ANALYSIS OF DEGRADABLE HOUSEHOLD KITCHEN WASTE AND HARNESSING BIOELECTRICITY FROM IT BY ENHANCING MICROBIAL FUEL CELL (MFC) PERFORMANCE

M.Sc. Thesis

Year 2023

Submitted to

Central Department of Biotechnology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

For partial fulfillment of the requirement for the Master of Science in Biotechnology

Submitted By

Pravesh Paudel

T.U Regd. No.: 5-2-37-818-2013

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RECOMMENDATION

This is to certify that the research work entitled **"Analysis of degradable household kitchen waste and harnessing bioelectricity from it by enhancing Microbial Fuel Cell (MFC) performance"** has been carried out by Mr. Pravesh Paudel under my supervision.

This thesis work was performed for the partial fulfillment of the Master of Science in Biotechnology under the course code BT 621. The result presented here is his original findings. I hereby, recommend this thesis for final evaluation.

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GLOSSARY ACRONYMS

AAS	Atomic Absorption Spectroscopy
APS	Ammonium per sulfate
BOD	Biological Oxygen Demand
CE	Columbic Efficiency
CEM	Cation Exchange Membrane
CNTs	Carbon Nanotubes
COD	Chemical Oxygen Demand
CTAB	Cetyltrimethyl Ammonium Bromide
CV	Cyclic Voltammetry
DET	Direct Electron Transfer
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleoside Triphosphates
DO	Dissolved Oxygen
FAO	Food and Agricultural organization
FMFC	Fabricated Microbial Fuel Cell
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
IRENA	International Renewable Energy Agency
ISWA	International Solid Waste Association
M	Molar
MET	Mediated Electron Transfer
MFC	Microbial Fuel Cell
MSW	Municipal Solid Waste

NAST	National Academy of Science and Technology
nm	Nanometer
OCV	Open Circuit Voltage
OLR	Organic Loading Rate
OMC	Outer Membrane Cytochromes
PANI	Polyaniline
PCR	Polymerase Chain Reaction
PEM	Proton Exchange Membrane
PVC	Polyvinyl Chloride
RE	Reference Electrode
RID	Refractive Index Detector
rpm	Rotation per minute
SCE	Standard Calomel Electrode
SHE	Standard Hydrogen Electrode
SLR	Sludge Loading Rate
TSS	Total Suspended Solids
UN	United Nations
UV	Ultraviolet
V	Volt
v/v	Volume per volume
VSS	Volatile Suspended Solids
W	Watt
WTE	Waste to Energy

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ABSTRACT

Today the world is facing a problem in solid waste management along with global energy depletion due to rapidly increasing population. Microbial Fuel Cell (MFC) technology, which transforms chemical energy residing in organic compound into electrical energy with the support of microbes, is regarded a promising alternative. In this study, dual chambered Microbial Fuel Cell (MFC) was operated to evaluate the potential for degradation of organic kitchen waste under anaerobic microenvironment using mixed consortia as anodic biocatalyst. The potential for bioelectricity generation from this system was also evaluated. Degradation potential of waste was evaluated by observing Chemical Oxygen Demand (COD), Ammoniacal-nitrogen and Phosphorus removal rates. Initially, the degradable kitchen waste had COD content of 26.75 ± 0.68 mg/g, Ammoniacal-nitrogen 0.017 ± 0.005 mg/g and Phosphorus 0.0956 ± 0.0068 mg/g. When multi-walled carbon nanotubes (MWCNT) coated graphite sheet electrode was used as anode, graphite sheet as cathode, organic kitchen waste as anolyte and phosphate buffer along with $KMnO_4$ as electron acceptor there was reduction in COD by 41.53 %, Ammoniacal-nitrogen by 64.12 % and Phosphorus by 45.25 %. Sugar analysis in waste sample was done by HPLC. The amount of glucose was 167.39 ± 10.21 mg/g and arabinose was 11.22 ± 0.69 mg/g. These sugars were not detected during end product analysis by HPLC which means we can say there was complete removal of these sugars. The maximum OCV observed was 558.0 ± 25.23 mV when multi-walled carbon nanotubes (MWCNT) coated graphite sheet electrode was used as anode electrode. Maximum power density of 323.59 ± 33.89 W/m^3 was obtained when an external resistance of 1000 ohm was used. The Microbial Fuel Cell (MFC) electrical performance was examined with cyclic voltammetry (CV). Bacteria from anode biofilm were isolated. Their morphological, biochemical and molecular characterization up to PCR were performed and submitted for sequence analysis. Biocompatibility test was performed to observe if the coated MWCNTs and neutral red had negative effect on anode biofilm. Resistance of the isolates to multiple drugs suggested proper management and disposal of domestic waste. End products analysis after MFC performance was done by using HPLC and concentration of each compounds were determined. Finally, this research conveys that microbial fuel cell (MFC) can be a promising alternative for organic waste management along with electricity generation.

Keywords: organic kitchen waste, Microbial Fuel Cell (MFC), Chemical Oxygen Demand (COD), Multi-walled Carbon Nanotubes (MWCNTs), Cyclic Voltammetry (CV), High Performance Liquid Chromatography (HPLC)

CHAPTER 1: INTRODUCTION

1.1 Background

Energy is everywhere. It is the force that drives everything. It powers our bodies, propels our vehicles and illuminates the entire universe. Planet earth is facing energy crisis due to an increase in global energy demand, a persistent reliance on fossil fuels for energy production and transportation as well as rapid increase in world population exceeding seven billions and rising steadily (Coyle and Simmons, 2014). Excessive burning of fossil fuels not only depletes the natural resources but also cause a constant rise in carbon-dioxide emission which experts believe is responsible for increasing average global temperature. Scientific communities and governments are in agreement that climate change is accelerating due to human activities and profound measures will be needed to mitigate its negative impacts (Coyle and Simmons, 2014).

Solid waste management is the principal challenge faced by authorities in developing countries. Municipal wastes are typically generated from variety of sources depending upon the type of human activities involved. The composition of waste varies from one municipality to another municipality as well as from one nation to another. These variations are primarily influenced by way of life, economic conditions of people, waste management laws and industrial structure. According to a number of studies municipal solid waste generated in developing countries are from households (55-80%) followed by markets or commercial sectors (10-30%) (Abdel-Shafy and Mansour, 2018).

Huge portion of municipal solid waste is comprised by food and kitchen waste. The organic portion of food and kitchen waste includes fruits, vegetables, cooked food waste and meats which are rich in carbohydrates, proteins, lipids and inorganic components. Several reports published by FAO shows that major proportion of food waste are generated during production, handling, storage, processing and consumption. Improper management of these wastes leads to several health hazards as well as critical environmental issues. However, different value added products like antioxidants, bio-actives, bioethanol, biogas, bioelectricity, biopolymers etc. can be produced from food and kitchen wastes. The main challenge in conversion of food wastes is their heterogeneous nature as well as high moisture content and low calorific value (Sindhu et al., 2019). Treatment of these organic wastes through the action of microorganisms and enzymes is possible in microbial fuel cells in addition to production of bioelectricity (Wang et al., 2013).

1.2 Techniques of organic waste management

1.2.1 Landfill

In many nations, landfills are the most widely used method of garbage disposal. The goal of this procedure is to bury the garbage in the ground. There are many problems associated with landfills, including infrastructure disturbance, road and environmental damage, including contamination of the soil and ground water. Extensive efforts have

been done to collect and treat landfill leachate before it enters groundwater. Methane is produced naturally when organic garbage decomposes in landfills. It is a strong greenhouse gas and can be dangerous due to its inflammatory nature and possibility for explosion in landfills. Additionally, rats and flies, which are transmitters for dangerous diseases, may make poorly managed landfills into a nuisance (Nabavi-Pelesaraei et al., 2017).

1.2.2 Incineration

Wastes are burned at high temperatures during the incineration process to create residues and gaseous products. The benefit of this procedure is that it can reduce the volume of solid waste to 20–30% of the initial volume, which lessens the area that the solid waste occupies and eases the pressure on landfills. The process, also known as thermal treatment, involves burning solid waste in incinerators to produce heat, gas, steam, and ash (Nabavi-Pelesaraei et al., 2017).

1.2.3 Composting

Organic wastes like plant remnants and kitchen scraps are transformed into nutrient-rich nourishment for plants through the simple and natural biodegradation process known as composting. Composting, which is typically utilized for organic farming, happens when organic materials are left in one location for months until microbes start to break them down. Despite the fact that it takes a long time and a lot of room, it is an effective way to dispose of garbage since it may turn hazardous organic waste into harmless compost. (Rawoteea et al., 2017).

1.2.4 Anaerobic digestion

In the absence of oxygen, microorganisms break down biodegradable materials through a number of processes known as anaerobic digestion. Waste management and/or fuel production are done using this procedure for domestic or commercial uses. In both domestic fermentation and industrial food and beverage production, anaerobic digestion is employed.

Bacterial hydrolysis of the input materials is the first step in the digesting process. The breakdown of insoluble organic polymers like carbohydrates yields soluble derivatives that are then accessible to other microorganisms. Acidogenic bacteria finally convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. The bacteria produce these acetic acids as well as more ammonia, hydrogen, and carbon dioxide. Methanogens ultimately transform these substances into methane and carbon dioxide. For the treatment of wastewater, methanogenic archaea populations are necessary.

The process of anaerobic digestion is utilized as a sustainable energy resource. It is used as a technique in the treatment of sewage sludge and biodegradable garbage. It reduces the quantity of landfill gas liberated into the atmosphere behaving as part of an integrated waste management system (Pang et al., 2015).

1.2.5 Recovery and recycling

Resource recovery is the practice of making use of recyclable garbage and making them of a particular use. The components and resources from these leftover items are eventually extracted, recovered, or converted into usable heat, power, or fuel. Recycling is the technique of converting discarded items into advanced ones so as to conserve energy and fresh raw material use. Recycling targets to preserve natural resources for future use and lowers energy consumption, landfill size, air and water pollution, and greenhouse gas discharge (Campos, 2008).

1.2.6 Waste to energy

Waste to energy is the process of transforming non-recyclable waste into usable heat, power, or fuel. Since non-recyclable waste may be recycled multiple times and used to produce electricity, this sort of energy source is renewable. By decreasing the demand for energy from fossil fuels, it can also help in reducing carbon discharges. The technique of converting waste into energy, either it can be heat or electricity, is said as waste to energy (Brunner and Rechberger, 2015).

1.3 Microbial Fuel Cell

The electrochemical activity was described for the first time in early 20^{Th} century employing bacterial/fungal (yeast) species and electrodes by Potter in which live cultures of Escherichia coli and Saccharomyces spp. utilized platinum macro-electrodes in a battery-like setup with sterile media to produce electricity. In 1931, Cohen proved this electrochemical procedure showing a voltage of 35 V at a current of 0.2 mA from a stack of bacterial fuel cells. Electro-microbiology come into discussion only when NASA space program demonstrated the capability to recycle and convert human excrement to electricity at the time of space flights in 1993. Pioneering work by Habberman and Pommer in 1990 showed long-term MFC. In this study the MFC run magnificently and deprived of maintenance for five years (starting in 1986) while utilizing municipal wastewater. Treatment of domestic wastewater and indirect electron transfer through soluble mediators was also described for the first time from that research. In 1999 it was exposed that mediators were not required for MFC configurations, so that MFCs could be designed without the cost of intermediaries (Slate et al., 2019).

Microbial Fuel Cell is bio-electrochemical system in which microorganisms transform chemical energy to electrical energy by the phenomena of oxidation of different carbon sources or organic residues (Angenent et al., 2004). The technology derives energy from microbes' metabolism and seems to be compelling enough to assist energy generation. The application of MFC as an alternative source for producing electricity is observed as a trustworthy, clean, and effective process. It results in the use of renewable resources and produces no hazardous byproducts. MFCs have thus approved to be a efficient technology for recovering and in-place transforming chemical energy into electricity in recent days (Logan, 2004).

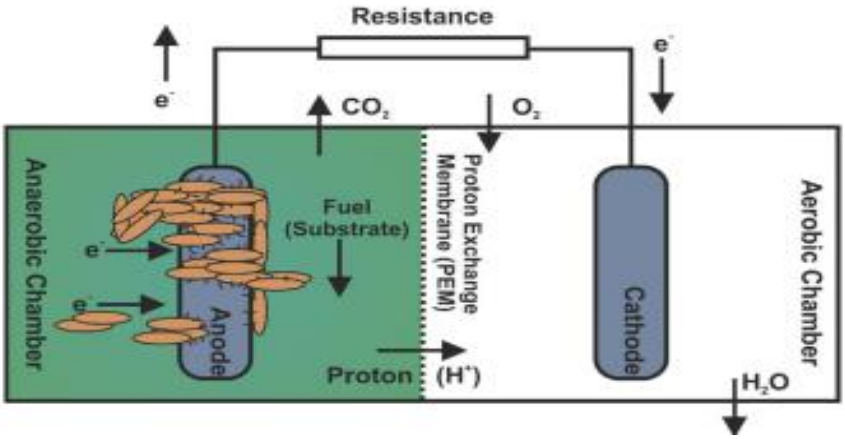


Figure 1.1: Schematic representation of Microbial Fuel Cell (Slate, 2019)

A classical MFC comprise of two chambers i.e. anodic and cathodic chambers with anode and cathode respectively. These chambers are parted by proton exchange membrane (PEM). In the anodic chamber, the microorganisms anaerobically break down the available substrate to generate electrons which are then transferred from anode to cathode through external circuit. The protons that are released from this process are then selectively transferred from the exchange membrane. Both of these byproducts, that the bacteria in the anodic compartment produced, move to the cathode and mix with oxygen to produce water (Slate, 2019).

Glass, polycarbonate, and Plexiglas can be utilized to construct MFC chambers. Anode electrodes can be prepared of materials such as carbon fabric, carbon paper, graphite, and graphite felt. To conserve the electrode's aerobic property, an air cathode is applied, and this can be built of platinum (Pt) or Pt-black catalyst materials (Zhang et al., 2011). The anode chamber consists of organic substrates that will be utilized by the microorganisms to produce electrons that will subsequently be gained by the solution in the cathodic chamber by the cathode. The ion exchange membrane permits the generated protons to move from the anode to the cathode. Ferricyanide ($[Fe(CN_6)]_3$) and permanganate (MnO_4) solutions can act as catholytes but are not considered appropriate (Wang et al., 2013).

1.3.1 Current studies on treatment of organic wastes by microbial fuel cell

The majority of municipal solid waste (MSW) produced in cities is disposed in open landfills or in poorly constructed and operated sanitary landfills leading to complications such as ground water pollution due to leachate percolation thus causing several health related problems. Food waste which comprises between 30-55% of MSW is more challenging to dispose because of its highly decaying nature (Rikame et al., 2012). Different technologies like composting and anaerobic digestion have been practiced for years to transform waste to energy. These techniques have their own advantages however taking present and future energy demand into account there is a need to generate direct useable energy from waste which can fulfill energy demand as well as help in waste management (Moharir and Tembhurkar, 2018).

MFC is a novel bio-electrochemical system which has the potential of using organic wastes and biomasses to generate bioelectricity utilizing microbial metabolism. The

technique has lately received a lot of attention as a potential strategy for producing sustainable energy along with bioremediation (Wang et al., 2015). Design of the reactor, electrode material, acclimatization of the biofilm, biomass/substrate, presence of electron donating mediators etc. are several factor that affect MFC performance (Li et al., 2018). Various organic compounds like cellulose, starch, simple carbohydrates, organic acids, amino acids and proteins, toxic waste chemicals like phenols, nitrobenzene, sulphides etc. have been reported to be utilized as oxidizable substrate to power MFCs (Hao et al., 2016). Sewage sludge, municipal waste, paper mill and food industry wastewater, metal contaminated wastewater etc. have also been utilized in laboratory scale MFC for bioelectricity generation (Kim et al., 2016). Although number of substrates have been reported to be utilized as substrate to power MFC very few reports are found on the use of kitchen waste in bio-electrochemical system (Adebule et al., 2018).

1.4 Research Hypothesis

Null Hypothesis (H_0)

The biodegradable kitchen waste cannot be utilized by microbes for waste degradation and bioelectricity generation in MFC.

Alternative Hypothesis (H_1)

The biodegradable kitchen waste can be utilized by microbes for waste degradation and bioelectricity generation in MFC.

1.5 Research Objectives

1.4.1 General Objective

- Application of Microbial Fuel Cell to observe degradation efficiency of kitchen waste by mixed culture of microbes and study the electricity generation efficiency.

1.4.2 Specific Objectives

1. Analysis of kitchen waste for its pH, Total Suspended Solid (TSS), Volatile Suspended Solids (VSS), Chemical Oxygen Demand (COD), ammonia nitrogen, Phosphorus, sugar contents by HPLC, metal ions and trace elements.
2. Isolation and molecular characterization of microbes from anode biofilm.
3. Operation of microbial fuel cell for enhancement of electricity generation using finely blended degradable kitchen waste sample.
4. Observation of waste degradation status and electricity generation in all successive waste degradation processes.
5. Analysis of byproducts generated in MFC by HPLC.

1.6 Rationale

Huge amount of waste is generated during production, harvest, sorting, packaging and utilization of fruits, vegetables and other food crops which ultimately pollutes the environment (El-Ramady et al., 2014). Also, with rapid increase in world population, the energy demand is accelerating thus subjecting the world to energy crisis and pollution. Thus, the main focus of the world has become organic waste management on one hand with utilizing organic waste as substrate for bioenergy generation on others. The use of MFC seems promising technology for waste treatment and energy production as it has mild working condition and also can use variety of organic waste as substrate. In recent years, research in this field has tremendously increased as the technology focuses on generation of green energy from organic waste. MFC has shown diverse applications such as bioelectricity generation, bio-hydrogen production, wastewater treatment, biosensors etc. Although there are several advantages of using MFC for waste treatment and bioenergy generation, the power generated is too low for commercial use, so researches have been continuously conducted to enhance MFC performance, to make it more efficient and cost effective (Maski et al., 2018).

1.7 Research Scope

MFC has variety of applications from bioelectricity generation to wastewater treatment, biosensor and bio-hydrogen production. Bioelectricity production from MFC technology can be a crucial form of bioenergy in coming days since it permits producing energy from wide varieties of soluble or dissolved complex organic residues and renewable biomass. Higher running cost, minimum power output from MFC and minimum cost of fossil fuels make it uncertain that bioelectricity from MFC can ever contest with present energy production techniques. Yet, it is undeniable that MFCs are carbon neutral, and in this case, cellulosic materials can be used to produce energy. So, improving power densities from MFCs, lowering the material and operation cost and global movement towards energy generation without net CO_2 emission may make MFC technology practical in future. MFCs are capable of converting biomass at low temperature and substrate concentration. But it relies on biofilms for mediator-less electron transport which is major downside of MFC. Identification of new acidophilic microbe which can magnify the electron transport from anode biofilm to cathode is desirable. Superbugs for MFC can be developed and exploited in future. Optimized microbial consortium with ability of better mass transfer and electron transfer but without mediator and biofilms are expected in future (Goswami and Mishra, 2018).

CHAPTER 2: LITERATURE REVIEW

2.1 Wastes to Energy (WTE) Technology:

Waste to Energy (WTE) is a method of obtaining usable heat, power, or fuel from waste materials. WTE technologies are currently viewed as the best solutions for resolving waste-related issues (Zhao et al., 2016). The global urban population is growing faster than the overall population. The majority of the world's population now live in cities, hence population increase, urbanization, and economic growth are the main causes of the MSW generation worldwide. Since generation rate depends on a country's economic and social potential, industrialized countries now have a higher per capita MSW generation rate than developing countries. The increase in MSW generation rate is mostly caused by changes in urban residents' eating habits, consumption patterns, and level of life (Khan et al., 2016). The biggest obstacle is still finding improved WTE technologies. Major obstacles for WTE technology include the emission of potentially harmful compounds, rising costs, and community opposition (Ren et al., 2016).

2.1.1 Present Scenario of Waste to Energy at Global Level

By 2025, it's predicted that there will be 8.1 billion people on the planet. According to United Nations (UN), the world population has so far touched 8 billion by November 15 2022. The increasing urbanization and industrialization brought on by the tremendous rise in world population and economic development have altered peoples' consumption patterns and, as a result, have caused MSW to proliferate at an alarming rate. According to an estimate by the International Renewable Energy Agency, the globe has the capacity to produce around 13 Gigawatts of energy from the WTE industry alone (IRENA, 2016). Particularly in industrialized nations, WTE technologies have seen significant modernization and advancement. According to the International Solid Waste Association (ISWA), more than 130 million tons of MSW are handled each year to produce power (ISWA, 2012) In densely populated nations like China, incineration is the most widely employed WTE solution (Liu et al., 2016). But on a smaller scale, developing nations like India, Vietnam, and Malasia have begun to extract energy from biological wastes. According to Nguyen et al., (2014), the anaerobic digestion process alone might produce enough biogas to supply up to 4.1% of Vietnam's electrical needs.

2.1.2 Importance of Waste to Energy Conversion

The world's energy demand is anticipated to increase by nearly six times by the end of this century (Kothari et al., 2010). The current available energy supply is much lower than the actual energy required for consumption in many of the developing countries. Fossil fuels currently account for around 84% of the world's electricity output, making them one of the main energy sources in use today. Since the world's reserves of fossil fuels are rapidly depleting, alternative energy sources like WTE are needed to prevent an approaching energy crisis (Charters, 2001). In many developing nations, there is a dilemma with how to dispose of the massive amounts of generated MSW and the need for a dependable source of renewable energy. MSW produces significant environmental degradation, therefore using it as a possible renewable energy source would help with

both garbage management and addressing the rise in energy demand. WTE technology has become a potential substitute, particularly for industrialized nations, thanks to technological development, enhanced pollution control methods, political incentives, and strict regulations. It offers a source of energy as well as additionally lessens any potential negative effects of trash on the environment. If equivalent CO2 emissions from fossil fuel-based power plants are also taken into account to generate the same amount of electricity, then 1.3 tons of CO2 equivalent emissions can be avoided for every tons of MSW that is burned for electricity generation rather than landfilling (without gas recovery) (ASME, 2008). When compared to fossil fuel-based power plants, waste incineration facilities with energy recovery facilities that use pre-treated MSW as a primary fuel have slightly lower net carbon emission factors (0.04 to 0.14 kg/MJ). When managing the same amount of garbage, WTE facilities require substantially less area than disposal facilities do (Jamasb and Nepal, 2010). Compared to a landfill for 30 million tons of MSW, a WTE plant processing one million tons of garbage annually has an average operational life of more than 30 years and uses less than 100,000 m^2 of land.

2.1.3 Organic Waste Status in Nepal

The composition of organic waste in municipal solid waste was highest i.e. 54.0% in the year 2075/76 in comparison to inorganic waste (33.3%) and other wastes (12.7%) according to waste management baseline survey of Nepal, 2020. Kalimati Fruits and Vegetable Market is a wholesale market of Kathmandu city, where 650 metric tons of vegetables arrive every day, out of which 12-25 metric tons of vegetables are discarded daily because they are found to be damaged as per the report published in Global Press Journal in 2014. In Nepal, biofuel production from waste material has not been industrialized yet; researches are being done on it in recent days. Bioethanol is produced from the red potatoes grown in hilly region of Nepal, optimum ethanol was 5.2 % acquired at a temperature of 30˚ C (Joshi, 2014). According to the report recently published in The Kathmandu Post in January 9, 2023, Kathmandu Metropolitan City produces about 73,000 tons of municipal waste annually among which 40,000 tons belong to organic waste. If this amount of waste could be screw pressed to dewater and reduce 80 percent of water and 50 percent of its volume, 8,000 tons of briquettes could be produced compensating the coal needed in Nepal, according to the report.

2.2 Role of MFC in Organic Waste Treatment

The bioreactors known as microbial fuel cells (MFCs) use microbial catalysis to transform chemical energy found in organic or inorganic compound substrates into electrical energy. MFCs have proven to be a superior choice for producing bioelectricity and recycling organic waste in order to preserve a clean and healthy environment. Manjrekar et al., (2018) studied the use of the sugarcane molasses and kitchen garbage as fuel in MFC electricity production. Kitchen trash (orange peels, banana peels, vegetable peels, leftover veggies, etc.) was utilized to operate the MFC and effectively digested to produce slurry before being added to the anode chamber, where 260 mV was observed to be the optimal potential. Municipal Solid Waste (MSW) is prominent classes of solid waste that is accumulated daily in large quantities and has the potential to be used as

fuel in bio-electrochemical systems (BES). Amid the solid waste researched food industry associated waste are more examined. Using a cylindrical single chambered MFC with an air cathode, Chandreshekhar and his coworkers investigated the degeneration of canteen-built food waste in solid state fermentation mode (Chandreshekhar et al ., 2015). Food waste collected from the canteen was used as substrate and anaerobic mixed culture was used as inoculums. Before adding it to the reactor, the oil proportion was removed by gravimetric separation, and 10% tap water was added to maintain moisture content. A maximum power density of 164 mW/m^2 was recorded, along with a hydrogen production rate of 21.9 mL/h and an ethanol production rate of 4.85% (w/w).

Agricultural waste products have been investigated as a substrate for bio electrochemical systems, including wheat straw, cattle dung, and corn straw (BES). Similar to this, because of their enriched organic percentage, highly biodegradable food wastes such vegetable residues, canteen-based garbage, yogurt waste, etc. can be simply used to generate biochemical energy (Srikanth et al., 2016). The widely produced, carbohydrate-rich vegetable leftovers from all around the world can be effectively used to recover energy utilizing MFCs. The application of vegetable waste in its natural state and its fermented effluents (from the process of producing hydrogen) in MFCs is proposed by Venkata Mohan and his coworkers (Venkata Mohan et al., 2010). Vegetable waste generated 57 mW/m^2 of electricity, whereas pre-fermentation increased that production to 111 mW/m^2 in MFC. Since complex polysaccharides and proteins are hydrolyzed to their corresponding monomers during fermentation, the treatment efficacy also showed good improvement with pre-fermentation (62 to 80%). Another study by Zhang et al. (2013) found that corn stover could be used to remove sulfide and generate energy in MFC, with the best power density reaching 744 mW/m^3, the best sulfide elimination reaching 91%, and the best COD elimination reaching 25%.

2.3 Working Principle: Dual Chambered Microbial Fuel Cell

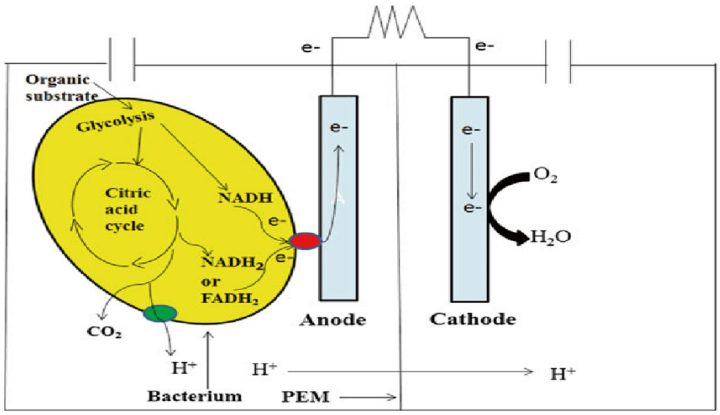


Figure 2.1: Working principle of MFC (Logan et al., 2006)

MFCs are a type of bio-electrochemical device that converts chemical energy found in organic or inorganic substances into electric current by using microorganisms as

biocatalysts (Aelterman et al., 2006; Bermek et al., 2014; Kumar et al., 2016). The anode and the cathode are the two chambers of a traditional double-chamber MFC. In order to allow the protons produced at the anode to migrate along itself to the cathode, a proton exchange membrane (PEM) is often provided between these two chambers. To build a complete MFC system, the cathode and the anode are connected via an electrical circuit, such as copper or titanium wires. The bacteria in the anode chamber oxidize the organic substrates, releasing carbon dioxide, protons, and electrons in the process. Redox-active proteins or cytochromes first transmit the electrons from the microbial metabolic activity to the anode surface, where they are then transferred to the cathode along the electrical circuit (Borole et al., 2011; Kumar et al., 2015). Electrons are reduced at the cathode chamber. At the cathode, an electron acceptor is typically given, such as oxygen or ferricyanide. Next, at the cathode, oxygen, protons, and electrons combine to form water. A platinum catalyst, for example, can also help this reaction. The established working procedure can be represented as below:

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

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

2.4 Microorganisms utilized in Microbial Fuel cell

As a result of the metabolism of organic materials, certain microorganisms have the ability to transmit electrons to the anode. Due to the ability of bacteria to reproduce on their own and behaving as self-sustaining catalysts for the oxidation of organic materials, the evolution of technologies that use bacteria to produce electricity marks a likely method for the production of bioenergy.

The most significant current-producing bacterium is *G. sulfurreducens*, which is also the most frequently identified exoelectrogenic species in MFCs inoculated with incredibly varied and concentrated microbial samples using media with a pH range of near neutral to moderately saline and low temperatures. *G. sulfurreducens* often predominates when the inoculum is obtained from sediments or waste water and the reactor is supplied with acetate in a bicarbonate-buffered or phosphate-buffered medium (Holmes et al., 2004). The marine bacteria Shewanella putrefaciens is gram-negative and facultative anaerobe which can metabolically reduce iron and manganese. *Shewanella putrefaciens* appeared first to generate electricity when there is lack of exogenous mediators in 1999 (Kim et al., 1999) *Shewanella* spp. consists of outer membrane cytochromes which can be used for direct electron transfer by contact, but they can also release out electrically conductive nanowires (Myers and Myers, 1992). *Pseudomonas aeruginosa* is a gram-negative, aerobic rod that belongs to the Pseudomonadaceae bacterial family and is a member of the Gamma Proteobacteria subclass of bacteria. Pyocyanin and related substances produced by *P. aeruginosa* are examples of self-made or autogenic/endogenous chemical mediators that have the capacity to transmit electrons to an electrode and produce energy in an MFC (Rabaey et al., 2004). *Escherichia coli* is widely used as a non-exoelectrogenic control because of its history as a non-exoelectrogen. *E. coli* can, however, be used in some circumstances to produce current. Certain strains have been seen to produce power densities of 3,800 mWm^2, which are comparable to strains of Geobacter or Shewanella following

prolonged cultivation or cultivation using electrodes treated with chemicals (Li et al., 2018). Different other microorganisms, like *Bacillus subtilis*, *Pseudomonas putida* and *Klebsiella aerogenes* , commonly generate comparatively low current densities but they possess distinctive responsibility in biofilm microbial ecology (Doyle and Marsili, 2018).

2.5 Electron Transfer Mechanism in MFC

2.5.1 Direct Electron Transfer (DET)

In this method, the electrons travel directly from the microorganisms to the electrode. This requires a physical engagement between the electrode and the bacterial cell membrane (Prathiba et al., 2022). There is absence of dissolved redox species taking part in this pathway for electron transport. DET is instead carried out by self-assembled nanowires, conductive pili, or outer membrane cytochromes (OMCs). It was found out that the protein possess self-assembling properties, making nanowires that might assist electron transmission. LSCV cyclic voltammetry, electron impedance spectroscopy and other techniques are applied to examine direct electron transfer in exoelectrogenic biofilms (EIS) (Winaikij et al., 2018).

2.5.2 Mediated Electron Transfer (MET)

Many microorganisms are not able to carry out DET because either they do not have the required mechanisms or because do not have the contact with the electrode surface. In such scenarios, they carry out electrons in an indirect way. In mediated electron transfer (MET), a redox carrier acts as a shuttle to transport electrons to the terminal electron acceptor (TEA).This redox mediator in MFCs carries electrons from bacterial cells, keeping them reduced and moves them to anode, oxidizing them during the process. The oxidized form is then free to begin the subsequent round of electron transfer. A good mediator should not be harmful to bacteria, be able to easily penetrate bacterial cell membranes, have a sufficiently high redox potential to aid in electron transfer, be better soluble in the anolyte, and be economical and easily available for commercial use (Aiyer, 2020).

2.6 Essential components of Microbial Fuel Cell

A PEM divides the anodic and cathodic chambers of a traditional MFC, which are constructed from a variety of materials. This is a list of the MFC's different components.

2.6.1 Anode

The anodic substance in the reactor solution should have conductive properties, biocompatibility, and chemical stability. Due to its harmful nature to bacteria, metals like copper cannot be used as anodes; however, metal anodes with non-corrosive stainless steel mesh can be. Carbon, which can be found as tightly packed graphite plates, rods, or granules, as well as fibrous material and glassy carbon, is the most flexible electrode material. In comparison, graphite plates, rods, or granules are less expensive, easier to handle, and have precise surface areas (Logan, 2008).

2.6.2 Cathode

The cathode material choice significantly alters performance of MFC and it is altered based on its application. Various types of materials have been tested as bio-cathode in MFCs, along with carbon paper, graphite fiber brush, graphite felt and stainless steel mesh. Carbon felt was shown to be more suitable for bio-cathode than stainless steel meshes, according to De Schamphelaire et al., (2010). The communication between microbial biofilms and electrode surfaces is demanding for overall cathode performance, along with microbial adhesion, electron transport, electrode resistance and electrode surface reaction rate.

2.6.3 Membrane

The anode and cathode compartments are separated by PEM in almost all MFC systems. The PEM that is used the most is nafion. Alternatives to Nafion, such Ultrex CMI-7000, are suitable for MFC applications and sometimes more affordable. It is crucial to understand that when a PEM is used in an MFC, it could be permeable to substances like oxygen, ferricyanide, other ions, or organic materials used as the substrate (Logan, 2008).

2.6.4 Catalysts/ Catholytes

The cathode chamber is a compartment where protons and electrons reunite and reduce an electron acceptor. Because of its high oxidation potential, availability, affordability, sustainability, and lack of a chemical waste product, oxygen is the best electron acceptor for an MFC. When the cathode consists of acceptable electron acceptor (for example, oxygen) and the cell's overall thermodynamics are suitable, electrons move concurrently along the external circuit to the cathode for the reduction processes. The cathode catalysts are classified into carbon-based complexes and biocatalysts. Likewise, into activated carbon, carbon nanotube, graphite/grapheme, metal oxides and others. Chemicals like as ferricyanide, potassium dichromate and potassium permanganate have been exploited successfully with results similar to those attained with platinum. These chemicals are cheaper than platinum however, the downside is that they are used up in the reaction and must be changed (He and Angenent et al., 2006). In comparison to MFCs with oxygen, those with potassium permanganate and potassium ferricyanide yield comparatively higher voltage. (Cai et al,. 2015).

2.7 Biofilm Anode

The biofilm-anode in MFC provides the framework for turning a chemical substrate into energy. By a sequence of protein-based redox processes, the biofilm metabolizes the substrate and sends the subsequent electrons to the anode. The ability of a bacterium's outer membrane proteins or cytochromes to transfer electrons to the anode determines how much electricity is actually generated by these reactions. Its ability is determined by two factors: 1) the bacterial electron transport chain's metabolic capacity; and 2) the activation resistance between the anode and the electron transport chain. The anode, which is the final electron acceptor in the biofilm-anode, cannot be improved by genetic

engineering, despite the possibility that it could improve the metabolic efficiency of the electron transport chain. However, by designing a better connection between the electron transport chain and the anode through the use of a catalyst or an altered electrode, the activation resistance seen in the terminal electron transfer might be reduced. The fact that protein-protein interactions have become functional but protein-electrode interactions are still being researched and developed supports this concept (Gerasimov et al., 2010; Alwarappan et al., 2010).

2.8 Designs of Microbial Fuel Cell

2.8.1 Two compartments microbial fuel cell system

In order to produce energy, two-compartment MFCs are often run in batch mode using a chemically delimited medium like glucose or acetate solution. Currently, they are only used in laboratories. Anodic and cathodic chambers are connected by a PEM or salt bridge in a typical two-compartment MFC. While allowing protons to move from the anode to the cathode, it also prevents oxygen from diffusing into the anode. The chambers may be equipped with a variety of useful constructions. These can be helpful for refueling autonomous sensors for extended operations in harder-to-reach places. Compared to their power generation, pumping fluid requires a lot more energy. Thus, their primary objective is wastewater treatment rather than power generation (Min and Logan, 2004).

2.8.2 Single compartment microbial fuel cell system

Although two-compartment MFCs can be utilized in batch or continuous modes, their complex architecture makes scaling up difficult. One-compartment MFCs are inexpensive and have a simple design. They typically don't require aeration in a cathodic chamber and just have an anodic chamber. A one-compartment MFC built by Park and Zeikus has an anode in a rectangular anode chamber connected to a porous air-cathode that is exposed to the air. There is transfer of protons from anolyte solution to porous air-cathode (Park and Zeikus, 2003).

2.8.3 Up-flow mode microbial fuel cell system

Other plans for an MFC that can run in continuous flow mode were provided by Jang et al. in 2004. Glass wool and glass bead films were used to divide a Plexiglas cylinder into two halves. These two sections performed analogously the roles of anodic and cathodic chambers. The reactor's bottom holds the cathode and an anode made of graphite that resembles a disk. Glass wool and glass beads were used to create a different MFC design based on the same general concept, but with a rectangular container and no physical separating. The anode's bottom receives the feed stream, and the top of the anode receives the effluent after it has passed through the cathodic chamber. The MFCs require a gradient of dissolved oxygen (DO) for proper operation, which is provided by a diffusion obstruction between the anode and cathode. As they are simple to scale up, up flow mode MFCs are more suited for wastewater treatment (He et al., 2005).

2.8.4 Paper microbial fuel cell system

Extremely affordable diagnostic tools that are also highly sensitive and selective are currently needed in low-resource communities all around the world. Paper-based diagnostic tools may be able to satisfy these requirements. To enhance sensitivity, paper-based diagnostic systems have lately begun to utilize a battery-operated luminescence detector (Wang et al., 2012). Conventional batteries, on the other hand, are costly and even uneconomical for this single-use, low-power device, as it only requires a few minutes of power to get yields from the devices. This hindrance gave rise to discover paper-based MFCs that may be utilized as a unified power source in paper-based diagnostic devices to power on-chip performance. Paper based MFC was developed which was flexible, lightweight and has minimum moisture amount. The anode/cathode electrodes were prepared of flexible carbon clothing and the paper chambers were characterized by photoresist hydrophobic barriers. In areas with minimal resources, low –cost, highly portable MFC is planned to be applied as a power source for single-use diagnostic instruments (Fraiwan et al., 2013).

2.8.5 Substrates of Microbial Fuel Cell

One of the most significant biological elements impacting the production of electricity in MFC is the substrate. Various types of substrates can be utilized for electricity generation in MFC. Bacterial population in the anode biofilm, power density, columbic efficiency and overall performance of MFC is impacted by the type of substrate (Chae et al., 2009). As acetate is naturally inert for many microbial processes, including fermentation and methanogenesis at normal temperature, it is a commonly used substrate in MFC. Acetate is a byproduct of other metabolic processes as well (Aelterman ,2009). Another generally utilized substrate in MFC is glucose but Lee et al,.2008 outlined that in MFC the energy conversion efficiency of acetate was much larger than that of glucose. All monosaccharides obtained from lignocellulosic biomass by hydrolysis were suitable substrates for power production in MFC. Microorganisms with the ability to degrade cellulose and having electrolytic properties are required for the production of electricity from those materials (Rezaei et al., 2009). Synthetic wastewater as well as chemical wastewater having particular composition can be utilized in MFC. Brewery wastewater, wastewater from starch manufacturing, leachate, and other waste streams are ideal substrates for MFC because they include essential organic matter that is present in both industrial and municipal wastewater (Rezaei et al., 2009).

2.9 Performance parameters of MFC

Power Density: Power Density is the power output of the cell per anode surface area. High internal resistances of MFCs limit the maximum power density that can be achieved using MFCs. Therefore, reactor configuration and electrolyte has to be in such a way to reduce the internal resistance in order to operate the full microbial potential (Kim et al., 2007).

Columbic Efficiency (CE): The proportion of electrons received from organic matter to the theoretical maximum through which all electrons go to current generation is known

as the columbic efficiency (CE). Following are the factors that reduce Columbic Efficiency (Logan and Regan, 2006).

- Fraction of the substrate is utilized for cell synthesis of electricity producing bacteria.
- If facultative, the same bacteria that produce electricity can use the oxygen used at the cathode which gets diffused through the membrane to the anode chamber for aerobic respiration. If mixed culture is used, other bacteria can use the oxygen as well.
- Substrate is lost to alternative electron acceptors if they reside in the medium (eg: Nitrate of Sulfate along fermentation and methanogenesis)

2.10 Factors affecting the performance of MFC

Power production from MFC is altered by different factors like type and amount of substrate utilized, microbes choice, pH, temperature, ionic strength, fuel cell configuration etc. The parameters can be controlled to improve the efficiency of MFC (Liu H et al., 2005). Some of the factors influencing MFC performance are as follows:

2.10.1 Effect of Electrode Material

The function of MFC can be improved by using electrode material with better performance ability. Anode and cathode fabrication with platinum and platinum black electrode is better than graphite, graphite felt and carbon cloth electrodes but those electrodes are very expensive. While no current flow was seen with non-modified carbon-cloth under similar conditions, Schroder et al. documented current of 2-4 mA with platinumized carbon cloth anode and conventional glucose medium.

2.10.2 Effect of pH buffer and electrolyte

At the time of biodegradation of organic substrate, protons are released. Some protons travel to the cathodic chamber via the PEM or salt bridge and get mixed with dissolved oxygen to give water while some get gathered up in anodic chamber thus there is acidification at anode and alkalization at cathode. According to Gil et al. current output increased by 1-2 times, and the pH shift between the cathode and anode was reduced from 4.5 to 0.5 when pH 7.0 phosphate buffer was utilized compared to that without buffering. Also, adding of NaCl enhanced the conductivity of both catholyte and anolyte thus increasing power output (Jang et al., 2004).

2.10.3 Effect of Temperature

In MFCs, the change in temperature can affect system kinetics, mass transfer, thermodynamics and also type and distribution of microbial community. Temperature is important parameter in MFC performance for COD removal and electricity production. Enhancement in power generation with increase in temperature might be because of betterment in microbial metabolism and membrane permeability and also decrease in ohmic resistance since there is enhancement in conductivity (Martin et al., 2010).

2.10.4 Effect of organic loading rate

At the time of start-up, the two key factors that determine the nature of sludge are the organic loading rate (OLR) and the sludge loading rate (SLR). The substrate conversion rate affects the power density and columbic efficiency of the MFC, but SLR or OLR play a significant role in regulating the performance of the MFC. To have the best results in terms of removing organic matter and producing power, MFCs should be regulated at an ideal SLR or OLR. As the rate of organic load increased, improvements in power production and substrate degradation were noted. Yet, if the organic load rate was too high, power generation was observed to be reduced even while substrate degradation increased (del Campo et al.,2013).

2.10.5 Effect of feed rate and shear stress

Before MFCs may be used effectively in wastewater treatment plants, critical aspects like flow rate, consecutive hydraulic retention time (HRT), shear stress etc. need to be acknowledged (Moon et al., 2005). A rise in flow rate results in an increase in power output. Power density decreases if a very high flow rate is used. This demonstrates that the best performance is attained when the microbial community has had time to mature and when nutrient utilization and substrate hydrolysis are at their optimal levels. Coulombic efficiency and COD elimination decrease as flow rate increases (Juang et al., 2011).

2.10.6 Effect of operating condition in anodic chamber

A MFC's performance is influenced by a number of factors, such as fuel variety, quantity, and feed rate. Power density varies significantly with different fuels for a specific microbe or microbial consortium. Many systems demonstrate how fuel concentration is necessary for both batch and continuous-flow mode MFCs to produce energy. A higher fuel concentration typically results in a higher power output over a wider concentration range. The increased feed rate may not have much of an effect on the flora if microorganisms are forming biofilms inside the electrodes. One reason could be that the anode is competing with other potential electron acceptors at the high feed rate to reduce output (Rabaey et al., 2003).

2.10.7 Effect of operating condition in cathodic chamber

The most often used electron acceptor in MFCs for the cathodic process is oxygen. The proportion of electron acceptor concentration has a significant impact on an MFC's power output. While it persisted below the air saturated level, according to a number of studies, dissolved oxygen (DO) was a major limiting factor. Surprisingly, a catholyte spurge with 38 mgL^{-1}DO of pure oxygen did not increase the power output compared to the water saturated with air (at 7.9 mgL^{-1} DO) (Min, B., & Logan, B. E. 2004). With ferricyanide serving as the electron acceptor in the cathodic compartment, power output is significantly improved. Ferricyanide was utilized in the cathodic chamber in all of the observed cases with extremely high power outputs, such as 7200 mWm^{-2}, 4310 mWm^{-2}, and 3600 mWm-2 (Schröder et al., 2003).

2.11 Types of Anode and their modifications

The anode plays an important role in MFC performance and is often the deciding factor for more power output. The anode's composition and structure have a direct impact on the bacteria's affinity towards it, electron transfer and substrate oxidation. The anode material should have high conductivity, non-corrosive, highly porous, non-fouling and cheap, easily created and made to greater sizes and should have more specific area (area per volume). The most significant variable of them is more electric conductivity since this few ohms of added internal energy can hugely lower the power and non-corrosiveness which boycott many metals.

The most popular MFC anodes are carbon cloth and carbon paper because of their stability in a microbial inoculum mixture, higher conductivity, and larger surface area. The relation between anode electrode and microbial inoculums has huge effect on MFC power density. There are some refinements done in anode electrodes by infusing it with various chemical catalysts. Research has demonstrated enhancement with the electron transfer between microbial cells to anode by utilizing microorganisms or mediators (Logan, 2008)

Enhancement of porous structure of the anode with greater specific surface area enhances the power density but it is seen that, the pores are blocked by the infiltrating bacteria causing the cell death and decrease in electrochemical reaction surface. Traditional carbon based anodes like carbon felts and porous carbon papers bear this problem. Evolution of a new nanostructure for anode material having more specific area suitable for both bio and electro catalytic procedures are most for improving the power density (Qiao et al., 2007)

2.11.1 CNT based anode material

Carbon nanotubes (CNTs) are allotrope of carbon in which the structure consists of enrolled tubular grapheme in the configuration labeled armchair (Slate et al., 2019). CNTs contain one or more layers of grapheme either single walled (SWCNT) or multi-walled (MWCNT) having open or closed ends (Komarov et al., 2004). Due to their significantly improved performance in electron transfer capacities, microbial adhesion, and substrate diffusion, MWCNTs with hydroxyl functional groups are likely an alternate anode material to commonly used carbon cloth (Thepsuparungsikul et al., 2014).

2.11.2 Polymer-carbon composites

The amalgamation of CNT with polymers could also bring in the new electronic characteristics depending on new morphological disposition or depending on the electronic cooperation or synergy between the two components resulting to new functionalities and usage (Cabezas, 2013). Mostly applied conductive polymers are conjugated polymers having heteroatoms in the principal chain as polyaniline (PANI), polypyrrole (PPy), polythiophene (PTh) and their derivatives. Conducting polymers such as PPy and PANI have a superior biocompatibility and are normally applied to immobilize enzymes and fabricating enzyme electrodes (Eftekhari, 2011). So, conductive polymers can be utilized as good supports for microorganisms in MFCs. The anode carbon supports have been overlayed with PANI (Lai et al., 2011) and PPy (Yuan and KIM, 2008). A conductive polymer and CNTs are said to work together to produce a symbiotic effect.

Also, it showed how a CNT-polyaniline composite enhanced electro catalytic properties and bacterial cell adhesion. They assumed that the considerable increase in surface area and polyaniline's protective effect were cause of the result (Qiao et al., 2007).

2.12 Application of Microbial Fuel Cell

2.12.1 Wastewater Treatment

Microbial Fuel Cell technology was used for the first time in early 1990s for wastewater treatment. He et al., 2017 have demonstrated MFC technology as likely technology for treatment of different types of wastewater in municipal, industrial and agricultural scenarios. A competent treatment system needs more operational sustainability and low material cost. MFCs are also used on a smaller scale in the field to monitor inaccessible sites and treat wastewater in coordination with other treatment units. Although MFC now only produces a little amount of electricity (the ideal power generation efficiency is around 60%), researchers are eager to improve MFC performance (Ramadan, 2017).

2.12.2 Generation of Bio-electricity

With the aid of microorganisms, microbial fuel cells can convert the chemical energy stored in the chemical components in a biomass into electrical energy. Logan argued that MFCs can be used as a viable energy source and since between 1999 and 2008, the power density observed in MFC research grew from 0.1 W/m^2 to 6.9 W/m^2 (Fan et al., 2008). Bigger reactors with significant energy output should be promoted in order to convert MFCs into useful energy generating units (Hu et al., 2017). Oh and Logan (2006) demonstrated that two-chambered MFCs can operate with anodes and cathodes of variable size and PEM with three distinct surface areas (3.5, 6.2, 30.6 cm2). According to the PEM size, the power density is boosted to the anode surface area. It has been shown that the PEM surface area limits power production when it is smaller than the electrode surface area due to an increase in internal resistance. Additionally, they advise boosting ionic strength and adding ferricyanide to the cathode to increase power output (Oh and Logan, 2006).

2.12.3 Bio-hydrogen Production

Besides electricity production using microbial fuel cell, it can also be utilized for hydrogen gas production. Normally, the two chambers (anode and cathode) are divided by proton exchange membrane and protons move through this membrane whereas electron passes along the external circuit. The proton interacts with oxygen and electron in cathode chamber and results water. If the cathode chamber is kept in an anaerobic condition and a minimal amount of external potential (to break thermodynamic barrier) is given, a thermodynamically agreeable reaction occurs in the cathode chamber. The protons (H^+) interact with electrons (e^-) to give hydrogen molecule (H_2). Hypothetically, only 110 mV is necessary to cross the thermodynamic barricade but approximately 1210 mV is necessary to break the water molecule (electrolysis). Nearly, about 8 to 9 mole H_2 produced for 1 mole of glucose which is 2 times more than traditional fermentation around 4 mole of H_2 produced for each mole of glucose (Liu et al., 2005).

2.12.4 Methane Production

When methanogen bacteria are used in MFC technology, methane can also be produced. The design has two compartments—an anode and a cathode—separated by a proton exchange membrane, similar to designs used for the production of hydrogen and electricity, however the mode of operation may vary. A small power source is applied for breaking water molecules in anode compartment in presence of anaerobic conditions lacking microbes. CO_2 is provided in cathode compartment and proton that is generated near anode moves through proton exchange membrane, reacts with CO_2 to produce CH_4 and H_2O. The methane that is generated is pure and can be used straight (Wanger et al., 2009).

2.12.5 Biosensors

MFCs can also be utilized as biosensors to distinguish the pollutants level in the environment. There is an interrelation between wastewater robustness and columbic output. Hence, it can be utilized as BOD (Biological Oxygen Demand) sensors. There is a linear correlation between BOD and Columbic output. The current rises continuously with rise in BOD. MFCs BOD sensors are more accurate when contrasted with other BOD sensors due to stability and precision. Also, MFC BOD biosensors have more lifetimes (over 5 years) without care/maintenance when differentiating with other sensors (LI et al., 2007).

2.13 Open Circuit Voltage (OCV)

The thermodynamic quantity known as the cell emf does not include internal losses. The cell voltage that may be recorded over time when there is no current is known as the open circuit voltage (OCV). The OCV should theoretically approach the cell emf. Due to different potential losses, the OCV is really remarkably lesser than the cell emf in real practice. For example, cathode using oxygen commonly has a recorded potential of around 0.2 V at pH 7. Since this value is remarkably lesser than the anticipated value of 0.805 V, a remarkable amount of energy is being missed at the cathode. The phrase "over potential," which refers to the difference between the potential under equilibrium conditions and the true potential, is frequently used to describe this energy loss. The real potential in this instance is 0.605 V (0.805 V - 0.2 V) (Logan et al., 2006).

2.13.1 Identifying factors that affect cell voltage

The measured MFC voltage is significantly lesser because of a number of losses like,

2.13.1.1 Ohmic losses: The ohmic losses in an MFC consist of the resistance to the flow of ions across the CEM (if employed) and the anodic and cathodic electrolytes as well as the resistance to the flow of electrons along the electrodes and interconnections. By utilizing a membrane with a low resistance, thoroughly inspecting all connections, and (if practical), increasing solution conductivity to a level that the bacteria can tolerate, ohmic losses can be reduced (Hoogers, 2003).

2.13.1.2 Activation Losses: The activation energy required for an oxidation/reduction reaction causes activation losses when electrons are transferred from or to a chemical

that is reacting at the electrode surface. This substance may function as a solution moderator, a component of the bacterial surface, or the ultimate electron acceptor working with the cathode. Minimum activation losses can be achieved by expanding the electrode surface area, enhancing electrode catalysis, raising the operating temperature, and creating an enriched biofilm on the electrode (Logan et al., 2006).

2.13.1.3 Bacterial Metabolic Losses: Bacteria use the electron transport chain to move electrons from a substrate with a low potential to the final electron acceptor with a higher potential in order to produce metabolic energy. The potential of the anode, which serves as the final electron acceptor in an MFC, serves as a measure for the bacteria's energy gain. Bacteria achieve more metabolic energy with rise in difference between anode potential and substrate potential but it reduces the optimum achievable MFC voltage. Thus, greater MFC voltage can be attained by maintaining the anode potential as minimum as possible i.e negative but too low anode potential can hinder electron transport chain thus giving higher energy to bacteria by fermentation (Logan et al., 2006).

2.13.1.4 Concentration Losses: Concentration losses occur when the mass transit rate of bacteria from or to the electrode controls the generation of current. The concentration loss occurs when the current density is greater because chemical species can only diffuse to the electrode surface for a finite amount of time. Losses at the anode concentration are caused by a finite supply of reduced species to the electrode or a finite discharge of oxidized species from the electrode surface. The opposite could occur at the cathode side, causing a drop in cathode potential (Larminie and Dicks, 2000).

2.14 Analysis of metal ions by Atomic Absorption Spectroscopy (AAS)

The estimation of trace metal impurities has always offered a confrontation to the analytical chemist. Atomic absorption spectroscopy (AAS) is a technology for measuring the concentration of metallic elements in different materials. The analytical application is based on the concept that metal atoms strongly absorb at distinct, unique wavelengths that coincides with the emission spectra lines of particular metals (Robinson, 1960).

2.15 Cyclic Voltammetry (CV)

Cyclic voltammetry (CV) is an effective and popular electrochemical technique for examining the reduction and oxidation events of molecular species. The use of CV is necessary to examine chemical reactions that have involved an electron transfer, such as catalysis. A three-electrode setup with a working electrode, counter electrode, and reference electrode is frequently used for cyclic voltammetry. While the current travels between the working electrode and counter electrode, the reference electrode is utilized to accurately estimate the applied potential as opposed to a continuous reference reaction (Elgrishi et al., 2018).

Working electrode: The electrochemical event of desire is conducted by the working electrode. The applied potential of the working electrode is controlled by a potentiostat as a function of the potential of the reference electrode. The working electrode's composition of redox inert material in the desired potential range is its most important characteristic. Several types of working electrodes may be used in different experiments to provide various potential windows or to reduce or increase the surface adsorption of various species of desire (McCarthy et al., 2014).

Reference electrode: An equilibrium potential that is distinct and stable exists in a reference electrode. It serves as a benchmark against which the potential of other electrodes in an electrochemical cell can be calculated. As a result, the applied potential is frequently referred to as "VS," a peculiar reference. Certain commonly used electrode assemblies have an electrode potential that is independent of the cell's electrolyte. Standard calomel electrode (SCE), standard hydrogen electrode (SHE), and the AgCl/Ag electrode are frequently used reference electrodes in aqueous conditions. Usually, a porous frit separates these reference electrodes from the solution (Trasatti, 1986).

Counter electrode: Current begins to flow when a voltage is applied to the working electrode to allow for the reduction (or oxidation) of the analyte. The counter electrode's objective is to finish the electrical circuit. As electrons move between the working electrode (WE) and counter electrode (CE), current is detected. The counter electrode's surface area must be greater than that of the working electrode in order to ensure that the reaction's kinetics is taking place there. The counter electrode is commonly a platinum wire or disk, although carbon-based counter electrodes are also available (Zaski, 2006).

2.16 Polymerase Chain Reaction (PCR) for Molecular Diagnosis

Polymerase chain reaction (PCR) is a remarkably significant tool for molecular diagnosis. It is a primer-mediated in-vitro method (Lo and Chan, 2006) that can produce an adequate supply of a specific segment of DNA (i.e., an amplicon) from only a small quantity of starting material (i.e., DNA template or target sequence). Each PCR assay needs the existence of template DNA, primers, nucleotides, and DNA polymerase. DNA polymerase is the crucial enzyme that connects individual nucleotides cooperatively to make the PCR product. The nucleotides contain the four bases—adenine, thymine, cytosine, and guanine (A, T, C, G)—that are present in DNA. These behave as the building blocks that are used by the DNA polymerase to create the PCR product. The primers in the reaction define the precise DNA product to be amplified. The primers are short DNA fragments with an demarcated sequence complementary to the target DNA that is to be ascertained and amplified. These work as an extension point for the DNA polymerase to construct on (Joshi and Deshpande, 2010).

PCR comprise of integrated amplification of a DNA fragment, and its principle is established on the mechanism of DNA replication in vivo: dsDNA is denatured to ssDNA, duplicated, and this process is replicated along with the reaction (Rodriguez and Hernandez, 2013). PCR is performed in a reaction mixture that comprise of the DNA extract (template DNA), Taq polymerase, the primers, and the four deoxyribonucleoside

triphosphates (dNTPs) in surplus in a buffer solution. The tubes consisting the mixture reaction are put through to repetitive temperature cycles multiple tens of times in the heating block of a thermal cycler. The apparatus allows the programming of the duration and the progression of the cycles of temperature steps. Each cycle embodies three periods of a few tens of seconds. The process of the PCR is sectioned into three phases as follows: a denaturation of the template at 90˚C to 95˚C succeeded by annealing with specific primers (45 ˚C to 60 ˚C), and an elongation of the hybrid by the function of DNA polymerase at 72˚C. The products of each synthesis step function as a template for the following steps, thus integrated amplification is attained (Kadri, 2019; Bhat and Rao, 2020).

2.17 Analysis of Analytes by High Performance Liquid Chromatography (HPLC)

2.17.1 Introduction to HPLC

Liquid chromatography was developed as an analytical procedure to separate colored chemicals in the beginning of the 20th century. A crude kind of chromatographic separation was used by Russian botanist Mikhail S. Tswett to separate mixtures of plant colors into their distinct pure constituents based on their interactions with stationary phase. The stationary phase consisted of chalk and alumina powder, and the mobile phase was a solvent (Touchstone, 1993). Each component in a mixture is separated, recognized, and quantified using the analytical chemistry technique known as high performance liquid chromatography (HPLC), also referred to as high pressure liquid chromatography (Lozano, 2018).

Applications of HPLC

HPLC is widely applied analytical separation technique (Mumt, 2020) which can have applications in following areas,

1. Pharmaceutical quality control, identification of counterfeit drug items, and self-life assessment of pharmaceutical products.
2. Determining the presence of phenols in drinking water.
3. Using fish bile analysis to detect PAH pollution in high altitude alpine lakes.
4. Determining the toxicity of tetracyclines and their breakdown products to microorganisms that are relevant to the environment.
5. Detecting anabolic steroids in urine, sweat, serum, and hair
6. Fabric dye forensic analysis.
7. An examination of antibiotics
8. Finding endogenous neuropeptides in the extracellular fluid of the brain.
9. Examination of fruit juice sugar content.
10. Industrial and fine chemicals quality control and purity.

2.17.2 Principle of HPLC

A sample is injected into a column packed with stationary phase and a mobile phase is pumped at huge pressure along the column. Based on variations in the sample's partition between the stationary phase and the mobile phase, the sample is separated based on variances in its rate of migration across the column. Elution of various components occurs at different times depending on how they behave during partition. The component having greater affinity with stationary phase will travel slower and shorter distance while that having lower affinity with stationary phase will travel faster and longer distance. The eluting sample is often exposed to UV light produced by the detector attached to the HPLC unit, which have a certain wavelength. When molecules of the eluting species acquire energy from ultraviolet light, they become excited. When they de-excite, energy is released, and the detector captures this energy (Dincer Z. et al., 2003). A signal corresponding to the energy change is produced and captured as a graph known as a chromatogram. Depending upon the functional group, absorption of UV light varies.

2.17.3 Different Types of HPLC

2.17.3 1 Normal Phase HPLC

Using polar stationary phase and non-polar mobile phase, normal phase HPLC separates analytes based on their polarity. Hexane, methylene chloride, chloroform, diethyl ether, and combinations of these are examples of the mobile phases. The stationary phase is often silica. As a result, polar samples stay on the polar packing in the column longer than less polar components. (Hamilton and Comai, 1988).

2.17.3.2 Reversed Phased HPLC

It relies on hydrophobic contact, which is brought on by repelling forces between a polar eluent, the comparatively non-polar analyte, and the non-polar stationary phase. It has a non-polar stationary phase and aqueous, mildly polar mobile phase. When the analyte molecule associates with the ligand in the aqueous eluent, the contact surface area around its non-polar section determines how much of the analyte will bind to the stationary phase (Hovarth and Melander, 1977).

2.17.3.3 Ion Exchange Chromatography

Ion exchange chromatography is based on the attraction between charged sites bonded to the stationary phase and solute ions. Same-charge ion exclusion occurs. Ion-exchange chromatography of proteins, high pH anion exchange chromatography of carbohydrates and oligosaccharides, and others are some of the main applications of this chromatography (Gallant et al., 1996).

2.17.3.4 Size Exclusion Chromatography

Particles are mostly separated via chromatography according to size. It is most helpful for figuring out the quaternary and tertiary structures of proteins and amino acids. The

method is primarily employed to characterize the molecular weight of polysaccharides (Oeyen et al., 2018).

2.17.3.5 Bio-Affinity Chromatography

Bio-affinity chromatography uses reversible interactions between certain proteins and ligands to separate samples. A bio-affinity matrix's solid support, to which ligands are covalently bonded, maintains protein by interaction with the ligands that are column-bound (Malviya et al., 2010).

2.17.4 Components of HPLC

2.17.4.1 Solvent Reservoir

The solvent reservoir holds the mobile phase in HPLC. Simply it is a reagent bottle or glass bottle. Solvent is pumped from the reservoir to the pump through the inlet line. Some HPLC systems have special compartments for holding more than one mobile phases. Also the reservoirs may have the feature to degas the mobile phase (Wiese et al., 2011).

2.17.4.2 Pump

The purpose of the pump is to force the mobile phase at a set flow rate, measured in milliliters per minute (mL/min). The typical flow rate for HPLC is about 1-2 mL/min. Up to 400–600 bars of pressure are possible. The pump can deliver either an increasing mobile phase composition (gradient) or a constant mobile phase composition (isocratic) during the chromatographic experiment.

2.17.4.3 Injector

The liquid sample is added to the mobile phase's flow stream using the injector. 5- to 20-microliter (L) samples are the norm. Additionally, the injector needs to be able to tolerate the high liquid system pressures. When there are numerous samples for analysis or when manual injection is impractical, an auto-sampler is the automatic version that the user can utilize.

2.17.4.4 Column

Columns typically have an internal diameter of 2 to 5 mm, range in length from 50 to 300 mm, and are constructed of polished stainless steel. They are frequently filled with a stationary phase that comprises particles that are 3 to 10 m in size. Micro-bore columns are typically described as having internal diameters smaller than 2 mm. During an analysis, the mobile phase and column temperatures should ideally remain constant. The stationary phase of the column, referred regarded as the "heart of the chromatograph," uses a variety of physical and chemical characteristics to separate the important sample components. At normal flow rates, the microscopic particles inside the column are what lead to the significant back pressure. To drive the mobile phase through the column, the pump must exert significant force, and this resistance results in a high pressure inside the chromatograph (Polite, 2000).

2.17.4.5 Detector

The HPLC detector at the end of the column detects the analytes as they elute from the chromatographic column. Electrochemical, UV, fluorescence, mass spectrometric, and evaporative light scattering detectors are frequently used detectors. The detector is able to identify each individual molecule that elutes (comes out) from the column. In order for the chemist to examine the sample components quantitatively, a detector measures how many of those molecules are present. The detector's output is sent to a recorder or computer, where it is converted into a liquid chromatogram (i.e., the graph of the detector response). A detector provides the required sensitivity as well as a targeted response for the components split up by the column. The composition of the mobile phase must not impact it (Garcia et al., 1997).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

All the reagents were provided by Central Department of Biotechnology, Tribhuvan University Laboratory. All the reagents were of analytical grades unless stated.

3.2 Methodology

3.2.1 Sample collection

During the month of December, kitchen based biodegradable organic waste was collected in zip lock bags from 5 different household kitchens in Kathmandu 16, Banasthali, Kharibot area. Five batches of sample were collected in 15 days, each batch collected for 3 days.

3.2.2 Sample processing

All the collected samples were mixed together. The composition of collected waste was identified by visual estimation and categorized according to its amount present in the collected waste. The sample was then grinded and kept in zip lock bag and stored at -20˚C for future use.

3.2.3 Physical Characterization of Sample

3.2.3.1 pH measurement (Estefan et al., 2013)

The sample was prepared- without dilution and other several dilutions like 1:5 dilutions, 1:10 dilutions, 1:15 dilutions and 1:20 dilutions. It was then allowed to stand for 30 minutes. Then the sample was stirred and after 1 hour pH was measured with calibrated pH meter.

3.2.3.2 Moisture Content and TSS (US. Environmental Protection Agency, 2001)

To determine the moisture content, the sample was taken in petriplate and weighed. It was then heated in hot air oven at 105˚C overnight. Again the weight was measured and finally the moisture content was calculated using following formula:

%Total Suspended Solid (TSS) = (dried sample weight/ fresh sample weight) ×100

% Total Moisture = 1- TSS%

3.2.3.3 Ash Content (US. Environmental Protection Agency, 2001)

Dried sample from moisture content determination was transferred to the crucible and weighed. These samples were then placed in muffle furnace at 550˚C for 2 hours. Final weight was measured after cooling of residue in desiccator and then ash content and volatile suspended solids (VSS) were calculated using following formula:

% Ash content = (weight of residue after heating/ weight of initial sample residue) × 100

% VSS = 1- % Ash content

3.2.4 Environmental Analysis of Sample

Digestion of sample with H_2SO_4- Salycyclic acid- H_2O_2 (Temminghoff and Houba, 2004)

A 50 mL volumetric flask was taken and 0.6 g of sample was put in it. To a flask, 3.3 mL of digestion mixture (Appendix I) was added, and some carborundum beads. The mixture was carefully swirled until the entire sample was soaked. It was then allowed to stand at room temperature overnight. Distilled water was used as blank solution. On the following day the volumetric flasks were transferred to hot plate at 180˚C for 1 hour. The flasks were removed from hot plate, cooled down and 5 drops of pure H_2O_2 were added. The flasks were again heated on hot plate at 280˚C for 10 min until the water was evaporated. The flask was removed from hot plate, allowed to cool down; again 5 drops of H_2O_2 were added and heated again for 10 min. The process was repeated until the digest turned colorless. The flask was taken away from the plate and cooled until it reached room temperature. About 10ml of distilled water was added slowly and mixed, swirled until majority of the precipitate got dissolved. The mark was made up with water, mixed well and filtered over course filter paper. The calibration blank solution was prepared in the same way as the sample prepared for further analysis. The final medium of thus prepared solution was 0.8 M H_2SO_4.

3.2.4.1 Determination of Total Phosphorus (Pisal A., 2003)

Standard curve for Phosphorus

Stock solution of 1000 mg/L PO_4 was prepared using potassium dihydrogen phosphate, and then 1mL, 2mL, 3mL, 4mL and 5 mL of the stock solution was pipetted into a 100 mL volumetric flask that already contained 40 mL ultrapure water. In one flask no stock solution was added. Then 4.5 mL of conc. H_2SO_4 was added to each volumetric flask and final volume 100 mL was made with addition of ultrapure water. After that a series of reference solution with concentrations 0 mg/L, 10 mg/L 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L were prepared.

Determination of Phosphorus

All the digested samples, prepared standard series and blanks were diluted in water at the ratio of 1:9 (v/v). After that, 1mL of each diluted samples were pipetted into a test tube followed by 3.8 mL of diluted mixed reagent [Appendix]. The solution was allowed to stand for 10 minutes then the absorbance was measured in spectrometer at 880 nm.

3.2.4.2 Determination of trace elements and heavy metals

The prepared digested solution along with blank was subjected to trace elements and heavy metals estimation at National Academy of Science and Technology (NAST).

3.2.4.3 Determination of Chemical Oxygen Demand (COD) (Pisal A., 2003)

Standard curve for COD

Stock solution (1000 mg/L) of hydrogen phthalate was prepared. This stock solution was used to prepare a series of reference solution with concentration ranging from 50 mg/L to 1000 mg/L were prepared. 2 mL of solution from each reference solution with water as a blank was taken in culture tube and 1.2 mL of digestion solution was added with mixing thoroughly. Then 2.8 mL of catalyst solution was added to each tube, cap tightly and shaken properly to mix the layers. Then culture tubes were taken to digester within oven at 150˚C for 2 hours. After that solutions were allowed to cool down and any precipitate to settle. Absorbance was measured at 600 nm using blank for background correction.

Determination of COD of sample

Similarly 2 mL of each sample were taken in culture tube and the same procedure was followed as in the standard curve preparation. Then COD of sample was calculated using standard curve.

3.2.4.4 Determination of Ammoniacal-nitrogen (Pisal A., 2003)

Standard curve of Ammoniacal-nitrogen

Ammonia nitrogen stock solution (100 mg/L) was prepared using ammonium chloride in distilled water. This stock solution was used to prepare a series of reference solutions with concentrations 0.1 mg/L, 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L, 1 mg/L, 1.6 mg/L and 2 mg/L by using appropriate volume of stock solution. Final volume was made 5ml. Then 2mL of Nessler's reagent was added to each tube and mixed thoroughly. The solution was left to stand for 20 minutes and observed for color development. The absorbance was observed at 425 nm with blank as background correction.

Determination of Ammoniacal-nitrogen

100 mL of each sample was taken in Kjeldahl flask and 5 mL of borate buffer was added. From mixed sample 30 mL of sample was taken and placed in heating gauge, distillate was collected in flask which already contained 5 ml of boric acid. There the tip of condenser was dipped into the boric acid. After collecting all the distillate final volume was made 50 mL with distilled water. 2.5 mL of collected distillate was taken in the test tube and 0.1 mL of Nessler's reagent was added into it. After that solution was allowed to stay for 20 minutes and observed for color development. Finally, absorbance was measured at 425 nm taking blank for background correction. Similarly, water was used instead of sample for determination of ammonia nitrogen in water.

3.2.5 Construction of Microbial Fuel Cell

Microbial Fuel Cells are made up of two chambers, anodic and cathodic chambers, two electrodes (anode and cathode), and a salt bridge. At anaerobic conditions, the anodic chamber contains a substrate and biocatalyst. At the cathodic chamber, an aerobic environment is maintained. Dual chambered fuel cell of 350 mL capacity was taken and

marked as an anode and cathode. In the anodic chamber, 325 mL of sample was used as an anodic inoculation, whereas in the cathodic chamber, 325 mL of phosphate buffer solution was used as cathodic solution. Proton exchange membrane (PEM) situated in between anodic and cathodic chambers. Graphite sheets were applied as electrodes with Nafion 177 applied to separate anodic and cathodic chambers functioning as proton exchange membrane.

3.2.5.1 Membrane Treatment (Najafpour et al., 2010)

Nafion membrane having diameter of 5 cm was treated in four steps. At first membrane was boiled in 3% H_2O_2 at 100˚C for 2 hours. Then membrane was taken in distilled water and bpiled for 2 hours at 100˚C. Again membrane was boiled at 100˚C in 0.5 M sulphuric acid for 2 hours and finally in distilled water for 2 hours at 100˚C.

3.2.5.2 Electrodes Treatment (Swain G.M., 2007)

Graphite sheets with measurement (10 cm × 2.8 cm) were taken as anode and cathode electrodes. The electrodes kept on each side of the chambers were connected externally by using copper wire. These electrodes were chosen because they were affordable and readily available. Before use, electrodes were ultra sonicated with 70% methanol, 70% acetone, followed by distilled water and finally 15 minutes under UV light.

3.2.5.3 Electrodes Modification with PANI/MWCNT nanocomposites (Abdulla et al., 2015)

3.2.5.3.1 Purification of MWCNT

MWCNT was first treated under reflux condition using an H_2SO_4/HNO_3 mixture to increase dispersion and surface reactivity, as well as to eliminate other carbonaceous compounds created during synthesis. A round bottom flask furnished with a condenser was taken and 0.1 g of MWCNTs was put into it after which it was treated with a mixture prepared with 3 mL of 3 M sulfuric acid and 1 ml of 3 M nitric acid (3:1 ratio). The mixture was ultra sonicated for 30 minutes, stirred for 30 minutes, and then refluxed for 8 hours at 120˚C. The resultant solution was diluted in water and cleaned until it reached a neutral pH. The sample was dried overnight in oven at 60˚C.

3.2.5.3.2 Synthesis of PANI/MWCNT nanocomposites

Synthesis of PANI/MWCNT nanocomposites was carried out using an in-situ oxidative polymerization technique. At 0˚C, 1 mg of MWCNT was combined in an aniline/HCL (1:1) solution, resulting in monomer adsorption on the CNT surface. The reaction time was set at 12 h. A solution of 0.1 M APS dissolved in 1 M HCL was used to drop the aforementioned mixture drop by drop. The polymerization process took 6 h at 0˚C. The PANI/MWCNT nanocomposite was created by centrifuging the material several times with deionized water and methanol until it turned into greenish black powder. After that, the material was vacuum dried for another 24 h at 60˚C.

3.2.5.3.3 Treatment of Graphite Electrode

For 6 h, the surface of the graphite electrode was modified using an ultra-sonication bath containing H_2SO_4 and HNO_3 (3:1 v/v). The Graphite Electrode was washed multiple

times with distilled water. The pH of the washing solution was checked till it reached 7. After that, it was air dried before used.

3.2.5.3.4 Coating of Electrodes with Nanocomposites

Finally, synthesized PANI/MWCNT nanocomposite was combined with a solvent like N-methyl-2 prrrolidone. After dipping a clean graphite electrode in the mixture and ultrasonicated for about 15 minutes. Then electrode was dried in an oven for about 12 h at 55˚C.

3.2.5.4 Immobilization of Neutral Red in Electrodes (Wang et al., 2011)

The graphite electrode sheet electrodes were immersed in 1% (w/v) solution of polyvinyl alcohol (100 ml) at 60˚C under vacuum condition for 3 hours and then those electrodes were dried at 100˚C for 12 hours in hot air oven. After that the electrodes were immersed in pure chloroform which contained 10% thionyl chloride and 0.01% neutral red. After 6 hours electrodes were washed with methanol to remove any unbound neutral red.

3.2.6 Cyclic Voltammetry

Hokuto-Denko HA151 potentiostat was used in combination with national instrument Lab View work station. During the stabilized period of operation, CV investigated the bio-electrochemical behavior of MFC during the power generation, measurement were performed in a three electrode arrangement. The working electrode was anode of MFC; graphite while reference electrode was Ag/AgCl reference electrode (RE). CV was carried out by applying potential ramp to the working electrode against the reference electrode (RE). The scan rate used was 10 mV/s over the potential range from -0.8 V to +0.8 V. All electrochemical experiments were carried out in situ in MFC, with the anode (graphite) working as the counter electrode against RE. The cyclic voltammetry was done for 3 cycles in Central Department of Chemistry, Tribhuvan University (CDC, TU). (Venkata Mohan et al., 2010). The current generated from the electrochemical cell was observed in different interval of potential applied in graphite electrode (anode).

3.2.7 Analysis of substrate and byproducts by High Performance Liquid Chromatography (HPLC)

3.2.7.1 Sugar analysis in the Sample (Van Wychen S. et al., 2015)

Digestion of sample and generation of calibration standard

Concentrations of 0.05 mg/mL, 0.25 mg/mL, 1.25 mg/mL, 2.5 mg/mL and 4 mg/mL of five different sugars namely Glucose, Fructose, Maltose, Xylose and Arabinose were prepared in different reagent bottle which were further used in making calibration curve. Similarly, 0.3 g of sample was taken in heat resistant reagent bottle. To all the reagent bottles, 3 ml each 72% H_2SO_4 was added and incubated in water bath maintained at temperature 30±3˚C for 60 minutes. All the bottles were stirred in every 10 minutes without removing from water bath. After that dilution of the concentrated

acid from 72 % to 4 % was carried out by adding 84 ml of deionized water. Mixing of the acid sample mixture was carried out by inverting reagent bottles several times so that there is no phase separation between high and low concentration acid layers. After properly mixing the sample they were then autoclaved at 121˚C for 1 hour and then cooled to room temperature before use.

Sample Preparation for HPLC Analysis

From hydrolysis liquor 20 ml was transferred to another flask and each sample neutralized up to pH 6 using calcium carbonate in stepwise swirling the samples and pH was checked periodically during the addition of calcium carbonate. After reaching pH, sample was allowed to settle and decant off the supernatant into tubes. The neutralized sample was centrifuged and supernatant was filtered through 0.2 mm syringe filter. Thus, filtered hydrolysate was placed in clean and labeled HPLC vial and capped tightly.

HPLC Analysis for Sugars

The calibration standard and sample were analyzed by using carbohydrate specific column; Zobrax carbohydrate analysis column (4.6 × 250 mm, 5 micron). The mobile phase used was the mixture of HPLC water and acetonitrile in the ratio 25:75 and the flow rate was set at 1mL/min. Column temperature was set at 25˚C and sugars were detected on the basis of refractive index detector (RID). Injection volume of sample was 20 µl.

3.2.7.2 HPLC Analysis for MFC end products

For standard calibration, organic acids like acetic acid, citric acid, propionic acid, succinic acid and lactic acid, sugars like glucose and fructose and ethanol were used. For quantitative study of the compounds produced in MFC, the concentrations of 0.1 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL and 15 mg/mL of all above mentioned components were prepared as standards. Thus prepared series of calibration standard were filtered through 0.2 mm syringe filter and taken in HPLC vial. Sample was taken in eppendorf tube from MFC and centrifuged. Supernatant was filtered using 0.2 mm syringe filter and kept in HPLC vial. The mobile phase used was 5 mM sulfuric acid with the flow rate set at 0.6 mL/min. Column temperature was set at 50˚C and the components were detected on the basis of Refractive Index (RID) detector. The sample injection volume was set 10 µL (Bio-Rad, 2012).

3.2.8 Isolation and storage of bacteria from anodic electrode

Isolation of Bacteria

When the Open Circuit voltage (OCV) started to drop after reaching the peak point, the anode electrode was taken out from MFC, it was gently rubbed with sterile loop and streaked in Lurea Bertani Agar media. It was then kept in incubator set at 37˚C for 24 hours.

After 24 hours, the plate was taken, isolated and morphologically different looking colonies were marked and then sub cultured into the Nutrient Agar media. The streaked plates were sealed and kept in 37˚C incubator for 24 hours.

From the petriplate, gram staining of the isolates was carried out. They were observed under microscope and characterized. Similarly, biochemical test for the isolates were carried out and preliminary characterization of isolates was performed.

Maintenance of Bacteria

All the isolates were maintained in Nutrient Agar plate. The organisms were sub cultured at 15 days interval and were preserved using glycerol stock.

3.2.9 Molecular Characterization

3.2.9.1 Extraction of Genomic DNA from Bacteria (Nishiguchi et al., 2010)

For the extraction of DNA from liquid culture, clean, dry and sterilized eppendorf tube was taken and 1.5 ml of culture was taken in it. It was then centrifuged at 13000 rpm for 2 minutes. The supernatant was removed and to the pellete 567 µl of TE buffer was added. With the help of sterile micropipette tip, the pellete was resuspended by repeated pipetting. After resuspending 30 µL of 10% SDS as well as 3 µL of 20 mg/mL proteinage k solutions were added and mixed properly by pipetting. After that, 80 µl of CTAB/NaCl solution prepared with 0.7 M NaCl and 10% CTAB solution was added and mixed. The solution was incubated at 65˚C for 10 minutes. When incubation was completed, equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed. The resulting mixture was centrifuged for 5 minutes and the upper aqueous solution was shifted to the new tube. Again, centrifugation was done at 14000 rpm for 5 minutes and transferred the supernatant to the new tube. After that, 0.6 µl of isopropanol was added and mixed gently until the precipitation of DNA occurred. Afterwards, centrifugation was done to eliminate the isopropanol. 1 ml of 70% ethanol was added to wash the salt impurities away from the DNA. Again, centrifugation was performed to discard ethanol, dried and the pellete was resuspended in 50µl of TE buffer and kept at 4 ˚C. The obtained genomic DNA was subjected to gel electrophoresis and visualized under UV-transilluminator.

3.2.9.2 Polymerase Chain Reaction (PCR)

After the extraction of genomic DNA, PCR was performed. The PCR reaction mixture and thermocyclic condition of PCR is given in table below respectively.

Table 3.1: PCR Reaction Mixture

Reagents	Amount (µl)
Master Mix	5
Forward primer	0.4
Reverse primer	0.4
Taq polymerase	0.05
$MgCl_2$	0.2
Nuclease Free Water (NFW)	2.95
Template	1
Total	**10**

Table 3.2: Thermocyclic conditions of PCR

stage	cycle	step		Temperature (˚C)	Time
1	1	1	Enzyme activation	94	2 min
2	35	1	Denaturation	94	1 min
		2	Annealing	55	30 sec
		3	Extension	72	1 min
3	1	1	Final Extension	72	10 min
		2	Hold	4	Hold

3.2.10 Biocompatibility Test of coated neutral red and PANI/MWCNT on isolates (Mohamad et al., 2017)

Biocompatibility of the coated PANI/MWCNT and Neutral Red on isolates was qualitatively investigated by the agar well diffusion test. The isolates were cultured in LB broth media at 37˚C. After proper growth, 100 µL of each culture broth was spread on solid Muller- Hinton Agar plates to prepare lawn of isolates culture. The plates were allowed to stand for 20 minutes for absorption of carpeted culture. Sterile borer was taken and wells were carefully punched into the agar plate. A drop of molten agar (0.8% MHA) was added to wells so that they were sealed and leakage was prevented from the bottom of the plate. With the help of micropipette, 100 µL (50 µg) of neutral red and PANI/MWCNT solution sample was transferred into the wells on the plates. The plates were kept in incubator set at temperature 37˚C for 24 hours after which size of the zone of inhibition was measured. A solvent blank was used as negative control and the antibiotic Tetracycline disc was taken as a positive control.

3.2.11 Drug Resistance Test for the Isolates

The isolates were tested for their Multidrug Resistance nature. Ciprofloxacin (CIP 5), Ampicillin (AMP 10), Vancomycin (VAN 30), Amikacin (AMK 30) and Ceftazidime (CAZ 30) were used to test multidrug resistance nature of isolates. The isolates were cultured in LB broth media at 37˚C for 24 hours. After proper growth, 100 µL of each culture broth

was spread on solid Muller- Hinton Agar plates to prepare lawn of isolates culture. The plates were kept to stand for 20 minutes for culture absorption. With the help of sterile forceps, the antibiotic discs were placed in the agar plate maintaining appropriate distance and slightly pressed from above. The plates were covered, sealed and placed in incubator set at temperature 37˚C for 24 hours. After 24 hours of incubation, zone of inhibition was measured and noted.

3.2.12 Construction of Fabricated Microbial Fuel Cell (Utomo et al., 2017)

The Fabricated Microbial Fuel Cell (FMFC) was designed similarly to the design of the conventional MFC available in Central Department of Biotechnology, TU. Volume of chambers was increased by using 1 liter capacity culture media bottle readily available in the laboratory. PVC pipe with internal diameter similar to conventional MFC was used to connect two bottles and arrangement was made so that Nafion membrane could be placed and there was no leakage. Two similar media bottles of 1 L capacity were taken and cleaned. On both the bottles, circles of 5 cm diameter were marked 1.5 cm above the base of the bottle. Two short pieces of PVC pipes were cut. One end of each pipes were inserted into the holes created in bottle. Leakage was checked by using silicon glue and rubber tubes. The next two free ends of pipes were connected by using valve and there was an arrangement for the placement of Nafion Membrane.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Biodegradable kitchen waste composition

Table 4.1: Composition of biodegradable kitchen waste

Composition	Percentage %
Vegetable peels and leftovers	54 – 70
Fruit peels and leftovers	16 – 22
Leftover cooked food	7 – 15
Other waste (leftover tea, straw etc.)	2 – 12

The composition of collected organic waste from kitchen was as shown in table 4.1. The waste consist highest amount of vegetable peels and leftovers i.e. 54 – 70 % followed by fruit peels and left over. Similarly, 7-15 percent of the waste was leftover cooked food and rest was other unidentified waste.

4.2 Determination of Environmental parameters of sample

Table 4.2: Different physical parameters of kitchen waste sample

Characteristics of Sample	
pH	4.72 ± 0.24
Total Suspended Solids (TSS) %	24.33 ± 0.51
Volatile Suspended Solids (VSS) %	12.7 ± 0.46
Moisture content %	75.67 ± 0.75
Ash content %	87.3 ± 0.46

Various physical parameters analysis of organic kitchen waste sample such as pH, Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Moisture content and Ash content were as shown in table 4.2. The result showed that the biodegradable waste from kitchen has low pH value and high moisture content. The low pH value might be due to presence of fruits and vegetable based waste that has low pH such as tomato, lemon etc. Also the food waste might have undergone acidic fermentation during collection, processing and storage. The average pH value of organic canteen waste was 5.99 while the total solid content was 14.00 % and volatile solid content was 99.26 %, according to Shrestha et al., 2017.

Table 4.3: Different analytical parameters of kitchen waste sample

Analytical parameters of sample	Concentration (mg/g)
Chemical Oxygen Demand (COD)	26.75 ± 0.68
Ammonical Nitrogen	0.017 ± 0.005
Phosphorus	0.0956 ± 0.007

Table 4.4: Analysis of different metal ions in kitchen waste sample by AAS

Element	Observed Value (ppm)
Zinc (Zn)	0.0078
Iron (Fe)	1.139
Copper (Cu)	0.006
Nickel (Ni)	0.127
Manganese (Mn)	0.063
Cadmium (Cd)	0.02

The waste sample contained variable amount of ammonical nitrogen, phosphorus and chemical oxygen demand (COD) as shown in table 4.3. It contained 26.75 ± 0.68 mg/g of COD, 0.017 ± 0.005 mg/g of ammonical nitrogen and 0.0956 ± 0.007 mg/g of Phosphorus.

Table 4.4 shows the concentration of various metals and trace elements in the waste sample. According to the result, the organic kitchen waste contained zinc 0.0078 ppm, iron 1.139 ppm, copper 0.006 ppm, nickel 0.127 ppm manganese 0.063 ppm and cadmium 0.02 ppm.

4.3. Sugar Analysis in Kitchen waste sample by HPLC

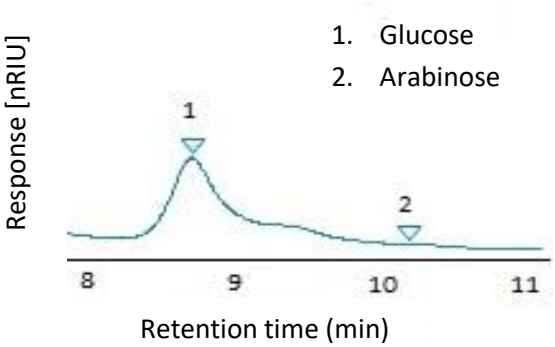


Figure 4.1: Chromatogram of sugar analysis of kitchen waste sample using RI detector

Sugar analysis in digested kitchen waste sample was carried out by HPLC using Zobrax carbohydrate specific column. Calibration standard were analyzed in HPLC according to which different peaks in chromatogram were identified on the basis of retention time. Two different sugars glucose and arabinose were identified in chromatogram on the basis of retention time as shown in the figure above.

Table 4.5: Concentration of different sugars in sample determined by HPLC analysis

Sugar	**Concentration (mg/g)**
Glucose	167.39 ± 10.21
Arabinose	11.22 ± 0.69

The concentration of glucose was 167.39 ± 10.21 (mg/g) and that of arabinose was 11.22 ± 0.69 (mg/g) as shown in the table above. Similar study was carried out by Xu et al., 2015 for determination of sugars like fructose, sucrose and glucose predominately found in molasses since it is predominantly used in bakery, used in preparation of animal feed additive and also as a fermentation feedstock. HPLC technology with Refractive Index (RI) detector was used and the column used was Agilent Zobrax Carbohydrate Column.

4.4 Working with Microbial Fuel Cell (MFC)

4.4.1 Optimizing various parameters in anode compartment affecting Open Circuit Voltage (OCV)

4.4.1.1 Effect of substrate concentration

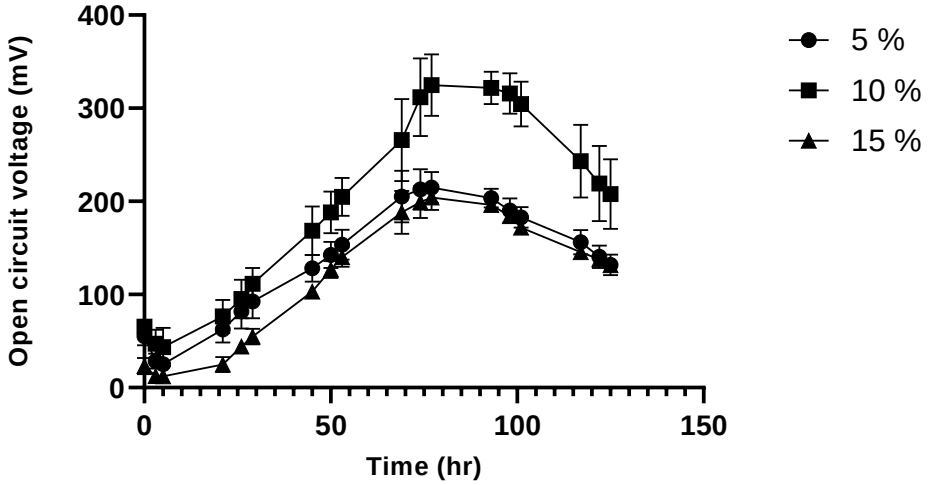


Figure 4.2: Effect of substrate concentration on open circuit voltage at different time

Figure 4.2 shows the time versus open circuit voltage curve obtained when different concentrations of substrates were used in MFC operation. The data showed that 10 % of substrate was best for operation with highest open circuit voltage (OCV) of 324.67 ± 33.80 mV after 77 hours of operation. 10 % substrate concentration means 10 g finely grinded waste sample in 100 mL of phosphate buffer solution. Ghoreyshi et al., 2011 studied on the effect of the type and concentration of substrate on the MFC performance. They took glucose as substrate over concentration range of 2-20 g/L. As the substrate concentration increased, the OCV value was minimal. This might be because at higher concentration substrate stress is created thus most of the substrate might have remained unconsumed. At lower concentration, lower OCV value might be due to limitation of substrate for microbial growth. Higher concentration of substrate adversely affects MFC operation (Gurung et al., 2012). The 10 % of waste biomass as substrate was taken for all MFC operation.

4.4.1.2 Effect of preparing sample in different media

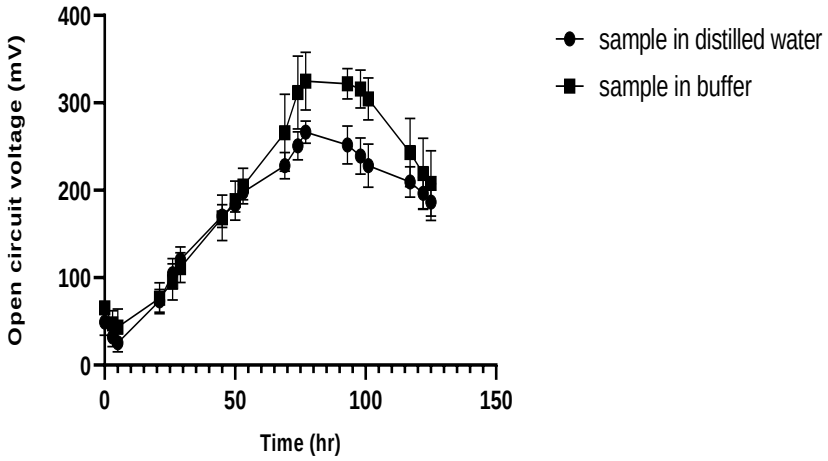


Figure 4.3: Effect of sample preparation in distilled water and phosphate buffer

Figure 4.3 shows time versus open circuit voltage curve obtained when sample preparation was done in different media. In one case neutral pH was maintained by preparing sample in phosphate buffer while in another case sample was prepared in distilled water and neutral pH was maintained by adding anhydrous sodium carbonate. This experiment was carried out to observe if better result could be obtained in latter case so that MFC operation could be made more economic. The maximum OCV obtained when sample was prepared in buffer was 324.67 ± 33.80 mV while that in distilled water was 266.72 ± 13.46 mV. Mohan and Chandrasekhar, 2011, used sodium carbonate to adjust pH to neutral while harnessing bioelectricity from food waste fermentation in solid phase MFC. Significant positive change was observed in fuel cell performance.

Na_2CO_3 on reaction with water produces carbonate ions and Na+ ions. The carbonate ion thus produced has substantial affinity for a proton and forms bicarbonate on combination with it which helps in conserving the buffering capacity of the system (Mohan and Chandrasekhar, 2011).

Since better result was not observed when sample was prepared in distilled water, in rest of the experiments, sample was prepared in phosphate buffer thus considering 10 % substrate concentration prepared in phosphate buffer with normal graphite sheet anode electrode as standard anode condition for this research work.

4.4.1.3 Effect of various other modifications in anode compartment

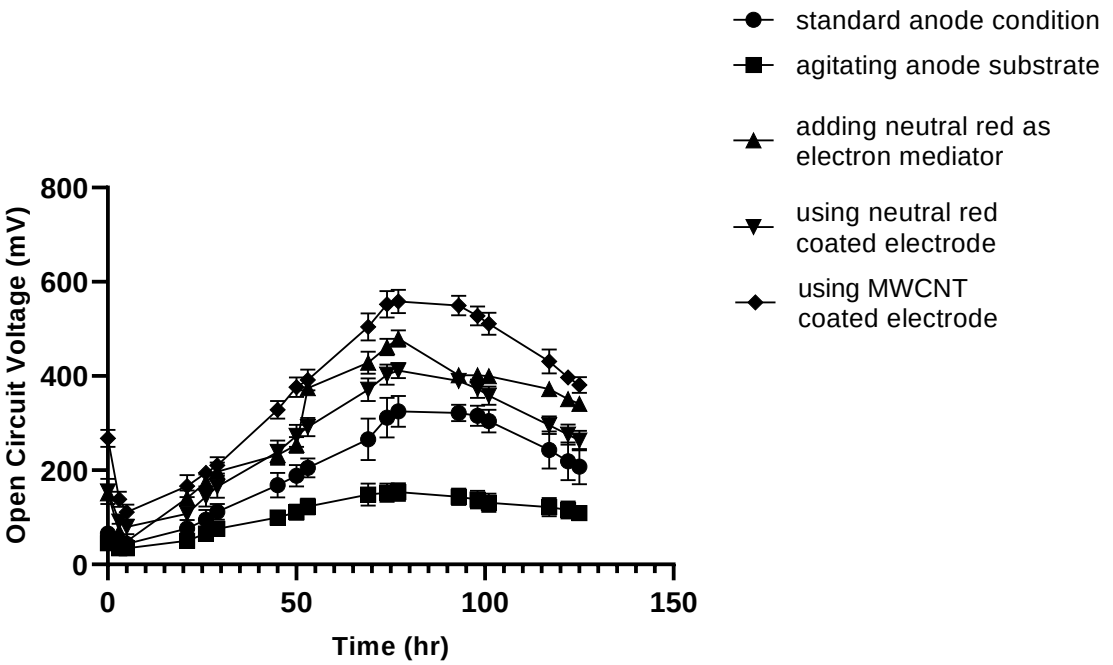


Figure 4.4: Effect of various modifications in anode compartment on OCV

Figure 4.4 shows time versus open circuit voltage curve obtained when various modifications were made in anode compartment. Maximum OCV obtained when agitating the anode substrate was 153.14 ± 18.86 mV. Similarly, maximum OCV obtained while adding neutral red (NR) as electron mediator was 479.67 ± 17.21 mV. On coating the anode electrode with neutral red maximum OCV was 411.33 ± 16.25 mV while that on coating with MWCNT maximum OCV was 558.0 ± 25.23 mV.

Agitation of anode substrate was carried out by circulating the substrate with the help of peristaltic pump (Major Science Company) at the rate of 50 mL/min and another. Low OCV was observed while agitating. This might be because there might have been disturbance in biofilm formation on anode surface or a chance of washing off of formed biofilm during continuous circulation of anode sample was also possible.

Neutral red was added to the anode media at the concentration of 100 µM. There was enhancement in OCV generation on addition of neutral red to anode media. It is clear that the transfer of electrons directly from microbial cells to electrode is not significantly efficient. An electron mediator is necessary for the passage of electrons from a microbial electron carrier to an electrode. For transformation of metabolic reducing power to electricity, the electron mediator should possess peculiar characteristics. It should be able to form reversible redox couple at the electrode as well as it should have affinity to link with NADH. Also, it should not break down during prolonged redox cycling and should be stable both in oxidized and reduced forms. The mediator should be soluble in aqueous systems at neutral pH. Either it should have the ability to move through the microbial cytoplasmic membrane or it should possess such property that it is absorbed by it. Neutral red (NR) has all of these general properties so can be used as better electron mediator (Park and Zeikus, 2000).

The anode electrode was coated with neutral red (NR) and Multi-walled Carbon nanotubes (MWCNT). There was improvement in open circuit voltage generation with the use of coated electrodes than normal graphite sheet electrode. Similar study was carried out by Hindatu et al., 2017 where anode modification was carried out to improve the performance of MFC operation. The ideal anode material for MFC will promote strong microbial adhesion, be biocompatible, and facilitate electron transmission. It should also have a wide surface area, low resistance, and strong electrical conductivity. It should also possess chemical inertness, anti-corrosion, anti-degradation, and appropriate mechanical strength and toughness. While all of these qualities are highly desirable, it is actually difficult to reach a compromise since the materials that are accessible vary in their physicochemical characteristics, susceptibility to microbial adhesion, and electron transport. However, in order to facilitate electron transfer and microbial adhesion, the physicochemical properties of the electrodes could be improved utilizing synthetic components with the appropriate attributes (Hindatu et al., 2017).

The biocompatibility of coated neutral red and MWCNT on bacteria isolated from anode biofilm is presented in latter part of the work.

4.4.2 Optimizing various parameters in cathode compartment affecting Open Circuit voltage (OCV)

4.4.2.1 Effect of using various catholyte solutions

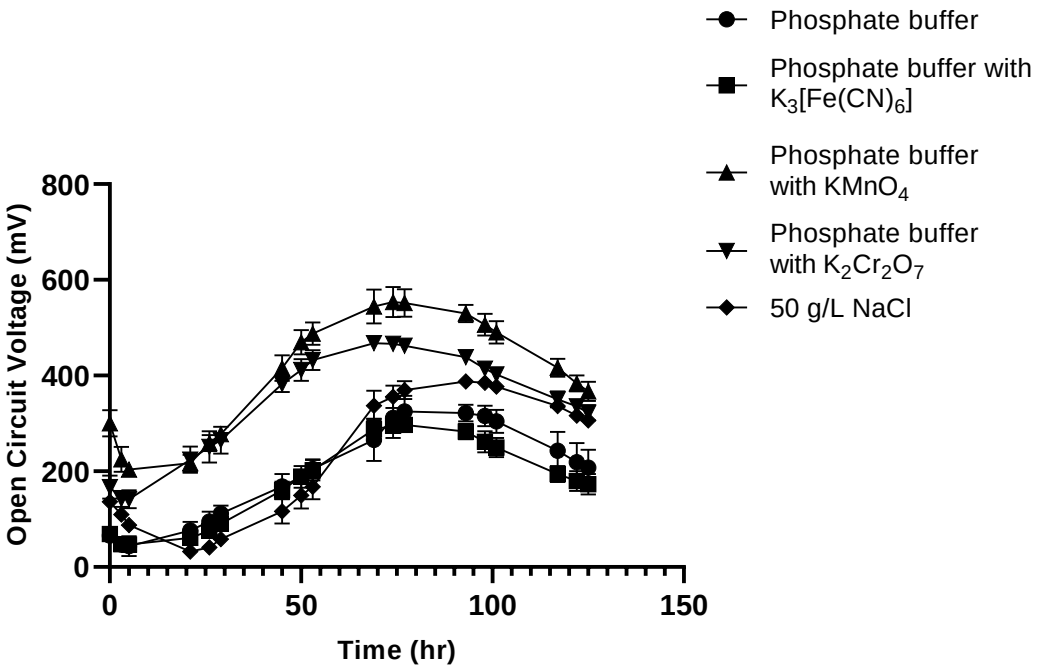


Figure 4.5: Effect of using various catholyte solutions on OCV

Figure 4.5 shows time versus open circuit voltage curve obtained when various catholyte solutions were used in cathode compartment. pH 7 phosphate buffer was considered as a control. The maximum OCV obtained when using pH 7 phosphate buffer was 324.67 ± 33.80 mV. When phosphate buffer was substituted with NaCl maximum OCV of 387.39 ± 12.18 mV was observed. Similarly, on addition of electron acceptors $KMnO_4$, $K_2Cr_2O_7$ and $K_3[Fe(CN)_6]$, the maximum OCV obtained were 553 ± 31 mV, 467 ± 15 mV and 296.67 ± 9.50 mV respectively.

Higher OCV was observed when phosphate buffer was substituted by NaCl catholyte. 50 g/L NaCl solution was used. The ion transport through the electrolyte affects the performance of MFC. Slower ion transport drastically bottlenecked the electron transport through electrode (Oliot et al., 2016). Utomo et al., 2017 studied the effect of substituting buffer with NaCl in MFC and higher OCV was observed. Higher conductivity of 50 g/L NaCl solution allowed the MFC set up utilizing NaCl as catholyte to perform better than that using buffer as catholyte (Utomo et al., 2017).

The open circuit voltage were observed when three different oxidizing agents $KMnO_4$, $K_2Cr_2O_7$ and $K_3[Fe(CN)_6]$ were added to phosphate buffer solution separately. MFC setup

using phosphate buffer as catholyte was taken as control. The concentration of these electron acceptors were 10mM added to catholyte. The highest OCV was observed using $KMnO_4$ as electron acceptor followed by $K_2Cr_2O_7$ and least OCV was observed while using $K_3[Fe(CN)_6]$ as electron acceptor. Similar type of work was carried out by Tardast et al., 2014 and above mentioned oxidizing agents was used as final electron acceptors in cathode chamber. Best result was obtained with $KMnO_4$ as electron acceptor followed by $K_2Cr_2O_7$ and $K_3[Fe(CN)_6]$. These materials can increase reduction reaction on the surface of cathode electrode. Cathode losses can also be minimized using this set of electron acceptor (Tardast et al., 2014).

From all above experiments, 10 % substrate concentration prepared in phosphate buffer as anode media, MWCNT coated graphite sheet as anode electrode and phosphate buffer added with $KMnO_4$ as electron acceptor as catholyte solution were suitable for enhanced OCV generation. So, in further experiment these conditions were used in power generation in MFC by applying different resistances.

4.4.3 Power Generation in MFC using different resistors

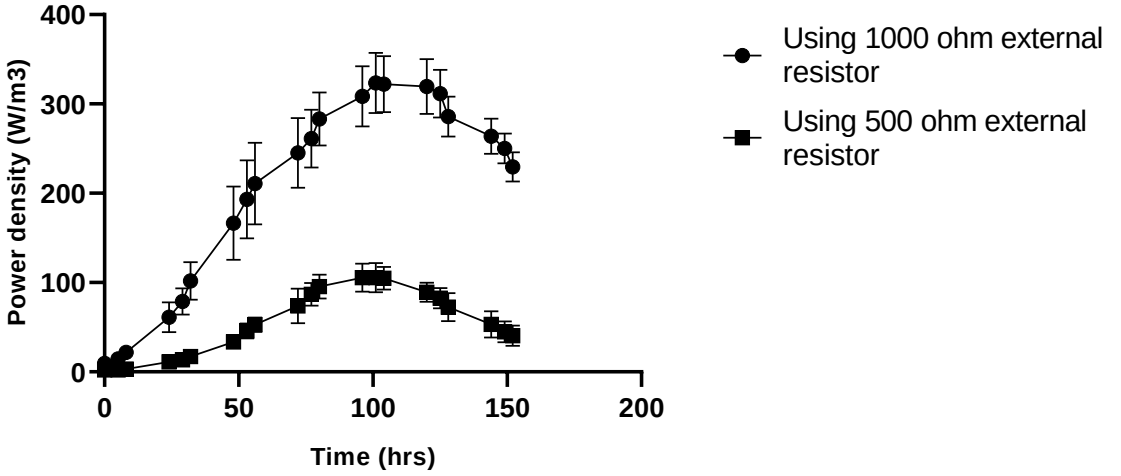


Figure 4.6: Effect of external resistor in power generation in Microbial Fuel Cell

Figure 4.6 shows the power generation in MFC during operation with 1000 ohm resistor and 500 ohm eternal resistors. The experiment was carried out to observe the effect of external resistance on overall performance of MFC. Phosphate buffer with $KMnO_4$ was used as catholyte and MWCNT coated graphite electrode was used as anode electrode. The maximum power density generated using 1000 ohm external resistance was 323.59 ± 33.89 W/m^3 and that with 500 ohm external resistance was 105.47 ± 16.40 W/m^3. Error bar represented standard error based on measurements from triplicate operation.

External resistance acts as an integral part of an electrical grid that controls the output of the fuel cell (Risamani et al., 2011). The higher power density while using higher external resistance might be because higher resistance might have lowered the internal resistance that overall improved the rate of reaction. The optimum external resistance usually correlates with internal resistance of MFCs. Internal resistance is not system constant, and depends on the external load applied to MFC (Manohar et al., 2008). A slight increase in internal resistance can dramatically decrease microbial fuel cell performance (He et al., 2005). External resistance could also relate to anode potential. Du et al., 2015, studied variations of the anode potential with external resistances. Changing in external resistance showed the highest anode potential. Higher anode potential means higher free energy which boosts up startup electricity generation (Goud et al., 2011).Power Density was calculated based on anodic volume liquid in order to better reflect three-dimensional properties of both electrodes and MFC reactors. Power Density was introduced in the unit watts per cubic meter (W/m3) (Rabaey et al., 2005).

4.4.4 Analysis of different components reduction after 7 days in MFC operation

Table 4.6: Reduction of COD with different modes of MFC operation

	Initial concentration mg/g	**Final concentration mg/g**	**% Reduction**
Using NaCl as catholyte	26.75 ± 0.68	18.16 ± 0.78	32.11
Agitation	26.75 ± 0.68	20.46 ± 0.75	23.51
Adding $KMnO_4$ in catholyte	26.75 ± 0.68	15.95 ± 0.17	40.37
Adding $K_2Cr_2O_7$ in catholyte	26.75 ± 0.68	16.21 ± 0.11	39.40
Adding $K_3[Fe(CN)_6]$ in catholyte	26.75 ± 0.68	17.61 ± 0.25	34.17
Using MWCNT coated electrode	26.75 ± 0.68	15.64 ± 0.42	**41.53**

Using neutral red coated electrode	26.75 ± 0.68	16.90 ± 0.31	36.82
Using neutral red as electronophore	26.75 ± 0.68	16.97 ± 0.33	36.56

The COD removal rate in different setups of MFC operations were determined using initial and final amount of COD in the sample. Maximum COD removal was found to be 41.53 % by using MWCNT coated graphite electrode at anode whereas minimum COD removal was found to be 23.51 % during agitation of anode sample by circulating with the help of peristaltic pump.

The COD removal rates are typically reported for the complete cycle in batch and fed-batch reactors based on initial and final COD concentration (Zhang et al., 2015). Chemical Oxygen Demand (COD) measures the amount of oxygen required to chemically oxidize the organic matter present in any sample. Higher COD signifies higher organic content. Das and Calay., 2022 studied the power generation and COD removal efficiency by air cathode Microbial Fuel Cell using *Shewanella baltica*. With the increase in initial COD value, there was increase in COD removal rate to certain limit after which increment in initial COD caused reduction in removal efficiency. COD removal efficiency of 94 % was reported in an up flow tubular MFC treating dairy (Marassi et al., 2020).

Table 4.7: Reduction of Phosphorus with different modes of MFC operation

	Initial concentration mg/g	**Final concentration mg/g**	**% Reduction**
Using NaCl as catholyte	0.017 ± 0.005	0.0122 ±0.0062	28.06
Agitation	0.017 ± 0.005	0.0136 ± 0.0016	20.18
Adding $KMnO_4$ in catholyte	0.017 ± 0.005	0.0110 ± 0.0029	35.23
Adding $K_2Cr_2O_7$ in catholyte	0.017 ± 0.005	0.0113 ± 0.0022	33.82

Adding $K_3[Fe(CN)_6]$ in catholyte	0.017 ± 0.005	0.0110 ± 0.0035	35.21
Using MWCNT coated electrode	0.017 ± 0.005	0.0093 ± 0.0006	**45.25**
Using neutral red coated electrode	0.017 ± 0.005	0.0125 ± 0.0011	26.64
Using neutral red as electronophore	0.017 ± 0.005	0.0126 ± 0.0034	26.11

Similarly, the removal of Phosphorus in waste sample after each MFC operation was determined. Maximum amount of Phosphorus reduction was found to be 45.25 % while using MWCNT coated electrode in anode whereas minimum reduction was found to be 23.51 % during agitation of anode sample by circulating with the help of peristaltic pump. The data shown by (Chaulagain, 2016) also revealed that CNT enhanced electrical performances. CNT actually have shown to be promising alternative material for MFC electrode because of their electrical conductivity, chemical stability, biocompatibility and high specific area (Mustakeem, 2015)

The phosphorus accumulating organisms (PAOs) can take up phosphorus far in excess of their requirements for growth and accumulate phosphorus in the form of polyphosphates (polyp). The influx of phosphorus into the water bodies leads to eutrophication of water bodies (Liu et al., 2018). Liu et al., 2018 carried out an experiment to evaluate the phosphorus removal efficiency by preparing synthetic wastewater in laboratory. Cultured *Pseudomonas putida* was added into the synthetic wastewater. 96 % phosphorus removal was observed confirming the highly effective phosphate removal by *Pseudomonas putida*.

Table 4.8: Reduction of Ammonia- Nitrogen with different modes of MFC operation

	Initial concentration mg/g	**Final concentration mg/g**	**% Reduction**
Using NaCl as catholyte	0.0956 ± 0.0068	0.0364 ± 0.0067	61.92
Agitation	0.0956 ± 0.0068	0.0404 ± 0.0074	57.64
Adding $KMnO_4$ in catholyte	0.0956 ± 0.0068	0.0361 ± 0.0108	62.23
Adding $K_2Cr_2O_7$ in catholyte	0.0956 ± 0.0068	0.0365 ± 0.0044	61.94
Adding $K_3[Fe(CN)_6]$ in catholyte	0.0956 ± 0.0068	0.0374 ± 0.0061	60.89
Using MWCNT coated electrode	0.0956 ± 0.0068	0.0343 ± 0.0039	**64.12**
Using neutral red coated electrode	0.0956 ± 0.0068	0.0365 ± 0.0054	61.82
Using neutral red as electronophore	0.0956 ± 0.0068	0.0371 ± 0.0091	61.19

Removal of ammonia-nitrogen in waste sample after each MFC operation was determined. Maximum amount of ammoniacal-nitrogen reduction was found to be 64.12 % while using MWCNT coated electrode in anode whereas minimum amount of ammoniacal-nitrogen reduction was 57.64 % during agitation of anode sample by circulating with the help of peristaltic pump. Li et al., 2022 carried out an experiment for optimization of ammonia nitrogen and phosphorus removal performance. After optimizing the reaction conditions, the actual removal of NH_4^+-N was 94.88 %.

4.4.5 Analyzing the anode media

4.4.5.1 Cyclic Voltammetry on 3rd day of MFC operation

MFC set up was prepared with pH 7 phosphate buffer as catholyte, 10 % substrate prepared in phosphate buffer as anode media and graphite sheet electrodes as both

cathode and anode electrode. MFC was allowed to operate for 3 days and on 3rd day of operation cyclic voltammetry test was carried out to study the redox activities of the components involved in the bio-chemical system in both solution and components bound to bacteria. Cyclic voltammetry was conducted in the potential range from -0.8 to 0.8 V at a low scan rate of 10 mV/s for three cycles. The observed cyclic voltammogram was as shown in figure 4.7.

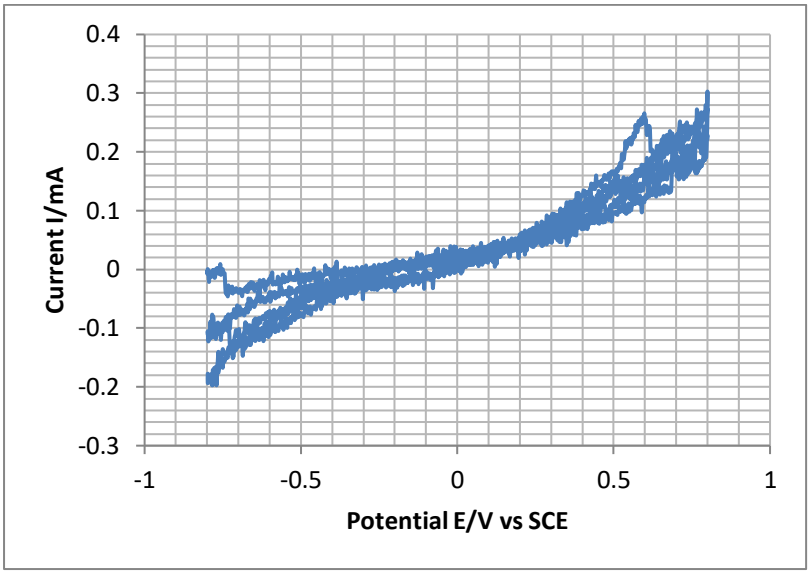


Figure 4.7: Cyclic Voltammogram on 3rd day of MFC operation

During metabolic processes, protons (H^+) and electrons (e^-) are created and consumed continually in the cell. A potential difference is created between the cell and the surrounding medium when an external potential is applied, causing the e^- to travel towards the working electrode and deposit, resulting in voltammogram. The cyclic voltammogram shows a redox reaction occurring at about 0.60 V. According to Martin et al., 2011, peak of carbon was found around 0.55 V. Thus in above voltammogram the peak observed between 0.5 V to 0.6 V might be of typical carbon electrode showing the peak of electroactive oxide/hydroxide. Maximum current of 0.3 mA (forward scan, 0.8 V) was visualized during stable phase of operation.

4.4.5.2 Anode media analysis by HPLC after completion of MFC operation

MFC set up was prepared with pH 7 phosphate buffer as catholyte, 10 % substrate prepared in phosphate buffer as anode media and graphite sheet electrodes as both cathode and anode electrode. MFC was allowed to operate until the OCV reached maximum and started to decline. Then, various components present in anode media were analyzed by HPLC technique. 1 ml of anode sample after 7 days of operation was taken, syringe filtered and subjected to HPLC using Aminex HPX-87H column. 5 mM H_2SO_4 was used as mobile phase. Components were separated on the basis of refractive

index and detected by RI (Refractie Index) detector. The observed peaks were compared with the standard peaks and identified peaks were labeled. The observed chromatogram was as shown in the figure 4.8.

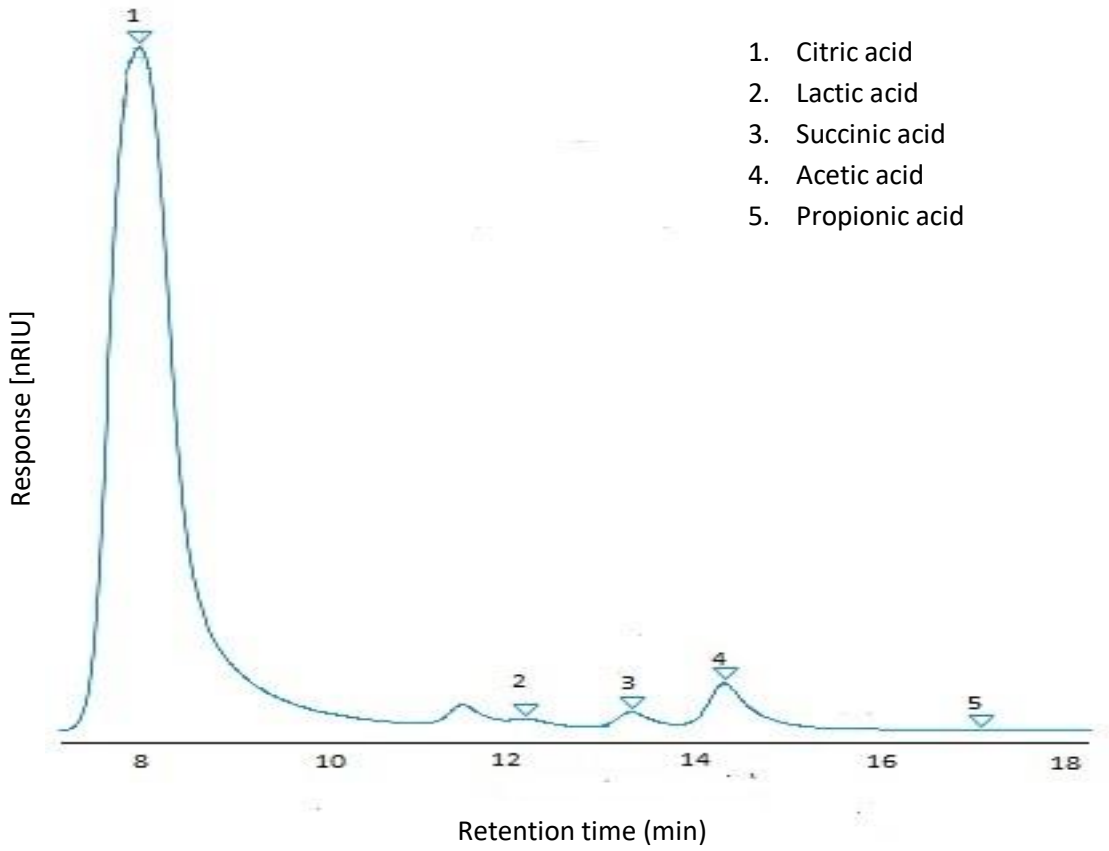


Figure 4.8: Chromatogram showing various components in anode media

Several Organic acids were detected by HPLC analysis of anode media after completion of MFC operation which are presented in table 4..

Table 4.9: Different organic acids detected by HPLC technique

Components	**Concentration (mg/mL)**
Citric acid	5.859 ± 0.052
Lactic acid	0.0115 ± 0.0003
Succinic acid	0.0613 ± 0.002
Acetic acid	0.0347 ± 0.002
Propionic acid	0.000278 ± 0.000

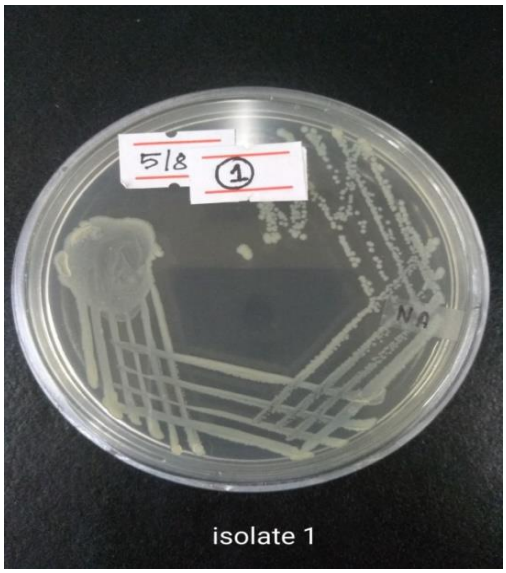
Organic acids such as citric acid, lactic acid, succinic acid, acetic acid and propionic acid were detected in the anode media and the concentrations were determined based on

peak area of the calibration standards. Highest concentration of citric acid was detected. The column used for HPLC analysis can detect the sugars like glucose, xylose organic acids like citric acid, lactic acid, succinic acid, acetic acid, propionic acid, butyric acid and alcohols like ethanol in methanol in fermentation samples. Initially during sugar analysis of sample by HPLC technique, glucose was detected in higher amount. But after completion of MFC operation no glucose was detected. The sugars present in anode media might have been utilized by microbes present in anode compartment to give acidic end products. The anodic reaction in bio electrochemical system produces organic acids like acetate, propionate, butyrate, lactate and alcohols like ethanol, glycerol etc. (Logan and Rabaey, 2012).

4.4.6 Working with anode biofilm

4.4.6.1 Isolation of bacteria from anode biofilm

MFC set up was prepared with pH 7 phosphate buffer as catholyte, 10 % substrate prepared in phosphate buffer as anode media and graphite sheet electrodes as both cathode and anode electrode. MFC was allowed to operate until the OCV reached maximum and started to decline. The anode electrode was asceptically taken; sterile loop was lightly rubbed into the surface of electrode and inoculated into the LB agar medium and inoculated. Morphologically different looking microbes were sub-cultured into the nutrient agar medium. Two different bacteria were isolated named as isolate 1 and isolate 2.



(i)

(ii)

Figure 4.9: (i) Colony morphology of isolate 1 on nutrient agar (NA) plate. (ii) Colony morphology of isolate 2 on nutrient agar plate

The colonies of isolate 1 were circular in shape, smooth, moist, greyish white in colour and with translucent disc like appearance. The colonies of isolate 2 were smooth, large, translucent, flat colonies with irregular margins, and greenish colouration of the medium was observed.

4.4.6.2 Microscopic structures of isolated strains

Table 4.10: Microscopic structure of isolates

S.N	Strain	Gram Staining	Shape
1.	Isolate 1	Negative	Rod
2.	Isolate 2	Negative	Rod

Isolated microbial strains were observed under microscope as shown in appendix. When Gram staining was performed and observed under microscope, both the strains were gram negative and rod shaped.

4.4.6.3 Biochemical characteristics shown by isolated strains

Table 4.11: Biochemical tests of the isolates

S.N	Test Name	Isolate 1	Isolate 2
1.	Sulphur Indole Motility (SIM)	Negative	Negative
2.	Methyl Red (MR)	Positive	Negative
3.	Voges-Proskauer (VP)	Negative	Negative
4.	Citrate Utilization	Positive	Positive
5.	Oxidase	Negative	Positive

The isolated strains were subjected to various biochemical tests as shown in the table above. The isolate 1 gave negative Sulphur Indole Motility (SIM) test, positive Methyl Red (MR) test, negative Voges-proskauer (VP) test, positive Citrate Utilization test and negative oxidase test. Similarly, isolate 2 gave negative Sulphur Indole Motility (SIM) test, negative positive Methyl Red (MR) test, negative Voges-proskauer (VP) test, positive Citrate Utilization test and positive oxidase test.

Figure 4.10: Positive oxidase test shown by isolate 2

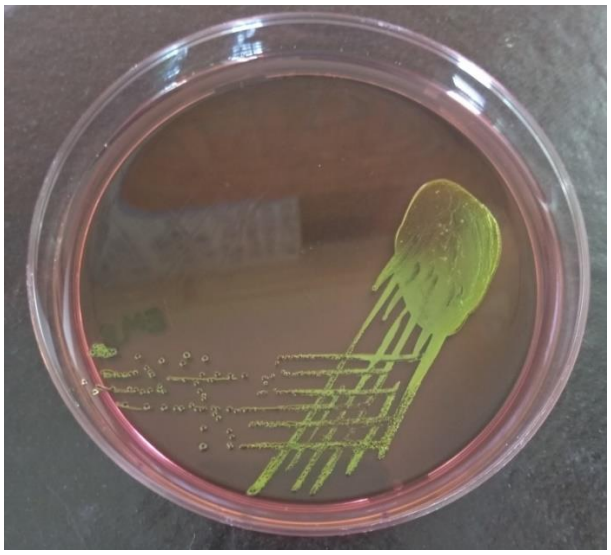


Figure 4.11: Green metallic sheen shown by isolate 1 on Eosin-methylene Blue (EMB) agar

From all above tests, gram negative rod shaped bacteria that formed circular, smooth, greyish white disc like colonies on nutrient agar plate, which gave positive methyl red (MR) test, negative Voges-Proskauer (VP) and oxidase tests and which formed green metallic sheen in Eosin-methylene blue (EMB) agar plate might be *E. coli* bacteria. Similarly, gram negative and rod shaped bacteria that formed smooth, flat colonies with irregular margins with green coloration in nutrient agar plate, which gave negative indole , methyl red (MR) and Voges-Proskauer (VP) tests and positive citrate utilization and oxidase tests might be *Pseudomonas* spp (Shah et al., 2013).

These two bacteria have been already proved by different researches to have exoeletrogenic characteristics. The endogenous chemical mediators like pycocyanin and related compounds produced by *Pseudomonas* can transfer electrons to an electrode and generate electricity in MFC (Rabaay et al., 2004). Similarly, certain strains of *E. coli* after extended cultivation or cultivation with chemically treated electrodes have been reported to produce high power densities comparable to that produced by *Geobacter* or *Shewanella* strains (Li et al., 2018).

4.4.6.4 Genomic DNA extraction and PCR amplification of isolate 1 and isolate 2

On the basis of colony morphology and various biochemical characteristics two bacterial strains were selected. The genomic DNA was extracted by CTAB (Cetyl trimethyl ammonium bromide) method. Distinct bands of DNA were observed by gel electrophoresis in 0.8 % agarose gel which was then used as a template for PCR (Polymerase Chain Reaction).

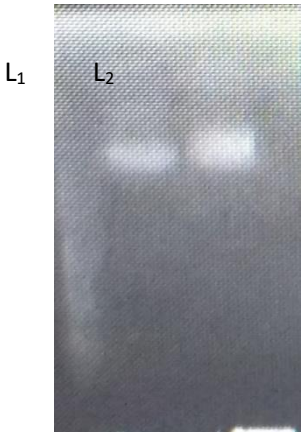


Figure 4.12: Gel electrophoresis in 0.8 % agar of bacterial genomic DNA using CTAB method. L1: isolate 1: L2: isolate 2

The two samples were successfully amplified using conventional PCR. The preliminary experiment evaluated the effectiveness of the primers Fd1/rP2 targeted to amplify a region of 16s rDNA of the isolated species, which produced amplified product of 1500 bp.

The success of PCR amplification reaction was observed by performing agarose gel electrophoresis. The amplified bands of DNA fragments in the gel were observed under UV illumination. The visible bands correspond to the positive results. Negative control (reaction mixture with no template) and DNA ladder were used to compare the size.

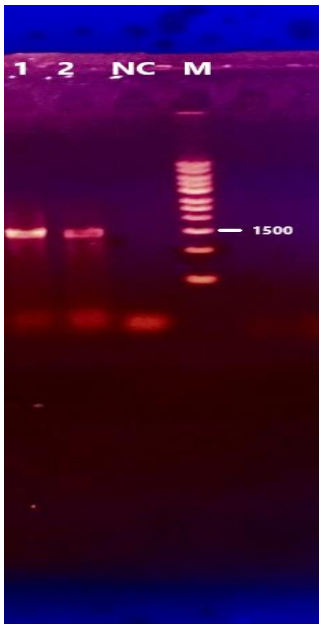


Figure 4.13: Agarose Gel Eletrophoresis of PCR products after amplification of the 16S rRNA gene, on 1% agarose gel. M: DNA ladder DirectLoad of 1kb; lane: 2 and 3 samples and NC: negative control.

4.4.6.5 Biocompatibility test for the isolates

Biocompatibility test was carried out to observe if the Multi-walled Carbon Nanutubes (MWCNT) and neutral red coated on the anode electrode have inhibiting effect on the isolates. The test was carried out by agar well diffusion method. The result showed that both the materials used for coating the electrode did not have any inhibiting effect on the microbial biofilm on anode electrode.

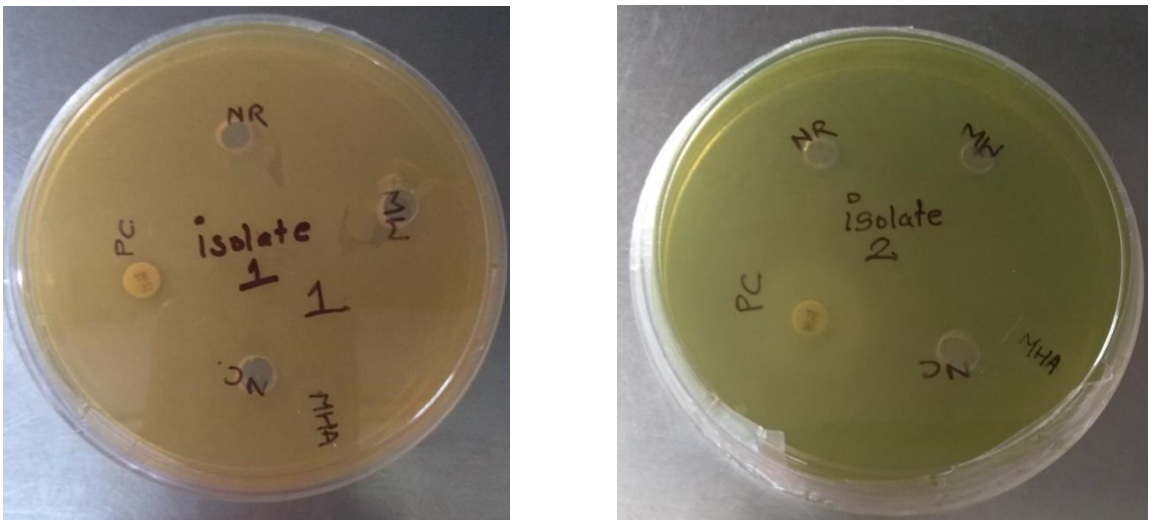


Figure 4.14: Biocompatibility test of neutral red and MWCNT on isolates 1 and isolates 2. NR: neutral red, MW: Multi-walled Carbon Nanotubes, NC: negative control (sterilized distilled water), PC: positive control (Tetracycline disc).

4.4.6.6 Drug Resistance Test for the isololates (Akter et al., 2019)

While conducting biocompatibility test, isolate 1 demonstrated resistant to tetracycline disc used as a positive control. Thus, both the isolates were tested for their multi-drug resistance characteristics. Five drugs- Vancomycin (VA 30), Ciprofloxacin (CIP 5), Ampicillin (AMP 10), Ceftazidime (CAZ 30) and Amikacin (AK 30) were used.

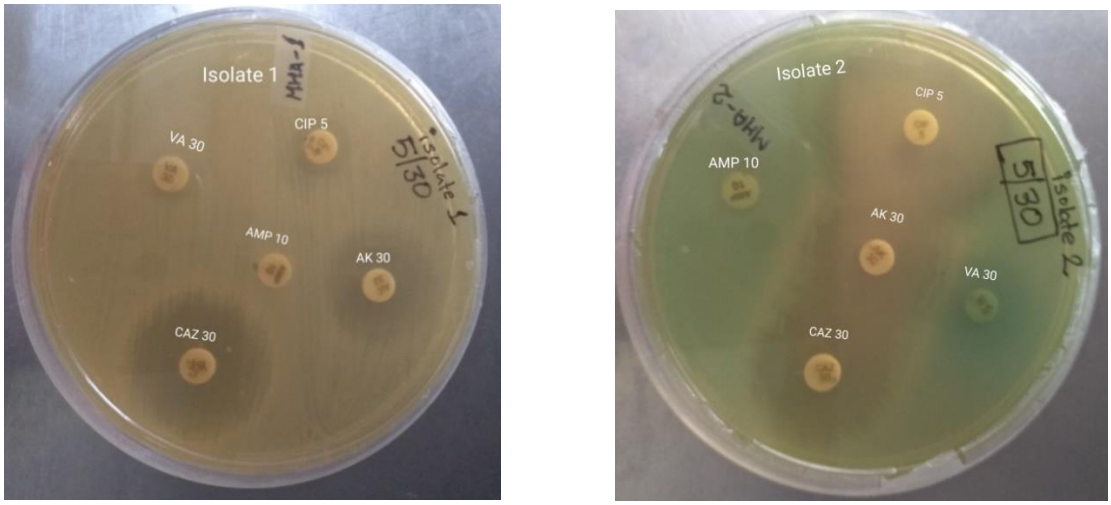


Figure 4.15: Drug resistance test for isolates 1 and 2. VA 30: Vancomycin, CIP 5: Ciprofloxacin, AMP 10: Ampicillin, CAZ 30: Ceftazidime and AK 30: Amikacin.

The result showed that the isolate 1 was resistant to vancomycin (VA), ampicillin (AMP) and ciprofloxacin (CIP). Similarly, isolate 2 was resistant to ampicillin (AMP) and vancomycin (VA). Akter et al., 2019 carried out an experiment to find out the occurrence of drug resistant bacteria in household waste sample. Eight different bacterial strains were isolated. Among the isolates hundred percent resistance was documented against cefuroxime and amoxicillin whereas all the isolates were sensitive to meropenem, amikacin and ceftriaxone. Presence of pathogenic bacteria with antibiotic resistance traits in organic kitchen waste portrayed serious public health threats. Thus, present study critically raises the requirement for proper management and disposal of the accumulated domestic wastes by the municipal and government authorities (Akter et al., 2019).

4.4.7 Laboratory fabrication of MFC for scale-up purpose

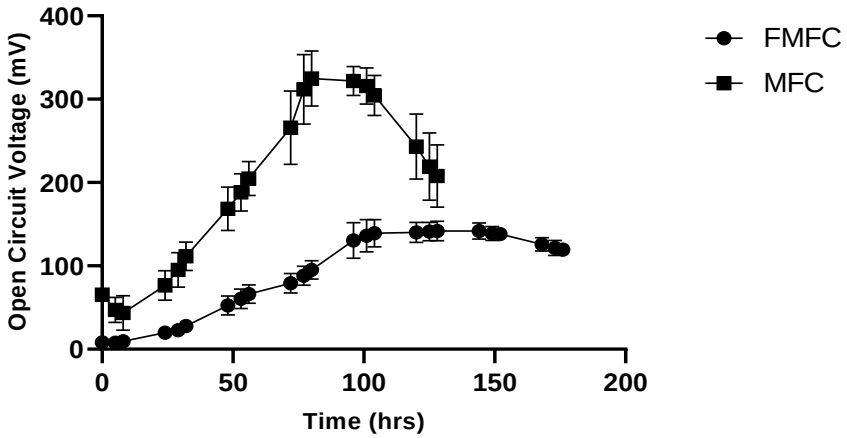


Figure 4.16: Open circuit voltage obtained in FMFC and MFC

Fabricated MFC was constructed in laboratory. The purpose was to construct economic MFC with higher volume for laboratory purpose. Open Circuit Voltage reading was taken. The highest OCV obtained was 141.67 ± 9.71 mV. Better result was expected with double volume of anode sample and catholyte as well as larger surface area of electrode. The result might be because the PVC pipe used for connecting two chambers protrudes inside the chamber thus reducing the contact surface area of anode sample and catholyte with the membrane. Similar research was carried out by Utomo et al., 2017 where fabricated Microbial Fuel Cell was constructed and applied for voltage generation. Better performance was observed with fabricated MFC than normal MFC. They concluded that better result was due to the FMFC's larger chamber size resulting in higher amount of bacteria and substrates being present thus producing a higher voltage than the MFC.

CHAPTER 5: SUMMARY

The MFC is a sustainable and renewable technology that converts biodegradable substrates into electricity utilizing microorganisms as a biocatalyst. MFC is a perfect solution for waste treatment and energy generation since bacteria can oxidize the substrates to produce power.

General introduction of this work was described in chapter first. Solid waste management has become the serious problem in every society with rapidly increasing population around the world. In such scenario, MFC technology has developed as promising technology for organic waste management along with the generation of electricity.

Second chapter describes about the literatures reviewed related to this work. The review found that the content and type of biomass, nature and surface area of electrode used as well as the microbial species involved during the process influence the performance of MFC. Optimization of operational parameters can be carried out to enhance electricity production from MFC.

Third chapter described about materials and methods involved in research work. Degradable kitchen waste was used as sample. Dual chambered MFC was constructed using graphite sheet electrodes. Anode and cathode chambers were separated with proton exchange membrane (PEM). Anode chamber contained 325 mL waste sample whereas cathode chamber contained 325 mL phosphate buffer. The mixed consortia present in waste acted as biocatalyst. Optimization for the enhancement of OCV was by finding out the best concentration of substrate to be used, by replacing phosphate buffer with NaCl, by adding 10 mM of various electron acceptors such as $K_3[Fe(CN)_6]$, $K_2Cr_2O_7$ and $KMnO_4$.Electrode modification was done by coating neutral red (NR) and multi-walled carbon nanotube (MWCNT) in anode electrode. After optimizing the parameters two different resistors were used for determination of power density. The end products generated in anolyte at the end of MFC operation were analyzed by HPLC analysis.

Chapter four described about the findings of the research work. Composition of degradable kitchen waste sample was determined. Different physical and analytical parameters of waste sample were determined. Construction of MFC was done using appropriate electrode and PEM. Optimization of MFC parameters were performed by finding the best concentration of the substrate, substituting the phosphate buffer with 50 g/L NaCl solution, adding different electrons acceptors like as $K_3[Fe(CN)_6]$, $K_2Cr_2O_7$

and $KMnO_4$ to catholyte and coating anode electrode with neutral red and MWCNT. MWCNT coated anode electrode gave best OCV of 558.0 ± 25.23 mV. Maximum power density of 323.59 ± 33.89 W/m^3 was obtained using 1000 ohm external resistance while using $KMnO_4$ added phosphate buffer as catholyte and MWCNT coated anode electrode. Morphological, biochemical and molecular characterization of isolates from anode biofilm were carried out. Along with that biocompatibility of coated materials on anode electrode and drugs resistance test for isolates was also performed. Sugar analysis in waste sample was carried out by HPLC and also end products analysis in anodic sample was performed after completion of MFC operation by HPLC. Citric acid, lactic acid, succinic acid, acetic acid and propionic acids were determined. Various end products generated during operation of MFC were analyzed using HPLC and concentrations were determined using calibration standard.

CHAPTER 6: CONCLUSION

Environmental parameters analysis of degradable kitchen waste was determined as its pH, moisture content, TSS, VSS, ash content, chemical oxygen demand (COD), ammoniacal-nitrogen, and phosphorus, different metal ions such as zinc, iron, copper, nickel, manganese and cadmium. Sugar analysis in sample was carried out by HPLC. Microbial fuel cell (MFC) was operated using waste sample in anode, phosphate buffer in cathode, graphite sheet electrode in both anode and cathode. Optimization of MFC parameters for enhancement of electricity generation was carried out. 10 % substrate concentration in anolyte gave best result so it was further applied in MFC operation. Phosphate buffer was replaced by NaCl and gave better result but it couldn't be further used because of semipermeable nature of nafion membrane to water molecules against osmotic pressure. Different electron acceptors were added to catholyte to enhance MFC performance. Addition of $KMnO_4$ gave the best result among all. Modification of anode electrode with neutral red and MWCNT was carried out and later one gave better result. Among all these operations highest OCV was observed with MWCNT coated anode electrode which was 558.0 ± 25.23 mV. Using $KMnO_4$ added phosphate buffer as catholyte and MWCNT coated anode electrode maximum power density of 323.59 ± 33.89 W/m^3 was obtained using 1000 ohms external resistance. The main purpose of this research was to optimize MFC parameters for management of degradable waste along with enhanced electricity production.

CHAPTER 7 RECOMMENDATION

This research can be recommended to numerous industries for further improvement, production and/or policy implementation based on yield and process efficiency. Some of them are listed below.

This research could be useful to municipalities and other institutions working for waste management and electricity production.

It is recommended to perform scale up of this process to industrial applications.

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APPENDICES

Appendix I: Composition and Preparation of different Microbiological Culture Media and Reagents.

A. Culture Media

Nutrient Agar (NA)

Peptic digest of animal tissue	5.0 g
Beef extract	1.5 g
Yeast extracts	1.5 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	1 L

Mueller Hinton Agar (MHA)

Beef extract	2.0 g
Acid hydrolysate of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Distilled water	1 L
Final pH at 25°C	7.3 ± 0.1

Luria Burtani broth (LB-broth)

Yeast Extract	5.0 g
Sodium Chloride	10.0 g
Enzyme casein hydrolysate	10.0 g
Distilled water	1 L

Nutrient Broth (NB)

Peptic digest of animal tissue	5.0 g
Sodium Chloride	5.0 g
Beef extract	1.5 g
Yeast extracts	1.5 g
Final pH at 25°C	7.4 ± 0.2

Eosin Methylene Blue (EMB) agar

Peptic digest of animal tissue	10.0 g
Dipotassium phosphate	2.0 g
Lactose	5.0 g
Sucrose	5.0 g
Eosin Y	0.4 g
Methylene Blue	0.065 g
Agar	13.5 g

B. Reagents

1. Digestion Mixture for phosphorus

100 mL of 96 % sulfuric acid mixed with 18 mL water. Then 6 g of salicyclic acid powder was added to the mixture.

2. Stock solution of PO_4

1.432 g potassium dihydrogen phosphate was dissolved in 900 mL of distilled water in volumetric flask and volume was made 1 L by adding distilled water.

3. Ascorbic Acid Solution

1.76 g of ascorbic acid was dissolved in 100 mL of ultrapure water and mixed (used freshly prepared).

4. Ammonium Molybdate Solution

40 g of ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 900 ml ultrapure water and final volume was made 1 L.

5. Potassium Antimony Tartarate Solution

0.274 g Potassium Antimony Tartarate was dissolved in 100 mL of ultrapure water.

6. Sulfuric Acid solution (2.5 mol/L)

140 mL of sulfuric acid was diluted in 500 mL of ultrapure water, allowed to cool and final volume was made 1 L.

7. Mixed Reagent

50 mL of sulfuric acid, 15 mL ammonium molybdate solution, 30 mL ascorbic acid and 5 mL potassium antimony tartarate were mixed in a reagent bottle. Then 80 mL of mixed solution was diluted with 300 mL ultrapure water.

8. Hydrogen Phthalate stock solution

0.085 g of KHP was mixed in 80 mL of ultrapure water in volumetric flask and volume was made up to 100 mL. Stock solution of 1 g/L was prepared.

9. Digestion solution

1.02 g potassium dichromate, 16.7 mL sulfuric acid and 3.32 g mercury sulfate were dissolved in 50 mL of distilled water and final volume was made 100 mL with water.

10. Catalyst solution

0.09 g silver sulfate was mixed in 100 mL of conc. Sulfuric acid and left overnight.

11. Ammonium nitrogen stock solution

3.819 g ammonium chloride was dissolved in 900 mL of distilled water and volume was made up to 1 L by adding water.

12. Ammonia nitrogen working solution

10 mL of stock solution was diluted up to 1000 ml with distilled water.

13. Borate Buffer (pH 9.5)

88 mL of 0.1 mol/L NaOH was added to 500 mL of 0.025 mol/L sodium tetraborate solution and final volume was made 1000 mL with distilled water.

14. Boric acid solution

20 g of boric acid was mixed with 900 mL of distilled water and final volume was made 1000 mL.

15. Nessler's Reagent

100 g of mercuric iodide and 70 g of potassium iodide was taken in small amount in water. Then mixture was added to a cooled solution of 160 g NaOH in 500 mL of distilled water and dilute up to mark with distilled water in a 1000 mL volumetric flask.

16. Phosphate Buffer (pH 7)

1000 mL volumetric flask was taken with 800 mL of distilled water. 9.343 g of Potassium phosphate dibasic was added. 6.309 g of Potassium Phosphate Monobasic was added to the solution. It was mixed well and volume was made up to 1000 mL.

Appendix II: Standard Curves

1. Standard Curve of Spectrophotometer analysis

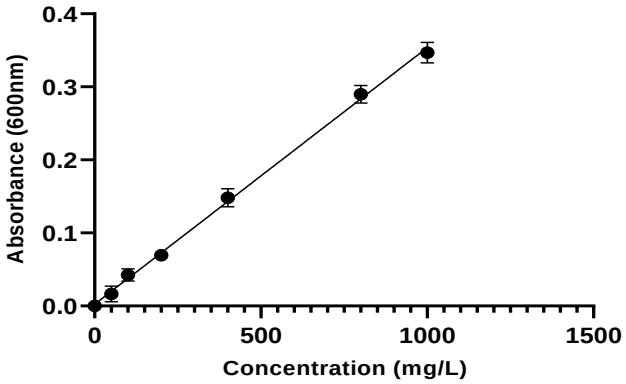

Figure 1: Standard curve of chemical oxygen demand (Y = 0.003512X + 0.0025)

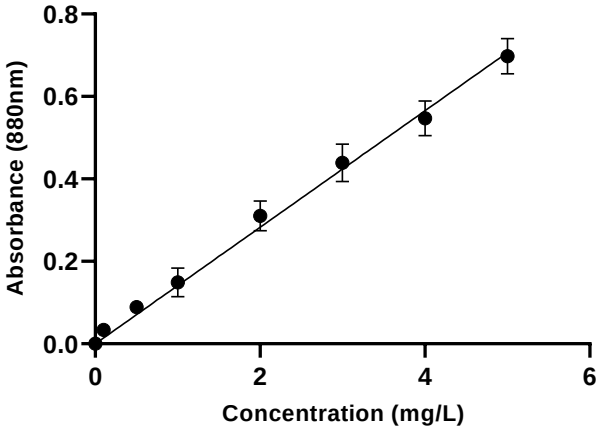

Figure 2: Standard curve of Phosphorus (Y = 0.1414X + 0.00)

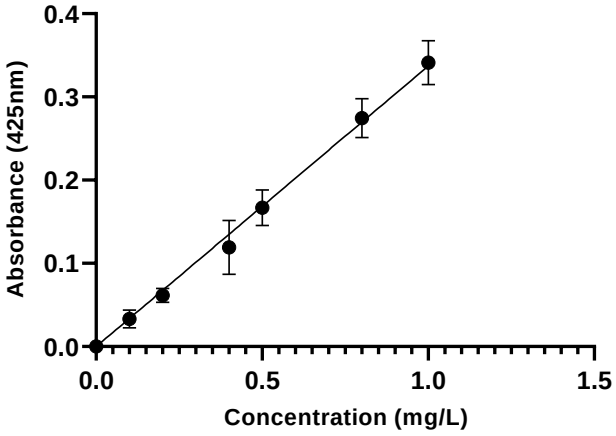

Figure 3: Standard curve of Ammonia Nitrogen (Y = 3.371X + 0.000)

2. Standard Curve of sugar analysis in HPLC

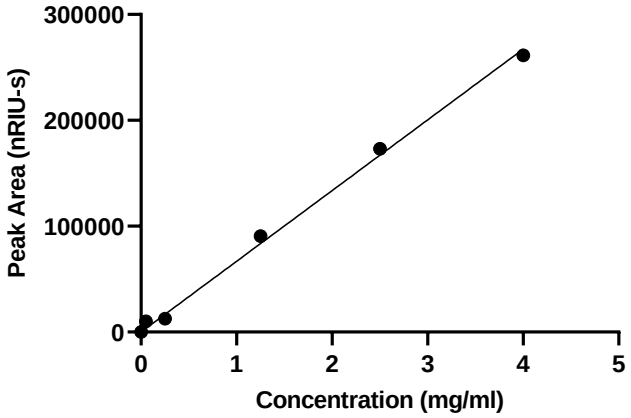

Figure 1: Standard curve of glucose (Y = 66813*X + 0.000)

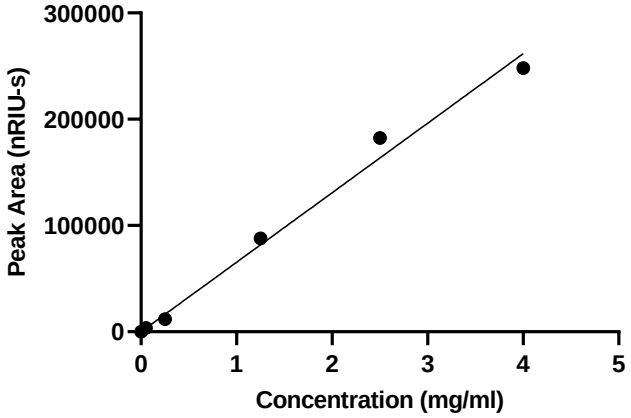

Figure 2: Standard curve of Arabinose (Y = 65414*X + 0.000)

3. Standard curve of end product analysis in HPLC

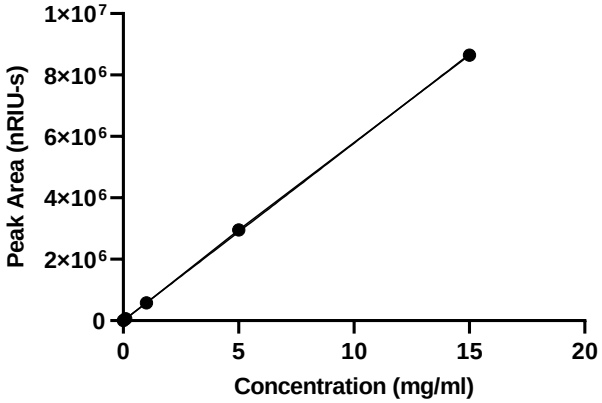

Figure1: standard curve of acetic acid (Y = 577906*X + 0.000)

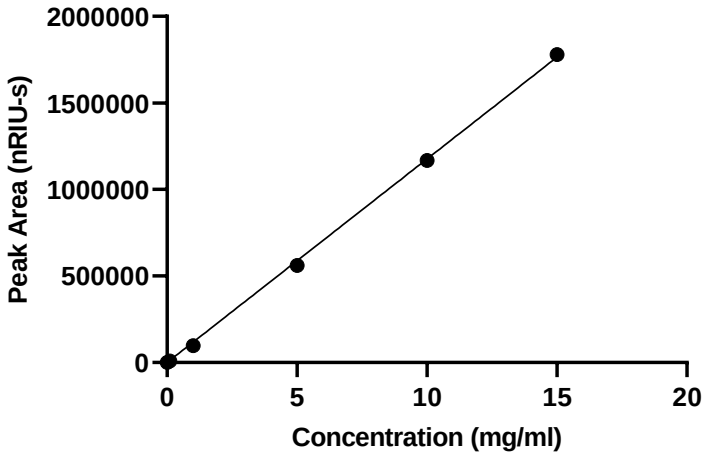

Figure 2: Standard curve of citric acid (Y = 117589*X + 0.000)

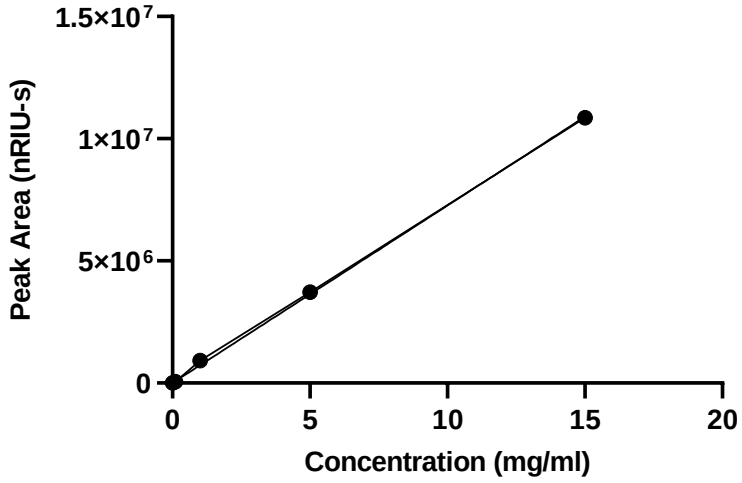

Figure 3: Standard curve of lactic acid (Y = 726461*X + 0.000)

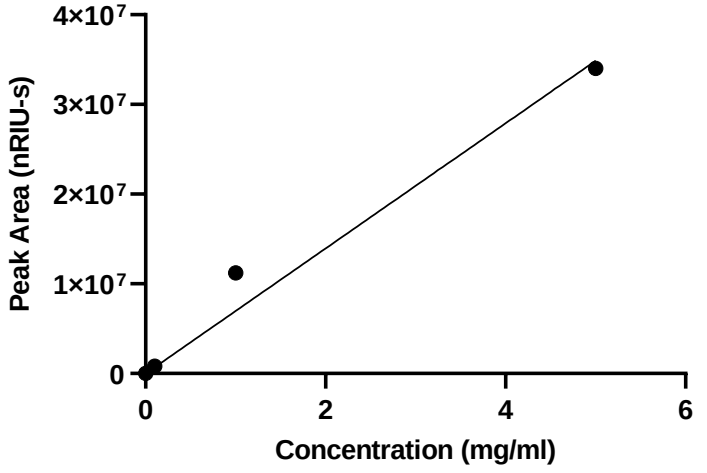

Figure 4: Standard curve of propionic acid (Y = 6975402*X + 0.000)

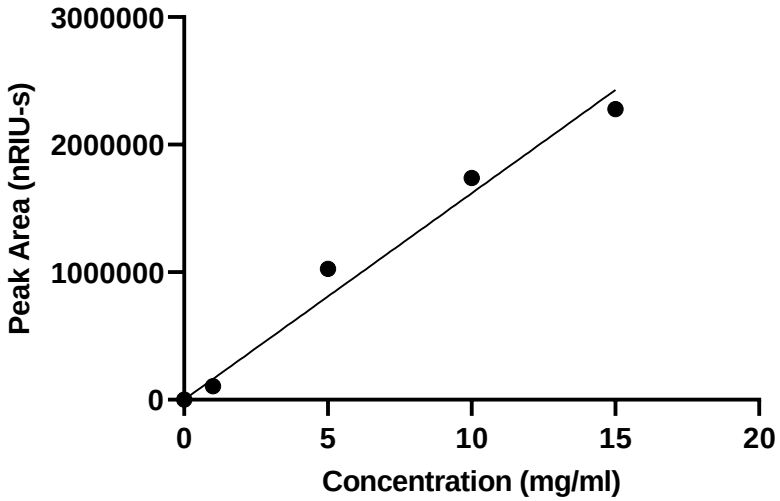

Figure 5: Standard curve of succinic acid (Y = 161861*X + 0.000)

Appendix III: Primer used for PCR (Sigma Company)

Forward Primer (Fd1)	5’ – AGTTTGATCGTGGCTCA
Reverse primer (rP2)	5’ – ACGGCTACCTTGTTACGACTT

Appendix IV: Photographs of Laboratory work

Figure 1: Sample preparation for MFC operation

Figure 2: Dual chambered MFC operation at Biofuel lab of CDBT

Figure 3: Performing sample digestion for determination of phosphorus and trace elements

Figure 4: Colour observed after digestion during COD determination

Figure 5: Carrying out distillation during ammoniacal- nitrogen determination

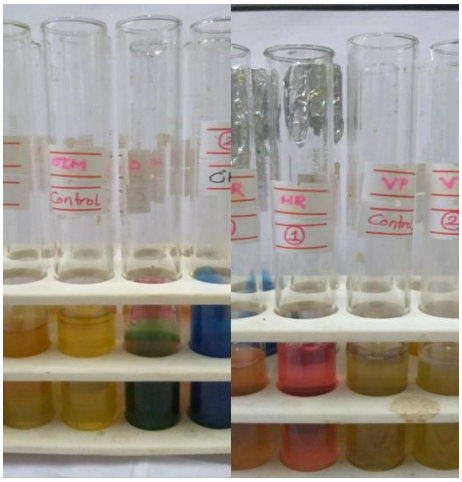

Figure 6: Biochemical tests performed for isolates

Figure 7: Operation of fabricated microbial fuel cell (FMFC)

Appendix V: Publication

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Characterization of Ethanol Producing Yeasts for their Efficiency in Ethanol Production, Salt Tolerance, and Utilization of Glucose and Xylose

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Abstract

Yeast is the mainstay in ethanol production industry. Search for efficient salt tolerant as well as hexose and pentose utilizing yeast strains is important in fermentation industry. In this regard, 12 yeast strains, *viz.*, CDBT1-12, were isolated from various sources and characterized. Molecular characterization of the yeast strains was done by sequencing their D1D2 region of 26S rRNA gene. Out of 12, 10 were found to be *Saccharomyces cerevisiae*, 1 was *Wikerhamomyces anomalous* (CDBT7), and the other was *Cyberlindnera fabianii* (CDBT8). All of the strains were found to be good ethanol producers. CDBT2 was found to have tolerance for high salt (up to 15%) and ethanol (up to 16%) concentrations. CDBT7 was both salt tolerant (up to 15%) as well as utilizes glucose and xylose without compromising on ethanol production efficiency. CDBT2's ethanol production efficiency was further enhanced by application of low voltage. Under such conditions alcohol dehydrogenase (ADH1) and pyruvate decarboxylase (PDC1) mRNA levels were increased by 2.78 ± 0.80 and 1.12 ± 0.37 fold, respectively, in CDBT2. This observation is novel, it has not been reported previously.

Keywords: Yeast, Molecular Characterization, Alcohol Dehydrogenase, Pyruvate Decarboxylase, External Voltage.

Introduction

Yeast strains are the common dwellers of most of nutrient rich media/sources such as fruits, tree bark, soils etc. [1]. They form one of

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