitable PH for electricity generating bacteria and increase the solution conductivity. The optimum PH for phosphate buffer using the two chambered MFC is 7-7.8. According to Fan et al., 2007 and Nam et al., 2010 along with phosphate buffer biocarbonate and Borax buffer can increase the solution conductivity and hence increase the power production.

To generate the maximum voltage, 0.1M of potassium ferricyanide (2ml/min) was added to the phosphate buffer as an electron acceptor and chemical oxidizer. Thus with the mixed culture of bacteria and *Lactobacillus* strains the MFC showed the maximum OCV of 609.5mv and maximum power of 22.261W/m^3 on the fourth day using the 1000Ω resistor. According to Rangel et al., 2010; potassium ferricyanide has the potential for maximum OCV generation of 710mv and maximum power density of 0.92mw/m^2 using the resistor of 5KΩ using the anaerobic sludge. Though the potassium ferricyanide has significantly increased the power it is less in comparision to the data because strains of microorganisms and the biomass used is different.

The graphite electrodes were coated with CNT composite and treated in different forms like CNT ES, CNT EB and CNT treated with absolute ethanol. Out of which the CNT treated with ethanol showed the maximum power of 25.869W/m^3 with 1000Ω resistor on the fifth day using the phosphate buffer enriched with potassium ferricyanide. According to Mustakeem 2015, CNT is an alternative material for MFC electrode because of their electrical conductivity, chemical stability, biocompatibility, high specific area and also catalytic properties. The CNT treated with ethanol produced high power because CNTs have strong cell adhesion, cell attatchment and growth properties. According to Erbay etal., 2015 ; microbes growth over the CNT results in excellent charge transfer characteristics due to π-π stacking between the carbon atoms of the graphite and the pili of microorganisms.

The CNT treated in ES and EB showed the maximum OCV of 333.4mv on the fifth day and 333.3mv on the fifth day respectively. The less power might be produced due to the imbalance in the PH of both anode and cathode effecting the growth of microorganisms. This causes the dramatic change in the electrical properties of CNT composites according to Gajendran and Saraswathi, 2008. Also, for the CNT in ES form, the polymerization of aniline was done to make the polyaniline (PANI).Due to the inappropriate concentration of the mixture of HCL and Aniline, an improper polymerization might have happened resulting to the poor performance.

The cyclic voltammogram consisted of 3 anodic peaks. Two peaks were observed between 0.3V-0.1V. These peaks were probably metal oxides in the graphite electrodes. In the third peak at 0.5V the typical carbon electrode shows the redox peak of electroactive oxide/hydroxide. The corresponding cathodic peak were observed at 0.1V – (-0.5V). The separation between the anodic and cathodic peak does not show a reversible system rather a quasi reversible system. It may be due to the presence of metal oxide. There was no distinct cathodic peak for metal oxide. In the first cycle the anodic current was influenced by the surface activation at above 0.2V. In the later cycles the difference between the fourth and the tenth cycle was not much as compared to the first cycle. The electrode was found to be degrading with the cycle number. However the degradation was not much higher.

CHAPTER SIX

CONCLUSION

The results revealed the feasibility of bioelectricity generation and dairy waste treatment using a two chamber electron mediated MFC fabricated with graphite felt as an electrode and paneer whey as a substrate. Our result showed that the treatment in MFC resulted in reduction of reducing sugar by 95.06%, reduction of phosphorous by 65.67%, reduction of Ammonical – Nitrogen by 67.13% and reduction in COD by 58.27%. This indicates the efficiency of MFC in removal of pollutants and waste water treatment.

The inoculum possessing mixed culture of bacteria and *lactobacillus* sps showed the maximum open circuit voltage of 528.4mV and power of 13.510W/m^3 on the fifth day using resistor of 1000Ω; in comparision to single strain of *Klebsiella* sps and *Lactobacillus* sps. This indicates that the electrogenic mixed culture of bacteria are more electrochemically active than the single culture bacteria in MFC.

The cathodic chamber possessing phosphate buffer was able to produce maximum power of 13.510W/m^3 on the fifth day whereas sodium acetate buffer was able to produce maximum power of 5.754W/m^3 on the third day using the resistor of 1000Ω. The 0.1M phosphate buffer was found to be best in comparison to 0.1M of sodium acetate buffer.

When electron acceptor like 0.1M of potassium ferricyanide (2ml/min) was added to the phosphate buffer the MFC showed the maximum OCV of 609.5mV and maximum power of 22.261W/m^3 on the fourth day using the 1000Ω resistor.

The graphite electrodes coated with CNT composite treated with absolute ethanol showed the maximum power of 25.869W/m^3 with 1000Ω resistor on the fifth day using the phosphate buffer enriched with potassium ferricyanide. However, decrease in power after the fifth day might be due to the leakage of buffer in the anodic chamber which inhibited the growth of microorganisms. Although the power produced is less in comparision to other reports of electrodes coated with CNT; the result is significantly higher as compared to our MFC using graphite electrode. Thus the high electrical conductivity of CNT can be used as a better approach in power generation. The cyclic voltammogram showed that the graphite electrode used was reasonably stable.

Various parameters like maintenance of PH, selection of substrates, microorganisms, electrodes, buffer and external resistance cause the variation in results of MFC. Proper optimization in these factors must be done for efficient result of MFC. One of the biggest obstacles is the lack of investment in improved and advanced form of MFC. In laboratory we used the MFC which was made locally and this caused hurdle due to constant leakage of substrate and buffer. Also, there was increase in oxygen cross over from cathode to anode chamber. This was the major factor which automatically reduced the power production. Thus, overcoming these challenges MFC can be used as a renewable energy recovery while accomplishing the waste water treatment.

Summary

The MFC is one such renewable and sustainable technology that produces electrical energy using microorganisms as a biocatalyst to oxidize the biodegradable substrates. The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production.

The treatment of dairy industrial waste and potent power production using MFC has given a positive feedback for the upscaling and commercial application of MFC technology. MFC can be developed as the efficient sustainable source of energy which might help to reduce the current energy crisis scenario occurring all over the world. The exploration of various electrode materials with increase in surface area and electrical conductivity, selection of potent bacterial strains, and maintainance of PH using different buffers is the key to large scale application of MFC. Although energy production may not be the most important feature of MFC technology, it is still beneficial to further improve electricity production by optimizing MFC configuration, materials and other factors affecting the efficiency.

Recommendation

- Use of optimized protocol for the coating of electrode with CNT.
- Use of highly electrogenic bacteria which are electrochemically active.
- Variation in buffer solutions like borax buffer, bicarbonate buffer and Nacl
- Operation of MFC using various external resistances.
- Molecular identification of bacterial isolates.
- Use of improved version of MFC which might reduce the deficiencies caused by using the locally made MFC.

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Appendix A

Composition and Preparation of different Microbiological Culture Media and Reagents.

A. Culture Media

1. Nutrient Agar(NA)

Peptic digest of animal tissue	5.0 gm
Beef extract	1.5 gm
Yeast extract	1.5 gm
Sodium chloride	5.0 gm
Agar	15 gm
Distilled water	1000 ml

2. Mac-Conkey Agar(MA)

Peptone	20.0 gm
Sodium chloride	5.0 gm
Bile salt	1.5 gm
Lactose	10.0 gm
Neutral red solution (1% aqueous)	10.0 ml
Crystal violet	0.001 gm
Agar	13.5 gm
Distilled water	1000 ml

3. Luria Burtani broth (LB- broth)

Yeast extract	5.0 gm
Sodium chloride	10.0 gm
Enzyme casein hydrolase	10.0 gm
Distilled water	1000 ml

4. Nutrient Broth

Peptic digest of animal tissue	5.0 gm
Sodium chloride	5.0 gm
Beef extract	1.5 gm
Yeast extract	1.5 gm
Final p^H at 25°C	7.4±0.2

B. Biochemical Media

1. 1. MR-VP broth

Peptone	7.0 gm
Dipotassium phosphate	5.0 gm
Dextrose	5.0 gm

2. SIM agar

Peptone	30.0 gm
Beef extract	3.0 gm
Ferrous ammonium sulphate	0.2 gm
Sodium thiosulphate	0.025 gm
Agar	3.0 gm
Distilled water	1000 ml

3. Simmon’s citrate Agar

Magnesium Sulphate	0.2 gm
Sodium chloride	5.0 gm
Ammonium dihydrogen phosphate	1.0 gm
Dipotassium hydrogen phosphate	1.0 gm
Sodium citrate	2.0 gm
Bromothymol blue	0.08 gm
Agar	20 gm
Distilled water	1000 ml

4. Triple Sugar Iron Agar

Peptone	15.0 gm
Protease peptone	5.0 gm
Beef extract	3.0 gm
Yeast extract	3.0 gm
Lactose	10.0 gm
Sucrose	10.0 gm
Dextrose	1.0 gm
Sodium chloride	5.0 gm
Ferrous sulphate	0.2 gm
Sodium thiosulphate	0.3 gm
Phenol red	0.024 gm
Agar	12.0 gm
Distilled water	1000 ml

5. Urea broth

Urea broth base	950 ml
Sterile urea solution	50 ml

Urea broth base was prepared and sterilized by autoclaving at 121˚C for 15 minutes and transferred to a 50-55˚C water bath. The sterile urea solution was added aseptically and mixed well.

C. Staining and Reagents

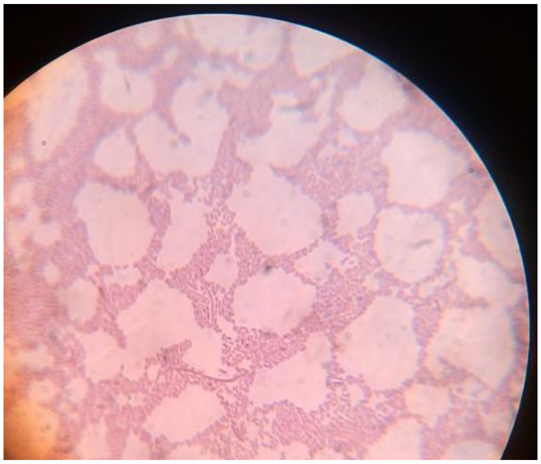
1. **Gram Staining Reagent:** Crystal violet solution(20 g crystal violet, 9 g ammonium oxalate and 95 g ethanol in 1000ml of distilled water), Gram’s Iodine, Acetone-alcohol Decolorizer and safranin.

2. **Barrit’s reagent A** (5% α-napthol solution): 5 gm of α-napthol was dissolved in 95 ml ethanol with constant stirring.

3. **Barrit’s reagent B**: 40% aqueous solution of KOH was prepared.

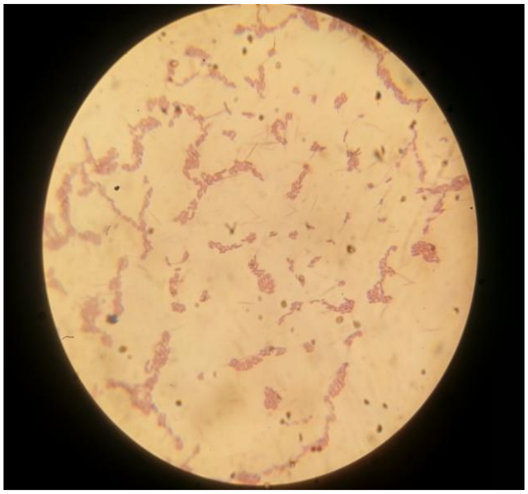
4. **Catalase reagent:** 3% H_2O_2 was prepared.
5. **DNS reagent**: 1 gm of 3, 5-Dinitrosalicylic acid was dissolved in 20 ml of 2N NaOH at room temperature and then 50 ml of D/W was added. To this, 30 gm of Rochelle salt (sodium potassium tartarate) was added and volume of 100 ml was made with D/W.
6. **Kovac's reagent:** 2 gm of para-dimethylaminobenzaldehyde was dissolved in 30 ml of isoamyl alcohol. 10 ml of conc. HCl was added and mixed well and stored in clean brown bottle.
7. **Methyl red solution:** 0.05 gm of methyl red was dissolved in 28 ml of ethanol and 22 ml of water and stored in clean brown bottle.
8. **Oxidase reagent:** 0.1 gm tetra methyl p-phenylenediaminedihydrochloride was dissolved in 10 ml of water. The filter paper is dipped in the solution and dried and stored.
9. **Phosphate buffer**: 8.02 gm of KH_2PO_4 and 7.0368 gm of K_2HPO_4 was dissolved in 1000 ml of distilled water to make 0.1M solution of P^H=7.6.
10. **Sodium tetraborate solution 0.025mol/ltr:** 5gm of anhydrous sodium tetraborate in was dissolved in 1000ml distilled water.
11. **Digestion solution:** 10.2 gm of potassium dichromate, 167ml of conc. sulfuric acid and 33.3gm of mercury sulfate was dissolved in 500ml distilled water and diluted to 1000ml with distilled wate.
12. **Catalyst solution**: 22gm of silver sulfate was added to 4.09 kg bottle of concentrated sulfuric acid and let it stand for 2 days until dissolved.
13. **Borate buffer**: 88ml of 0.1mol/ltr of sodium hydroxide solution was added to 500ml of 0.025mol/ltr of sodium tetra borate solution and diluted the mark to 1000ml with distilled water.
14. **Boric acid solution**: 20gm of boric acid was dissolved in distilled water and made the mark upto 1000ml with distilled water.
15. **Potassium hydrogen pthalate stock solution**: 0.850gm of potassium hydrogen phthalate was dissolved in 800ml of distilled water and diluted the mark upto 1000ml in volumetric flask.

Microscopical structure of bacterial Plates

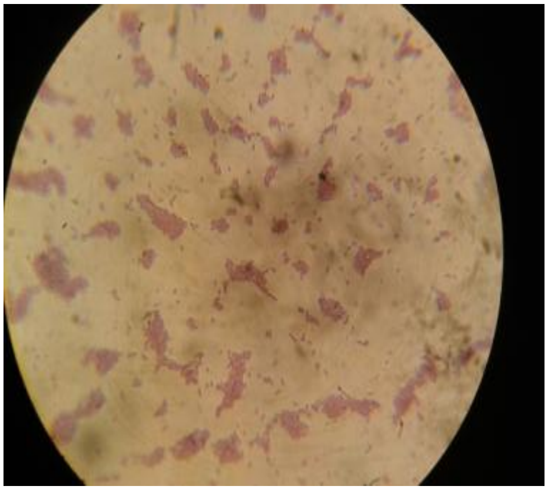


Klebsiella **sps**

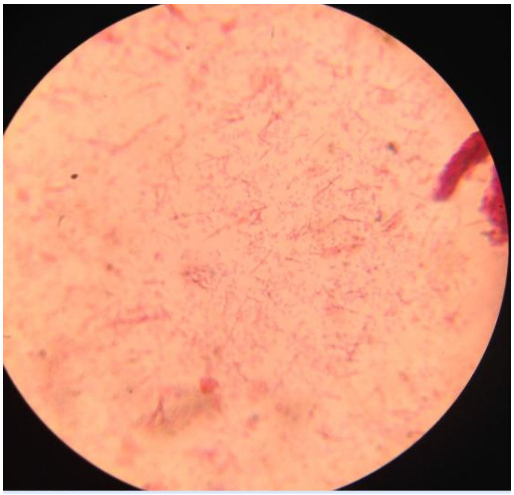
Pseudomonas **sps**



E.coli



Lactobacillus



Salmonella sps

Biochemical tests

Analysis of COD, Phosphorous, reducing sugar, and Nitrogen

Analysis of reducing sugar