Performance of Magnetic Iron Nanoparticle Decorated Electrodes in Single Chambered MEC fed with Combined Leachate and Dairy Industry Wastewater

A Project Submitted to the Central University of Punjab

For the award of

Master of Science

In

Environment Science and Technology

By

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June, 2017

DECLARATION

I declare that the project entitled “Performance of Magnetic Iron Nanoparticle Decorated Electrodes in Single Chambered MEC fed with Combined Leachate and Dairy Industry Wastewater” has been prepared by me under the guidance of Dr. Yogalakshmi K N, Assistant Professor, Centre for Environment Science and Technology, School of Environment and Earth Sciences, Central University of Punjab. No part of this project has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

I certify that DEEPIKA has prepared her project entitled “Performance of Magnetic Iron Nanoparticle Decorated Electrodes in Single Chambered MEC fed with Combined Leachate and Dairy Industry Wastewater” for the award of M.Sc. Degree of the Central University of Punjab, under my guidance. She has carried out this work at the Centre for Environment Science and Technology, School of Environment and Earth Sciences, and the Central University of Punjab.

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ABSTRACT

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Key words	: Microbial Electrolysis Cell, Membrane-less MEC, Dairy industry wastewater, Leachate, Magnetic Iron oxide nanoparticles, nanoparticle decorated electrodes

Abstract

Increased human activity and consumption of natural energy resources have led to decline in the stock of fossil fuels. The current technologies used for energy generation are not environment friendly. Microbial electrolysis cell (MEC) represents a new approach for harnessing the energy contained in the organic matter of wastewater. It is a type of bioelectrochemical systems in which chemical energy stored in organic compounds are converted to biogas such as hydrogen through biocatalytic oxidation by microorganisms. But it still suffers from the lack of efficiency in terms of hydrogen production and current generation. Previous studies have demonstrated that the electrodes coated with nanoparticles such as Fe, Au, Pd, and Ni nanoparticles have the potential to enhance energy recovery in MEC. Hence, the present study aims to use single chambered membrane-less microbial electrolysis cell with magnetic iron nanoparticle coated electrodes for treating combined leachate and dairy industry wastewater. The performance of the MEC was assessed through COD removal, current and biogas generation at an applied voltage of 0.8 V and HRT of 48 hours. Results demonstrated that the maximum current density achieved by nanoparticles decorated electrodes was 3.86 times higher than

generated by plain electrodes. The highest COD removal efficiency of 96.5% was achieved at OLR equal to 17.14 gCOD/L/d. The maximum coulombic efficiency of 155% represents the conversion of maximum chemical energy stored in the combined wastewater into electrical energy. The hydrogen production rate of 3.192 L/L/d was achieved in this study. The results shows that magnetic iron nanoparticle coated electrodes enhance the current generation and COD removal in single chambered MEC operated with combined leachate and dairy wastewater treatment.

Deepika Dr.Yogalakshmi K.N.

ACKNOWLEDGEMENT

It is a great pleasure for me to acknowledge the kind of help and guidance received by me during my research work. First of all, I would like to offer my humble thanks to Almighty for his grace, kindness and blessings that gave me opportunity, determination and motivation during my research work.

I would like to express my sincere gratitude to my supervisor, Dr. Yogalakshmi K N, Assistant Professor, Central University of Punjab for the continuous support during my M.Sc. study and related research, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this dissertation.

I would like to give my sincere thanks to Prof. R.K. Kohli, Vice-Chancellor of the university, Prof. P. Ramarao, Dean of academic affairs. Without their constant help, support and encouragement, this dissertation would not have been possible. I would also like to thanks, Dean and CoC Prof. V. K. Garg, Dr. Dhanya M S, Dr. Puneeta Pandey, and Dr. Sunil Mittal of Centre for Environment Science and Technology for pleasant atmosphere of knowledge. I would like to extend my humble thanks to the Central University of Punjab, the staff of Computer lab and library for providing me all kind of materials and dealing with all matters of the concern kindly. I am very thankful to the officials of Verka Milk Plant, for providing me with dairy industry effluent for my research work.

I got immense pleasure to express my thanks to all my friends Gurvir Kaur, Kamaljeet Kaur, Tejinder Singh, Fahreen Fazal, Bindu Bala and my seniors Jaskiran Kaur, Gini Rani, Anamika Das, Jatinder Randhawa and Vijay Jaswal for always supporting and believing in me with enormous affection and valuable suggestions.

Last but not the least, I will never forget to pay a deep gratitude to my parents, and thank my adorable brothers Nishant and Vishal, and special thanks to Minesh Kumar Srivastava for supporting me spiritually throughout writing this dissertation and my life in general.

Deepika

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LIST OF ABBREVIATIONS

Sr. No.	Full form	Abbreviation
1.	Activated Carbon Fiber Felt	ACFF
2.	American Public Health Association	APHA
3.	Anion Exchange Membrane	AEM
4.	Bio Chemical Oxygen Demand	BOD
5.	Bio Electrochemical System	BES
6.	Bio-Electrochemically Assisted Microbial Reactor	BEMAR
7.	Carbon Nanotubes	CNTs
8.	Cation Exchange Membrane	CEM
9.	Chemical Oxygen Demand	COD
10.	Hydraulic Retention Time	HRT
11.	Microbial Desalination Cell	MDC
12.	Microbial Electrochemical Technology	MET
13.	Microbial Electrodialysis Cell	MEDC
14.	Microbial Electrolysis Cell	MEC
15.	Microbial Fuel Cell	MFC
16.	Microbial Solar Cells	MSC
17.	Million Litres per Day	MLD
18.	Organic Loading Rate	OLR
19.	Plant Microbial Fuel Cell	PMFC
20.	Proton Exchange Membrane	PEM
21.	Terminal Electron Acceptor	TEA
22.	Total Dissolved Solids	TDS
23.	Total Solids	TS
24.	Total Suspended Solids	TSS
25.	Volatile Fatty Acids	VFA

CHAPTER I

INTRODUCTION

1.0 General

Energy resources are broadly classified into three categories: non-renewable, renewable and nuclear energy sources (Akdeniz *et al.,* 2002). Fossil fuels come under non- renewable energy resources whose depletion has led to world energy crisis because in recent years, global energy consumption has been repidlyincreased. Moreover, consumption of fossil fuel also results in carbon dioxide emission to the environment, leading to global warming and climate change (Reddy *et al.,* 2007). A sustainable society entails reduction of pollution and fossil fuel consumption. Henceforth, there is an urgent need for alternate energy sources that should be clean, reusable and economical.

Industrial operation produces huge volume of wastewater every year. Many treatment processes have been developed for removal of inorganic and organic pollutants of wastewater, but these technologies are very expensive and energy consuming (Oh *et al.,* 2010). Activated sludge process is a classical and most widely used technology for biological wastewater treatment. It involves aerobic micro-organism that metabolizes the organic matter into carbon dioxide and water in the presence of oxygen (Logan, 2008). However, these systems have limited use in recent times due to high energy cost, high sludge production and lack of efficiency in nutrient, heavy metal and other complex pollutant removal (Kim *et al.,* 2007). According to Goldstein and Smith, (2002) treatment of one cubic meter of wastewater requires 0.349 kWh energy accounting for 21 billion kWh of electricity consumption per year in USA.

The organic content of wastewater contains more internal energy than the amount of energy required to treat the wastewater. Energy potential of organic matter present in wastewater can exceed by 10 times the electricity used to treat it and it can meet up to 12% of national electricity demand (https://bcwa.org/.../Renewable-Energy-Recovery-Opportunities-fromDomestic-Waste). Likewise, the power density of industrial wastewater was reported to be in the range of 4 to 15 W/m^3 (Cheng *et al.,* 2006). Hence, efficient tapping of the energy by a suitable

technology would minimize the requirement of the energy generated from fossil fuels.

The search of a new, alternative, more promising, sustainable and eco-friendly technology led to the development of bio-electrochemical system (BES). They are unique systems with ability to convert the chemical energy of organic matter present in wastewater and lignocellulosic biomass into electricity or hydrogen in microbial fuel cells (MFCs) or microbial electrolysis cells (MECs), respectively (Pant *et al.,* 2012). MECs are analogous to microbial fuel cells (MFCs) expect for a minor difference. MFCs produce electric current from the microbial decomposition of organic matter while MECs generate biogas from organic matters. The MEC is based on modifying a microbial fuel cell (MFC) in two ways: adding a small amount of applied voltage (>0.2 V) to that produced by bacteria at the anode and not using any oxygen at the cathode. For any reaction to occur spontaneously, the Gibbs free energy of the reaction must be negative but in MEC the conversion of organic matter to hydrogen yields positive Gibbs free energy. So, the reaction is not spontaneous and requires external energy to be supplied for hydrogen production (Logan *et al.,* 2008). The addition of the applied voltage makes it possible to produce pure hydrogen gas at the cathode. Moreover, the MEC system is operated in completely anaerobic reactor (Logan, 2008). As mentioned earlier, MECs serves multiple purpose of energy generation, wastewater remediation and production of various value added compounds, it outperforms fuel cells. Hence unlike fuel cells which uses different chemicals (electrolytes) to produce electric current, MEC utilises wide varities of substrates such as marine sediments, anaerobically digested sludge, food industry wastewater, leachate, municipal and domestic wastewaters along with micro-organisms as catalyst for energy generation (Min *et al.,* 2005). Bacterial species such as *Geobacter, Shewanella, Pseudomonas, Clostridium* and many more are used in MECs to oxidize the substrates e.g. acetate, ethanol, lactate, butyrate (Niessen *et al.,* 2006).

Most research on MECs still stays in laboratory, because it has some limitations such as low power output, high cost, and less satisfactory wastewater treatment effects. There are various factors that influence the performance of microbial electrolysis cell such as high internal and external resistance, structure of the MEC, properties of substrates, electrode materials and proton exchange

membrane performance (Zho *et al.,* 2015). But in recent years many studies have been conducted to improve the efficiency of microbial electrolysis cell, to enhance their energy output and reactor development.

The key feature of microbial electrolysis cell is anode process in which microbes catalyze electron transfer by decomposition of organic matter into CO_2, electrons and protons. Enhancing the electron transfer from microbes to the anode is a critical step for improving the efficiency of the system, which in turn depends on the performance of electrochemically active microbes and the properties of the anode. In recent years, research efforts have been made on the modification of anode surface to enhance the anodic performance (Park and Zeikus, 2003; Niessen *et al.,* 2004; Cheng and Logan, 2006; Crittenden *et al.,* 2006). Nanoparticles have received much attention from researcher due to their unique physical, chemical and electrical properties which facilitate the interaction between bacteria and electrode surfaces (Liu, 2006). Previous studies have demonstrated that electrodes decorated with nanoparticles (Au, Pd, Fe and carbon nanotubes) showed enhanced efficiency of MEC in terms of current generation, COD removal and biogas production (Qiao *et al.,* 2008; Quan *et al.,* 2005).

The present study will focus on energy generation and treatment of combined leachate and dairy industry wastewater in MEC with magnetic iron nanoparticle decorated electrodes. . As India is a largest producer of milk and dairy products in the world with annual milk production crossing 85 million tonnes in the year 2002 (Ramasamy *et al.,* 2004). Some 200 million tonnes of wastewaters are generated annually from the Indian dairy industry (Dawood *et al.,* 2011). Dairy industry wastewater is a good source of organic material as it contains carbohydrates, proteins and fats originating from milk (Vidal *et al.,* 2011). Hence, it is a good feedstock for MEC for its treatment as well as energy generation.

Likewise, leachate are liquid discharges from landfills. It is a strong wastewater that contains high concentrations of organic matter and inorganic salts. It also might be considered a good feedstock for MECs because of its relatively high conductivity and buffering capacity (Zhang *et al.,* 2008). But most of the studies on the landfill leachate could not show acceptable results due to the large portion of poorly biodegradable organics in the leachate's COD. Henceforth, in present

study, a step is taken for simultaneous energy recovery and treatment of combined leachate and dairy industry wastewater.

1.1 Need for the study

Increased human activity and intensive use of natural resources for energy generation have led to decrease in the stock of fossil fuel. The current methods of energy production are not so compatible with the environment. Use of fossil fuels possess serious threats to the environment due to the production of greenhouse gases resulting global warming, climate change. So, there is a need for implementation of new methods for energy production by using natural and renewable carbon resources. In recent times, bioenergy has been focused more to combat the problem of carbon dioxide emission in addition to search of an alternative cleaner fuel. Moreover, energy generation from wastewater and waste materials are also extensively studied, as a step to manage the humongous generation of wastewater and various pollution problems associated with them. Bio-electrochemical systems have been developed as a solution to these problems.

Leachate a highly toxic but organic rich discharge generated from the landfills or waste dumping and processing sites. High COD, NH_4-N and VFAs. High COD and NH_4-N content and low BOD/COD ratio makes biological treatment of landfill leachate very difficult. Co-treatment of landfill leachate with other wastewater can be an alternative option to unlock the organic richness into energy using Bio-electrochemical systems.

Microbial electrolysis cell is a bioelectrochemical system that can convert the chemical energy stored in organic matter into electrical energy. This system produces energy and treats wastewater minimizing the effect on the environment. It is considered as a new green technology with the advantages of high efficiency, low energy consumption and production of cleaner fuel. Although MECs have shown better treatment efficiency and biogas production still it’s upscaling is limited due to various reasons. The performance of the MEC depends on the electrode, electrode material and the reactions involved within the fuel cell. Recent research has involved nanomaterial in the electrode material to improve the MEC performance. Nanoparticles have unique physical, electrical, and chemical

properties which facilitates the study of interactions between bacteria and electrode surfaces.

Henceforth, the objective of the present study is to focus on the energy recovery and treatment of combined leachate and dairy industry wastewater using magnetic iron oxide nanoparticle coated electrodes in single chambered membrane-less MEC.

1.2 Objective of the study

The scope of the present study is to investigate the performance of magnetic iron nanoparticle decorated electrodes in enhancing the efficiency of single chambered membrane less MEC operated with combined leachate and dairy industry wastewater. The efficiency was assessed through COD removal, current and biogas generation. The scope is achieved through the following objectives which include

- Collection and characterization of dairy industry wastewater.
- Preparation and characterization of simulated leachate
- To investigate the feasibility of MECs in treating the combined leachate and dairy industry wastewater.
- To assess the performance of magnetic iron nanoparticle coated electrodes in MEC operated with combined leachate and dairy industry wastewater in terms of COD removal, current and biohydogen generation.

CHAPTER II

LITERATURE REVIEW

2.0 Introduction

In last few years, bioelectrochemical systems have gained much attention of researchers for wastewater treatment and energy production. Among different types of bioelectrochemical systems, microbial electrolysis cell is investigated more due to its potential to generate energy as hydrogen (Rozendal *et al.,* 2007). It produces biohydrogen from organic matter present in wastewater. The technology was developed by Liu *et al.,* (2005). In MEC microbes oxidize organic matter to produce CO_2, electrons and protons. Microorganisms transfer electrons to the anode and protons remains in solution and then electrons travel through a wire and combine with protons at cathode and produce hydrogen. This occurs in completely anaerobic condition.

In recent years, MEC as a new source of renewable energy production has been extensively reviewed and various modifications and developments have been made in order to increase the efficiency of these systems. This section will review different electrode material (anode and cathode), separators or membranes used in MEC. Architectures, performance, microbial community, different types of substrates in MEC, mechanism of electron transfer, thermodynamics of hydrogen production, applications of MEC are also discussed in detail in later sections. Several challenges to scale up MEC technology such as cost of electrode materials, membranes, MEC configuration and syntrophic interactions among microorganisms are described in detail in following section.

2.1 Bioelectrochemical system (BES)

A device which converts chemical energy of fuel into electricity, is called fuel cell (Steele and Heinzel, 2001). But bioelectrochemical system (BES) is a device that employs biocatalyst for production of energy, instead of metals oxide catalyst that are used in fuel cell (Mokhtarian *et al.,* 2012).

2.2 Classification of BES

Based on their mode of application BES can be further sub-divided into: -

1. Microbial Fuel Cells (MFCs)
2. Microbial Electrolysis Cell (MECs): explained below in detail
3. Microbial Desalination Cells (MDCs)
4. Microbial Solar Cells (MSCs)
5. Enzymatic Fuel Cells (Pant *et al.,* 2012).

2.2.1 Microbial Fuel Cells

Potter was the first person to generate electricity in 1911 from living culture of *Escherichia coli* and *Saccharomyces* (Potter 1912). This technique was not so known until 1980s but later it was discovered that mediators can be used to enhance the efficiency of MFCs. Hydrogen was produced by the fermentation of glucose by *Clostridium butyricum,* but this was unreliable due to unstable nature of hydrogen production from the micro- organisms. Karube in 1976 proposed the current design concept of MFC and resolve the issue of hydrogen unstability. M.J. Allen and H. Peter in early 1980s build an understanding about the functioning of MFCs. Then Barnet Cohen created microbial half fuel cells and connected them in a series by which 37 volts energy was produced. The first microbial fuel cell that treated domestic wastewater and generated electricity was developed by Habermann and Pommer in 1991. In 1999, it was recognized that MFCs can be operated without mediators (Kim *et al.,* 1999). Liu et al. (2004) firstly initiated the use of MFC in wastewater treatment to reduce the cost of energy and demonstrated that domestic water can be used as a substrate in MFCs. MFCs consist of anode and cathode chambers that are separated by proton exchange membrane (Ghasemi, et al., 2012). In a MFC, micro-organisms oxidize or degrade the substrate and produce electrons and protons that provide energy for the cell in the form of ATP. The generated electrons are then conveyed to the terminal electron acceptor (TEA) which accepts the electrons and gets reduced. Further the protons moves to the cathode chamber through the proton exchange membrane (Rahimnejad *et al.,* 2011). Protons and electrons reacts in cathode chamber and forms water (Sharma and Li, 2010).

The typical reaction at the electrode is summarized in the following equations.

Anodic reaction

$$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \quad (2.1)$$

Cathodic reaction

$$2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O \quad (2.2)$$

2.2.2 Microbial Desalination Cells

Oceans, which contains 97% of earth's water is not used for drinking purpose due to its salt content. Finding a feasible technique to remove the salt and convert it into potable water will fulfil the need for freshwater in large. Microbial desalination cell is a technique that can remove salts from water and make it potable. This approach can be used in areas where seawater is available and drinking water sources are limited (Khawaji *et al.,* 2008). MDC is an extension of microbial fuel cell, which are designed for desalination of water and electricity production simultaneously (Saeed *et al.,* 2015).

It consists of three chamber, anodic chamber, cathodic chamber, and an additional desalination chamber in the middle of both chamber by inserting anion exchange membrane (AEM) next to the anodic chamber and cation exchange membrane (CEM) before the cathodic chamber (Luo *et al.,* 2012). Organic matter degradation occurs at anode with the help of bacteria that releases CO_2, hydrogen ions and electrons. The electrons flow through the electrical circuit towards the cathode, and a current across the cell is established. This maintains a potential gradient across the cell and the ions present in salt water in desalination chamber move in cathode and anode chamber through the AEM and CEM. The anions such as Cl^- migrates from the desalination chamber into anode chamber through AEM, and cations such as Na^+ and Ca^{2+} move across the CEM into the cathode chamber. This process can remove 99% salinity from sea water and there is no external energy required for operating this system (Forrestal *et al.,* 2012).

2.2.3 Microbial Solar Cells

MSCs are another modified form of MFCs, which integrate photosynthetic and electrochemically active organisms to produce electricity or chemicals such as methane, hydrogen, ethanol and hydrogen peroxide (Steinbusch, 2010). In this system, photosynthetic organisms use sunlight and produce organic matter that is converted into electricity through MFCs (Strik, 2010). The basic principles of MSCs

are photosynthesis, transport of organic matter to the anode, oxidation of organic matter by micro-organisms and cathodic reduction of oxygen.

2.2.4 Enzymatic Fuel Cells

This system employs enzymes as catalysts for oxidation of fuels in anodic chamber or reduction of oxidants at cathodes to generate electrical power (Leech *et al.,* 2012).

2.2.5 Microbial Electrolysis Cells (MECs)

MECs are one of the BESs that are developed firstly by Liu *et al*. (2005) for hydrogen production from acetate and other fermentation end products by the process of electrohydrogenesis. It is just a modified form of MFCs. MECs are operated in completely anaerobic condition. In MECs, organic matter is converted into hydrogen, CO_2 and electrons by micro-organisms. These electrons move to the anode and then flow towards the cathode through the electrical circuit, in which power is supplied externally. Protons move from anode to cathode inside the cell where its reduction occurs and hydrogen is produced (Jeremiasse *et al.,* 2010). Figure 2.1 depicts the mechanism of hydrogen generation in MEC. Overall reaction in MEC is endothermic, but an external voltage (in the range of 0.4-0.7) is applied to drive hydrogen production reaction (Call & Logan, 2008). It is also known as Bio-electrochemically Assisted Microbial Reactor (BEMAR) (Ditzig *et al.,* 2007), and a Bio-catalyzed Electrolysis Cell (BEC) (Rozendal *et al.*, 2006).
The reactions that occur in the electrodes when acetate is used as a substrate in MECs is summarized in the following equations

Anode: -

$$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \quad (2.3)$$

Cathode: -

$$8H^+ + 8e^- \rightarrow 4H_2 \quad (2.4)$$

MECs has two main advantages over other hydrogen producing processes. One is that a great variety of organic substrates can be used in MECs as a fuel which are oxidized by microbes (Ren *et al.,* 2007). Secondly, non-fermentable substrates

can also be oxidized to CO_2 in MECs resulting in high conversion yields of 67-91% (Cheng and Logan, 2007).

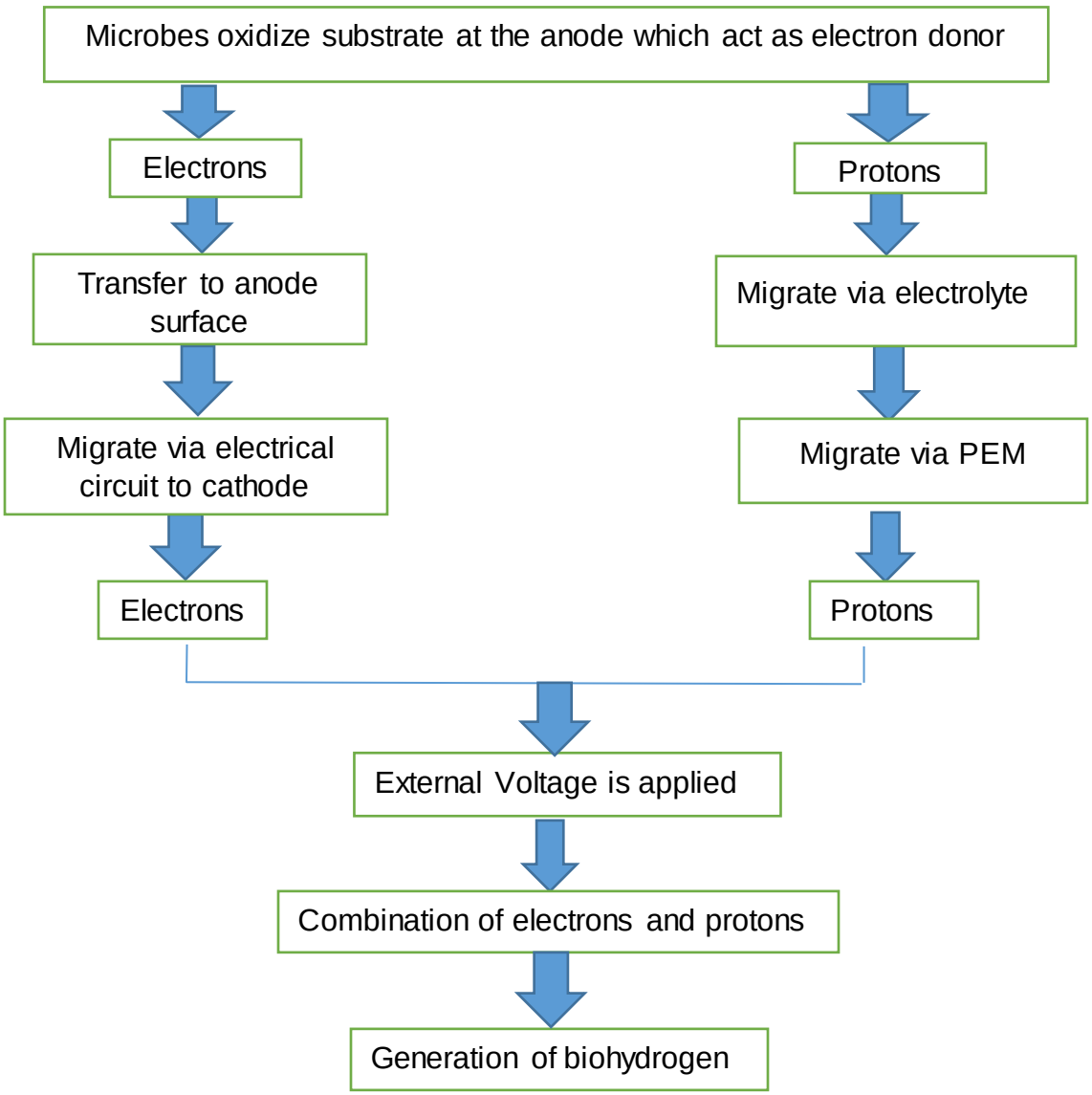


Figure 2.1 Schematic representation of process of hydrogen generation in MECs

MECs are analogous to MFCs except slight modification. MFC require some oxidants at cathode but MECs remain in completely anaerobic condition and produce hydrogen at cathode. The second factor is that in MFC we do not require any external supply of energy but in MEC to make negative potential at cathode we apply external voltage which is enough to generate hydrogen (Lee *et al.*, 2009). Two redox reactions occur in MECs. They include (1) redox reaction is oxidation of substrate by anode respiring bacteria and the transfer of electron to

the anode and (2) reduction that occurs on cathode by transfer of electron through external circuit that react with H^+ ions and produce hydrogen (Lee *et al.,* 2009).

2.3 Components of Microbial Electrochemical Technologies (METs)

In this portion several portions of Microbial electrochemical technologies including anode, cathode and separator, which play important role in METs design, are discussed.

2.3.1 Anode

Anode is an important component of METs. All the conditions which are required for degradation of organic matter are given in this chamber. It is maintained in anaerobic condition, and contains substrate, mediator (optional), bacteria, and the electrode (Rahimnejad *et al.,* 2015). In this chamber, bacteria function as catalysts and lowers the activation energy of anodic reaction (Virdis *et al.,* 2011). Anodic material is most effective factor that influence the performance of METs. An ideal electrode must consist features such as good electrical conductivity, low resistance, biocompatibility, chemical stability, anti-corrosion, large surface area and mechanical strength (Logan *et al.,* 2006). Different anode materials are used to improve the efficiency of system. The most commonly used electrode materials are made up of carbon material such as graphite fibre brush, carbon cloth, graphite rod, carbon paper, reticulated vitreous carbon and carbon felt. Carbon materials are commonly used because they show stability in microbial cultures, high electrical conductivity, and large surface area (Logan *et al.,* 2006). Metal electrodes consisting of non-corrosive stainless steel mesh can also be used, except copper because this is toxic to bacteria (Tanisho *et al.,* 1989). Many modifications of anode electrode are done to improve the performance of METs. Qiao et al., (2007) showed that carbon nanotubes (CNTs) can improve the electron transfer feasibility and electrode surface area. Electrocatalytic material such as polyanilins (PANs) / Pt composites, PAN / titanium dioxide and CNT / PANs composites are also used as anode electrode material (Watanabe, 2008).

Table 2.1 summarizes the different anode electrode material used in microbial electrochemical technologies with different substrates. It is clear from the table that Pt/PAN composite electrode which showed higher power density (6000 mW/m^2) with sewage sludge as a substrate, could be an excellent anode in METs (Franks

and Nevin, 2010). Graphite/PTFE composite electrode showed a high power density (760 mW/m^2) than simple graphite electrode (16 mW/m^2) with glucose as a substrate. Other anode electrode material which can be used in METs are Teflon treated carbon fibre paper, non-wet-prof carbon paper, graphite with Mn^{4+} etc.

Table 2.1 Different anode material used in Microbial electrochemical technologies with different substrates

S.No.	Substrates	Anode Material	Power density (mW/m^2)	References
1.	Glucose	Carbon paper	40.3 ± 3.9	Jung and Regan, (2007)
2.	Glucose	Graphite	16	Rahimnejad *et al.,* (2011)
3.	Marine sediment	Noncorroding graphite	25.4 - 26.6	Gil *et al.,* (2003)
4.	Sewage sludge	Graphite with Mn^{4+}	91	Park and Zeikus, (2002)
5.	Sewage sludge	Graphite with neutral red	152	Park and Zeikus, (2002)
6.	Sewage sludge	Pt/PAN composite electrode	6000	Franks and Nevin, (2010)
7.	Glucose	Graphite/PTFE composite electrode	760	Rahimnejad *et al.,* (2011)
8.	Glucose	Teflon treated carbon fibre paper	15.2	Rahimnejad *et al.,* (2011)
9.	Cellulose	Non-wet-prof carbon paper	188	Logan andRegan, (2006)
10.	Glucose	Graphite plates	283	Potter, (1911)

2.3.2 Different Cathode Material and Catalyst

Cathode is a very important part in microbial electrolysis cell because the production of hydrogen and other chemical compounds occurs in this chamber. In recent times, researchers are focussing more on the development of low cost

cathode or cathode catalyst for practical implication of MEC for hydrogen production. The base material of cathode and the catalyst on it play an important role in MEC. The hydrogen evolution reaction mainly takes place on cathode and this reaction is very slow when we use only plain carbon electrodes as high overpotential is required for hydrogen production. To minimize this over potential catalyst are used in MECs**.** The performance of different cathode material in METs are discussed in Table 2.2**.**

2.3.2.1 Platinum as catalyst in cathode

Commonly used catalyst for cathode is platinum because it has low overpotential (0.05 V at 15 Am^{-2}) for hydrogen evolution reaction (Jeremiasse *et al.,* 2009). Some commercially available platinum catalyzed electrodes are ETEK (USA), Magneto Special Anodes (The Netherlands), Alfa Aesar (Germany) but it can also be prepared in laboratory by mixing commercially available platinum with some chemical binder such as 5% Nafion solution or 2% PTFE solution (Cheng *et al.,* 2006). But there are many disadvantages of using platinum as a catalyst such as its high cost and negative environmental impacts by mining/extraction (Freguia *et al.,* 2007). It can also be poisonous by combining with chemicals such as sulfides which is commonly found in wastewater**.**

2.3.2.2 Microbial Biocathode

This concept was developed in 1960s in which microbes act as a biocatalyst (Lewis, 1966). The microbes which helps in producing hydrogen are present in environments in large variety (Schwartz and Friedrich, 2006) and these microbes contains hydrogenases enzymes which catalyze the following reaction

$$2H^+ + 2e^- \leftrightarrow H_2$$

On carbon cloth purified hydrogenases enzyme have been used as a catalyst for hydrogen production (Vignais, Billoud, & Meyer, 2001). They are more beneficial because they decrease the need of expensive catalysts and mediators such as platinum (Watanabe, 2008)

2.3.2.3 Stainless Steel as Cathode

Due to the high cost of platinum some other alternatives are find out in many researches and it came to know that first row transition metals can act as catalysts

on cathode. The main characteristics of these metals are their stability, abundance in nature, low cost and less toxic to living organisms. The most commonly used metals are nickel and stainless steel alloys because of their low cost, availability, low overpotentials and stability in highly alkaline solutions (Selembo,*et al.,* 2009).

2.3.2.4 Tungsten Carbide

Although it is not as good as platinum but it is also used as a catalyst for cathode because of its low price and it does not react with hydrogen sulphide and carbon mono-oxide present in wastewater (Harnisch *et al.,* 2009).

2.3.2.5 Nickel and Nickel Alloys

These are efficient, cost effective and stable cathodes that are developed by different research groups. These cathodes were developed by electrodepositing the NiMo and NiW on carbon-fibre-weaved cloth material and then these cathodes were observed for hydrogen production (Hu *et al.,* 2009). It is reported that NiMo and NiW increases the surface area of catalyst coating and decrease cathode overpotential (Navarro *et al.,* 2005).

2.3.2.6 Cathode coated with Nanoparticles

Nanomaterial are very stable in nature, structurally as well as electrical and chemical properties (Fan *et al.,* 2011). Palladium can be used as nanoparticles to improve the current generation in MECs because it is just like the platinum and has a very good catalytic property and high abundance (Kundu *et al.,* 2013). Nickel based nanoparticles can also be used as cathode catalyst in MECs (Mitov *et al.,* 2012). NiFe-, NiFeP and NiFeCoP nanoparticles were electrodeposited on the carbon felt and highest current production rate of 1.7 ± 0.1 $m^3 m^{-3}$ reactor liquid per day was observed with NiFeCoP/carbon felt electrode in the range of -0.60 and -0.75 V.

Table 2.2 Different cathode material used in METs with different hydrogen production rate

S. No.	Cathode material and catalyst	Cathode potential (V)	Hydrogen production rate m^3m^{-3} reactor liquid per day	Cathodic hydrogen recovery rH_2 cat (%)	References
1.	Carbon cloth with Pt catalyst	0.8	3.12 ± 0.02	96	Call and Logan, (2008)
2.	Titanium mesh with Pt catalyst	1	0.3	-	Rozendal *et al.,* (2007)
3.	Carbon cloth and mixing of black carbon with Pt catalyst	-	1.6	-	Selembo *et al.,* (2010)
4.	Biocathode	-0.7	0.63	49	Rozendal *et al.,* (2007)
5	Biocathode	0.8	0.03-0.04	17-21	Jeremiasse *et al.,* (2010)
6.	Stainless steel A286	0.9	1.50 ± 0.04	61 ± 3	Selembo *et al.,* (2010)
7.	Stainless Steel brush 304 with high nickel	0.6	1.7	84	Call *et al.,* (2009)
8.	Stainless steel A286 with nickel oxides	0.6	0.76 ± 0.16	52 ± 4	Selembo *et al.,* (2010)
9.	Stainless steel with bicarbonate buffer	0.9	1.40 ± 0.13	-	Ambler *et al.,* (2011)
10.	NiMo on carbon fibre-weaved cloth	0.6	2	86	Hu *et al.,* (2009)
11.	Ni 210 (60 mg Ni in 267 µL Nafion on carbon cloth	0.6	1.3 ± 0.3	79 ±10	Selembo *et al.,* (2010)
12.	Pd nanoparticles on carbon cloth	0.6	2.6 ± 0.5	46.4 ± 8.5	Huang *et al.,*(2011)
13.	MoS_2 on carbon cloth	-	-		Rozendal *et al.,* (2008)

2.3.3 Proton exchange membranes (PEM)

Proton exchange membrane are used to separate the anode and cathode chambers. They are the important component in microbial electrochemical technologies and influence the performance of bioelectrochemical systems (Rahimnejad *et al.,* 2014). The performances of PEMs can be assessed by comparing their cost, ion and mass permeability. An ideal membrane should increase both the power density and coulombic efficiency of the system (Kim *et al.,* 2007). A good quality PEM should have some characteristics such as, it should conduct only protons, should have mechanical strength and it should be static in acidic environment at high temperature for long period of time.

2.3.3.1 Cation Exchange Membranes (CEMs)

Cation exchange membranes transport produced protons to cathode chamber in MECs. In this type of membranes groups with negative charges are attached to the backbone of membranes which only allow the cations to pass through them. Hence, they are known as proton exchange membranes.

2.3.3.1.1 Nafion

In microbial electrochemical technologies, Nafion membranes are most commonly used proton exchange membrane which is developed by Du Pont Company. It is made up of polytetraflurothylene, also known as Teflon, that consists of a fluorocarbon backbone and hydrophilic sulphonate group attached on to this backbone. The sulphonate group provides the high level of cation conductivity and is less affected by chemical attack (Peighambardoust *et al.,* 2010). The maximum conductivity achieved by Nafion is 0.001 S/m (Vishnyakov, 2006).

Electrons produced in anode chamber is transported to cathode chamber through the external circuit while equal amount of protons should be transported to cathode chamber via membranes to maintain the electroneutrality (Rozendal *et al.,* 2008). However, practicaly some cations (Na^+, K^+, Ca^+, etc.) present in inorganic salts that are essential for microbial growth move faster than proton through the membrane. Due to this transport of cation in cathode chamber, the pH of the solution increases in cathode chamber and decreases in anode chamber which leads to internal resistance and inhibits microbial activity (Rozendal *et al.,* 2006). This is the main problems associated with Nafion in addition to other

disadvantages such as high cost, oxygen and substrate crossovers and biofouling (Li *et al.,* 2011).

2.3.3.1.2 Sulfonated poly-ether ether ketone (SPEEK)

A cation exchange membrane can be made up of sulfonated poly-ether ether ketone which is less costly. This membrane is made of thermally and mechanically stable polymer (Vona *et al.,* 2006). It is a very good alternative of Nafion as it has negative surface charge which attracts only cations and rejects the passage of anion (Zhao *et al.,* 2006).

2.3.3.1.3 Sulfonated Polystyrene-ethylene-butylene-polystyrene (SPSEBS)

Another low-cost cation exchange membrane is SPSEBS, which is synthesized by sulfonating polystyrene-ethylene-butylene-polystyrene tri-block polymers which is a thermoplastic elastomer consisting of styrene blocks dispersed in an ethylenebutylene matrix. The power density produced by PSEBS is 600 mWm^{-2} which is twice the power density produced by Nafion membrane (Ayyaru *et al.,* 2012). These membrane shows higher proton conductivity and water swelling as compared to Nafion (Mishra *et al.,* 2012).

Other proton exchange membranes that are commonly developed include perfluorinated ionomer, also known as Hyflon. It has shorter side chain and are more stable than Nafion (Ghielmi *et al.,* 2005). Another alternative of Nafion is Ultrex CMI-7000, which is more cost effective than Nafion (Rabaey *et al.,* 2004).

2.3.3.2 Anion Exchange Membrane

These are polymer electrolytes that are made up of positively charged cationic group bound covalently to a polymer backbone and conducts anionic group such as OH^- and Cl^- (Varcoe *et al.,* 2014). It is reported that AEMs functions better than CEMs due to diffusion of OH^- through the membrane, and it has lower membrane resistance, less fouling and cathode resistance caused by the transportation of cations (Piao *et al.,* 2013).

2.3.3.3 Composite Membranes

By combining different polymeric membrane and other organic or inorganic materials composite membranes are formed and it is proved that their

performance is better than the Nafion and other single proton exchange membrane (Ghasemi *et al.,* 2013).

2.3.3.4 Salt Bridge

It is simpler and less expensive as compare to ion exchange membranes. In this, ions are conducted between two chambers by salt bridge that consists a glass tube filled with electrolytes. Most commonly used electrolytes in salt bridge are KCl solution and phosphate buffer. To avoid the intermixing of fluids, agar is most commonly added in this. But salt bridge always results in low power density due to high internal resistance as compare to membranes (Min *et al.,* 2005).

2.3.3.5 Porous Membranes

These membranes enable the non-ion selective charge transfer. J-cloth, non-woven cloth, glass fibre, earthen pot, ceramic, biodegradable bag and natural rubber are few examples of the porous membrane. Porous membrane can be categorised into two categories depending upon their pore sizes. They can be microfiltration and ultrafiltration membrane (Daud *et al.,* 2015).

Table 2.3 Membranes used in microbial electrochemical technologies

S.No.	Membranes	Power density (mW/m^2)	References
1.	Nafion	106.7	Ghasemi *et al.,* (2013)
2.	SPSEBS	600	Ayyaru *et al.,* (2012)
3.	Anion exchange membrane AMI-7001	610	Kim *et al.,* (2007)
4.	Carbon nanofiber (CNF)/Nafion composite membrane	57.6	Ghasemi *et al.,* (2011)
5.	SPEEK/Polyethersulfone composite membrane	150	Lim *et al.,* (2012)
6.	J-cloth	790	Zhang *et al.,* (2009)
7.	Non-woven fabric filter	97	Choi *et al.,* (2013)
8.	Earthen pot with stainless steel mesh cathode	70.48 Wm^{-3}	Behera *et al.,* (2010)

2.3.4 Tubing and Gas Collection Systems

It is mainly used in MECs for collection of hydrogen gas. Diffusion of small amount of hydrogen gas can cause a major problem in laboratory tests because hydrogen is a small molecule and can easily permeate through the connections and tubing. So, it is very important that the reactor design should be gastight with proper seals. For Teflon, the hydrogen diffusivities is 10^{-12} cm^2/s and for Viton it is 10^{-13} cm^2/s. Hydrogen can also diffuse through the membrane from one chamber to another and this hydrogen loss is dependent on the concentration of hydrogen across the membrane (Logan, 2008).

2.4 Reactor Configurations of MECs

The various MEC reactors that have been used in MECs for high hydrogen gas production and wastewater treatment are summarized in this section.

2.4.1 Double Chamber MECs Reactor

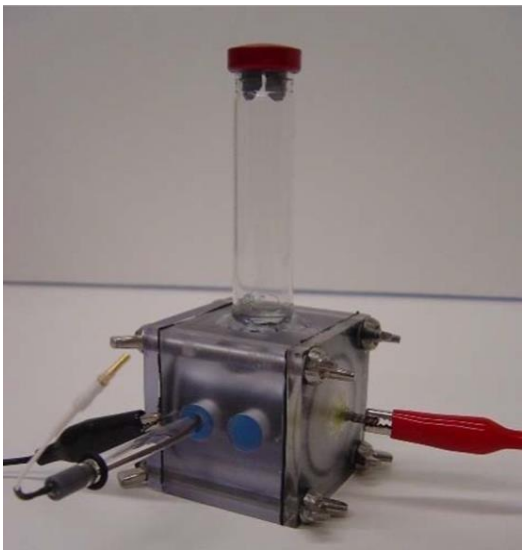
The first double chamber MEC system for hydrogen production was developed by Liu *et al.,* (2005). In this system they used two-bottle reactor that were separated by cation exchange membrane held in a tube between the reactors. The membrane is used in between two electrodes to improve the purity of hydrogen gas and to prevent consumption of hydrogen by microbes. Rozendal *et al.,* (2006) tested a cylindrical two chamber MEC system that was made up of two large disc-shaped anode and cathode chambers separated by a cation exchange membrane.

2.4.2 Single Chamber MECs

To reduce the cost of reactor and simplify reactor design single chamber MECs were developed. MECs are operated in completely anaerobic condition so there is no chance of introduction of oxygen at anode. So, the need for membrane between two chambers is not necessary and removal of the membrane will not cause any negative impact on MEC efficiency. To reduce the potential losses associated with membrane and increase the energy recovery of this process, single chamber MECs without a membrane was developed (Call and Logan, 2011). Figure 2.2 represents the single chamber MEC system with gas collection tube.

In this system, the hydrogen production rate was 0.53 $m^3m^{-3}d^{-1}$ based on liquid volume at the applied voltage of 0.6 V. But this hydrogen production rate is low. So, there is a need to improve this reactor design by increasing the electrode surface area and operating the reactor in continuous flow mode (Hu *et al.,* 2008).

Another problem associated with this reactor design is microbial hydrogen losses to methanogens, they will compete with electrochemically active bacteria for both substrate and product (Zhang and Angelidaki, 2014).



Source: Kadier *et al.,* (2016)

Figure 2.2 Single chamber MEC with glass collection tube

2.4.3 Continuous Flow MECs

The membrane less MEC operated in continuous flow mode consists of a gas phase cathode. This type of MEC was developed by Tartakovsky et al. (2009). In this reactor design anode was made up of carbon felt and cathode was a gas diffusion cathode. These two chambers were separated by using J-cloth. The maximum hydrogen production rate was 6.3 $LL^{-1}d^{-1}$ which is higher than the 0.53 $m^3m^{-3}d^{-1}$ value achieved in batch mode (Hu *et al.,* 2008).

In the gas collection chamber methane concentration remained below 2.1%, despite the presence of membrane. Then a membrane less single chamber up-flow biocatalyzed electrolysis reactor was designed (Wang *et al.,* 2012). It reduced the toxic chemicals in continuous feed to less or nontoxic chemicals in its cathode

zone. In this reactor design the influent enters through the cathode at the bottom to the anode zone at the top and this can convert nitrobenzene into aniline by applying external voltage of 0.5 V. But the problem with this reactor design is that we need a pump for wastewater treatment (Zhou *et al.,* 2013).

2.4.4 Integrated MEC Systems

To explore the scalability of MECs, a continuous flow MEC system was constructed with multiple electrodes. The maximum hydrogen production rate 0.53 $LL^{-1}d^{-1}$ was obtained with 144% energy efficiency relative to its electric energy input (Rader and Logan, 2010).

2.4.4.1 An MEC-MFC coupled System for biohydrogen production

It is possible to use MFC-MEC coupled system for hydrogen production. In this system MFC-MEC system are integrated in which MEC produced hydrogen and MFC produced electric current and this electric current is supplied to MEC for hydrogen production as MEC requires supply of external voltage for hydrogen production (Sun *et al.,* 2008).

2.4.4.2 Dye-sensitized solar cell (DSSC)-powered MEC

In this system, a dye-sensitized solar cell was used to provide an additional reductive power from light to an MEC. DSSC produced an open circuit voltage of 0.6 V which was supplied to the MEC and 400 mmol hydrogen was recovered from whole system within 5 hours with cathode recovery efficiency of 78% (Ajayi *et al.,* 2009).

2.4.4.3 Microbial reverse-electrodialysis electrolysis cells (MRECs)

This unique system was developed hydrogen production by combining a small reverse electrodialysis stack with microbial electrolysis cell. In MRECs, microbial oxidation of organic matter occurs at the anode. The salinity gradient between seawater and river water provides energy for hydrogen production and thus there is no need to provide any external energy (Kim and Logan, 2011). Other MEC integrated systems include microbial electrodialysis cell (MEDC), microbial saline-wastewater electrolysis cell (MSC), and microbial electrolysis desalination and chemical production cell (MEDCC).

2.5 Substrates used in MECs

Energy can be recovered in MECs in the form of hydrogen from various substrates such as acetate, glucose, butyric acid, lactic acid, cellulose and different type of wastewater. Some of the substrates and their influence on MECs are discussed below. In Table 2.4, different types of substrates used in MEC with different hydrogen production rate are listed.

2.5.1 Acetate

Acetate is most commonly used substrates in MECs because it cannot be directly converted into hydrogen through the process of dark fermentation (Saski *et al.,* 2012). It is a simple substrate that is used as a carbon source to induce electroactive bacteria (Bond *et al.,* 2002). It is used due to its inertness towards alternative microbial conversion such as fermentation and methanogenesis, at room temperature (Aelterman, 2009). Acetate is also an end product of various metabolic pathways for carbon sources (Biffinger *et al.,* 2008).

2.5.2 Lignocellulosic Biomass

Most abundant sources of lignocellulosic biomass are agricultural residues which are abundant and renewable. So, their abundance and renewable nature make them a cost-effective feedstock in MECs for energy production (Huang *et al.,* 2008). They cannot be directly converted by bacteria so pre-treatment is required to convert them into monosaccharides (Logan, 2004).

2.5.3 Brewery Wastewater

Brewery wastewater also can be used as a substrate in MECs due to its low strength, food derived nature of organic matter and it has low concentrations of inhibitory substances. The concentrations of brewery wastewater are in the range of 3000-5000 mg/L COD which is 10 times higher than the domestic wastewater (Vijayaraghav *et al.,* 2006).

2.5.4 Glucose

Glucose can also be used as a substrate in MECs to produce hydrogen by fermentation at mesophilic temperature but it is reported that the glucose can also produce hydrogen at lower temperature using microbial electrolysis cells. By using

single chamber MECs a yield of about 6-mol H_2/mole glucose was obtained from glucose at 4℃, and at a rate of 0.25 ± 0.03 → 0.37 ± 0.04 m^3 H_2/m^3 day (Lu *et al.,* 2012).

2.5.5 Domestic wastewater

Hydrogen production was examined using domestic wastewater as a substrate in MEC and it was observed that domestic wastewater with COD >350 mg/L showed maximum coulombic efficiency of 26% and hydrogen recovery of 42% (Ditzig *et al.,* 2007).

2.5.6 Starch Processing Wastewater

It contains high amount of carbohydrates (2300-3500 mg/L), sugars (0.65-1.18%), proteins (0.12-0.15%) and starch (1500-2600 mg/L), which makes them energy rich resource and can be used in MEC for energy recovery (Jin *et al.,* 1998). The COD of starch processing wastewater generally remains in the range of 6000-10000 mg/L (Jin *et al.,* 2002).

2.5.7 Swine Wastewater

Swine wastewater also provides a good source of organic matter for hydrogen production by using MEC. Hydrogen can be produced in MEC by the process of electrohydrogenesis. By using single chamber MEC with graphite fibre brush anode hydrogen production rate was 0.9-1 m^3 H_2/m^3/day using swine wastewater (Kadier *et al.,* 2014).

2.5.8 Food processing Wastewater

Food processing wastewater are nontoxic in nature and organic matter mostly present in the form of simple sugars and starch (Speece, 1996). The food processing contain high amount of complex carbohydrates (1940 mg/L), acetate and hydrogen production rate of 0.12 m^3/m^3-d is reported using food processing industry wastewater in MEC (Kadier *et al.,* 2014).

2.5.9 Landfill Leachates

Landfill leachates are highly polluted toxic liquid generated from waste processing and disposal sites. It mainly contains four groups of pollutants: dissolved organic matter, inorganic macro-components, heavy metals and xenobiotic organic

Table 2.4 Type of substrates used in Microbial electrolysis cells

Type of substrates	Concentration (g/L)	Hydrogen production rate (Q) (m^3 H_2/m^3/day)	Applied voltage Eap(V)	References
Acetic acid Glucose Butyric acid Lactic acid Propionic acid Valeric acid	1 (COD)	1.10 1.23 0.45 1.04	0.6	Cheng and Logan, (2007)
Acetate (sodium acetate)	2.72	50	1.0	Jeremiasse *et al.,* (2010)
Glucose	2	0.25 ± 0.03 0.37 ± 0.04	0.6 0.8	Lu *et al.,* (2012)
Synthetic effluent Lignocelluloses Cellobiose	1	1.11 ± 0.13 1.00 ± 0.19 0.96 ± 0.16	0.5	Lalaurette *et al.,* (2009)
Domestic wastewater	0.204-0.481	0.154 L H_2/g	0.5	Ditzi *et al.,* (2007)
Swine wastewater	2 (COD)	0.9-1.0	0.5	Wagner *et al.,* (2009)
Fermentation effluent	1	1.41	0.6	Lu *et al.,* (2009)
A de-oiled refinery wastewater	0.4-1	79%	0.7	Lijiao *et al.* (2013)
Industrial wastewater Food processing (FP) wastewater	0.386 ± 0.007	0.12 ± 0.02 0.08 ± 0.01	0.7	Tenca *et al.,*(2013)
Proteins	0.3	0.42 ± 0.07	0.6	Lu *et al.,* (2010)
Ammonia	-	0.01	0.6	Zhan *et al.,* (2014)
Lactate	-	0.25	0.6	Rosenbaum *et al.,*(2010)
Methanol	-	0.1	0.8	Montpart *et al.,* (2014)
Sodium bicanonate		45.27	-0.8	LaBelle *et al.,* (2014)

compounds (Kjeldsen *et al.,* 2002). Although, it can be considered as a good substrate for MECs it conatins relatively high conductivity, buffering capacity, COD and minimal solids (Zhang *et al.,* 2008) but it is reported that it showed low

coulombic efficiency and current density, it might be due to presence of poorly biodegradable organic matter. Kargi and Catalkaya, (2011) firstly used landfill leachate in MEC for hydrogen production and they achieved cumulative hydrogen production (5000 mL) and hydrogen yield of 2400 mL H_2 g^{-1} COD with COD removal efficiency of 77% at an applied voltage of 4 V.

2.6 Types of MECs.

2.6.1 MECs with mediators

Some bacterial species does not possess the ability to transfer electrons to electrodes through their electron transport systems because some cell surface structures are nonconductive in nature (Oh *et al.,* 2004). So, there is a need to add electrochemical mediators that helps in electrons transfer from the microbial cells to electrode. Mediators enter into the cell when they are in oxidized form and interact with the reducing agents within the cell. In reduced form, the mediators diffuse out from the cell and attach to the electrode surface (Rabaey *et al.,* 2003). Some exogenous mediators that are popularly used include neutral red (Park *et al.,* 1999), anthraquinone-2-6, disulfonate (AQDS), thionin, potassium ferricyanide, methyl viologen and others (Logan, 2004). A good mediator should have some properties such as, they should be able to cross the cell membrane easily, able to grab electrons from the electron carriers of the electron transport chains, soluble in anolyte and non-biodegradable and non-toxic in nature.

There are some microbes that need mediator for electron transfer. Some of the examples include *Actinobacillus succinogenes, Desulfovibrio desulfuricans, E.coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas fluorescens* (Du *et al.,* 2007).

2.6.2 Mediator-less MECs

Some microbes do not need any mediators for electron transfer and MECs having these bacteria are classified as mediator less MECs. There are mainly two mechanisms by which bacteria are known to transfer electrons to a surface, one is electron shuttling via self-produced mediators (e.g. pycocyanin and other compounds produced by the *Pseudomonas aeruginosa* (Rabaey *et al.,* 2004) and nanowires produced by some bacterial species such as *Geobacter* and *Shewanella* (Gorby and Beveridge, 2005).

2.6.2.1 Nanowires

These are electrically conductive bacterial appendages. There are mainly two bacterial species which produce nanowires, *Geobacter* and *Shewanella*, and contribute to electrons transfer. It is reported that nanowires produced by *Geobacter sulfurreducens* results in high efficiency of electron transfer (Lower *et al.,* 2001). *Geobacter* and *Shewanella* species exhibits promising capabilities for producing hydrogen in MEC system. Forty pascal (hydrogen partial pressure) hydrogen production by *Geobacter sulfurreducens* is reported after electron acceptor-limited growth with 20 mM fumarate and 20 mM acetate (Rozendal *et al.,* 2006).

2.7 Thermodynamics of Hydrogen Production (Logan *et al.,* 2008)

Many organic compounds are not suitable as substrates for fermentative hydrogen production due to some thermodynamic limitations. However, these organic compounds can be used in MECs for hydrogen production. Fermentation of glucose and cellulose produces different oxidized species which cannot be further broken down to hydrogen as microbes are not able to extract energy from those reactions. For any reactions to occur spontaneously, the Gibbs free energy of the reactions should be negative but conversion of most of the organic compounds to hydrogen yields a positive Gibbs free energy. If we take the example of acetate oxidation to hydrogen under standard biological conditions (T = 25℃, P = 1 bar, pH = 7), then the Gibbs free energy of the reaction $\Delta G_r^{\circ'}$ is (Thauer *et al.,* 1977)

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2$$

$$\Delta G_r^{\circ'} = +104.6 kJ/mol \quad (2.5)$$

This reaction results in positive Gibbs free energy causing acetate to not to fermented into hydrogen. So, there is a need to apply an external voltage for hydrogen production to occur and in MEC this added voltage is supplied by external power supply and hydrogen production occurs. For microbial electrolysis process the added voltage should be larger than $\Delta G_r^{\circ'}/nF$, where n is the number of electrons involved in reaction and F is Faraday's constant (96485 C/mol e^-). This value is known as equilibrium voltage, E_{eq}, and for acetate it is:

$$E_{eq} = \frac{\Delta G_r}{nF} = \frac{140.6 \times 10^3}{8 \times 96485} = -0.14 \text{ V} \quad (2.6)$$

The negative sign means that the reaction is not spontaneous and we have to add external voltage for reaction to occur.

The equilibrium voltage can also be calculated from theoretical anode and cathode potentials as

$$E_{eq} = E_{cat} - E_{an} \quad (2.7)$$

These potential values can also be calculated from Nernst equation. For example, for acetate the anode potential can be calculated as

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (2.8)$$

For anode, the theoretical anode potential E_{an}can be determined by

$$E_{an} = E_{an}^{0} - \frac{RT}{8F} \ln\left(\frac{[CH_3COO^-]}{[HCO_3^-]^2[H^+]^9}\right) \quad (2.9)$$

Where, E_{an}° equal to 0.187 V, R = ideal gas law constant (8.314 J/K mol), T (K) is the absolute temperature, F= Faraday's constant (96,485C/mol) (Logan *et al.,* 2006). Under standard biological conditions, the anode potential is equal to -0.279 V.

For cathode, the theoretical cathode potential is

$$2H^+ + 2e^- \rightarrow H_2 \quad (2.10)$$

$$E_{cat} = -\frac{RT}{2F} \ln\left(\frac{p_{H2}}{[H^+]^2}\right) \quad (2.11)$$

Where, p_{H2} = hydrogen partial pressure

Under standard biological conditions, the cathode potential is equal to -0.414 V. Therefore, the equilibrium voltage is

$$E_{eq} = (-0.414\ V) - (-0.279\ V) = -0.14\ V \quad (2.12)$$

This shows that cathode potential and equilibrium voltage also depends on the hydrogen partial pressure. Every 10-fold increase in hydrogen partial pressure increases the equilibrium voltage by 0.03 V (Logan *et al.,* 2008).

The electrode potential and equilibrium voltage are also dependent on pH. This is important when membrane is used in MEC because presence of membrane causes the pH difference between two chambers thereby affecting the MEC performance. And every pH unit difference between the two chambers increases the E_{eq} by 0.06 V which requires additional energy supply (Rozendal *et al.,* 2006).

But under operating conditions the applied voltage will always be greater than the equilibrium voltage due to some internal losses in the system. These losses are anodic overpotential φ_a, cathodic overpotential φ_c and Ohmic losses (IR_Ω). Therefore, anodic potential and equilibrium voltage are related by

$$E_{ap} = E_{eq} - (\sum \varphi_a + |\sum \varphi_c| + IR_\Omega) \quad (2.13)$$

2.7.1 Ohmic Losses

These are most important losses in an MEC that include both the resistance to the flow of electrons through the electrodes and the resistance to the flow of ions through the membrane (Larminie and Dicks, 2000).

2.7.2 Activation Losses

Activation energy are required for initiating the oxidation and reduction reaction. These losses occur due to transfer of electron from the cell terminal protein to the anode surface. These type of losses can be reduced by increasing the surface area of electrode, increasing the operating temperature and by enriched biofilm on anode (Logan,*et al.,* 2006).

2.7.3 Bacterial Metabolism Losses

For their metabolism bacteria gain energy by oxidation of substrate. In a MEC, anode is final electron acceptor and by measuring its potential the energy gained by bacteria can be determined. When there is larger difference between redox potential of substrate and anode potential, it means there is higher energy gained by bacteria but MEC voltage is low. To overcome this problem, anode potential should be low (Logan,*et al.,* 2006).

2.7.4 Concentration Losses

These are the lossses that arise when the rate of mass transfer of a species to the electrode or from the electrode limits current production. These are also known as mass transfer losses (Larminie and Dicks, 2000).

2.8 MEC Applications

2.8.1 MECs for wastewater treatment

Microbial Electrolysis Cells are new phenomenon, which is invented recently and requires extensive research into process engineering i.e., designs, scale-up, reactor control, operations, etc. MECs can be prove as reliable system for renewable energy production (Hydrogen), waste water treatment, reduction of solids production, hence lowering the sludge handling cost, and obviously limiting foul odour of the waste(Logan *et al.,* 2008). But, like every new technology MECs also requires deep research for its wide area applications like wastewater treatment. Cost efficiency is also a major factor for MECs to operate in large scale plants (Rozendal *et al.,* 2008).

The advantage with MECs are that they operate under anaerobic conditions resulting lower amount of sludge production (Rabaey and Verstraete, 2005). This also results in reducing sludge handling cost, unlike typical activated sludge system where almost half of the cost is related with sludge handling and treatment (Elissen *et al.,* 2006). Another important aspect of wastewater treatment is to prevent release of foul smell. Unlike AS process in which aeration disperse the odour into the air, MECs are completely enclosed processes and results in minimum odour release. Like, MFCs which can remove chemicals accompanying with the odour (Kim *et al.,* 2008) MECs can be further modified to remove the toxic chemicals from the odour.

2.8.2 MECs for renewable energy production

MECs works on the same principle of anaerobic digesters (ADs) with the capacity of producing higher value of gas than traditional ADs from the same amount of COD (Logan, 2004).

2.8.2.1 Biohydrogen production

MECs are mainly used for biohydrogen production and MEC is more advantageous over other processes such as thermochemical and electrochemical processes. Conventionally dark fermentation and water electrolysis (4% of global hydrogen production by this process) is mostly used in hydrogen production which are less efficient and energy consuming processes (Kothari *et al.,* 2008). In MECs 8-9 mol H_2/mol of glucose is produced while in fermentation process only 4 mol H_2/mol of glucose with acetate is produced (Prakash, 2016).

2.8.2.2 Methane production

Methane production from CO_2 by using micro-organisms as the catalyst with an external energy input is a recent developed application of MEC (cheng *et al.,* 2009). MECs can increase the fuel yield by converting CO_2 into methane, when a large amount of CO_2 is produced in biofuel production (Eerten *et al.,* 2012).

2.9 Challenges of the MEC technology

MECs have good potential for waste refineries. They are capable of converting organic matter present in wastewaters into renewable energy and products with high yields and high rates. But there are still many limitations associated with MECs which should be solved for applying MECs in large scale systems (Randolph and Studer, 2013).

2.9.1 Undesired electron sinks reduce hydrogen production in MECs

Hydrogen production is purpose for which MECs are generally set up. But, its production can significantly decreas due to several undesired electrons sinks of other metabolisms. For example, methanogenesis, which leads to the production of methane instead of hydrogen is a common phenomenon that generally occurs in single chambered MECs without separators (Cusick, *et al.,* 2011). Hydrogenotropic methanogens causes consumption of hydrogen and production of methane at an alarming rate. 1 mole of methane production results in the consumption of 4 moles of hydrogen, even at nano molar concentration. This is due to low acetate environment inside MECs, which causes acetoclastic methanogens to outcompete acetate oxidizing exoelectrogens (Lu *et al.,* 2012). This problem also occurs in the MECs with separators, because they just only slow down the methanogensis without really solving the problem (Lu *et al.,* 2010).

In the recent times, several strategies have been used to inhibit methanogensis in MECs, but they are inefficient in long run. For example, continuous addition of chemical inhibitors i.e., sodium bromomethanesulfonate (NaBES) results in new potential contamination. Exposure to oxygen causes damage to anaerobic exoelectrogens. Increasing flow rate and use of low temperature also have very limited effects on methanogen growth (Lu *et al.,* 2012). Employing these strategies are also not cost effective either.

2.9.2 Electrode Materials, MECs configuration and substrates used in the reactor

For large scale production of hydrogen, the process should be cost effective with major focus on high conductivity for low overpotential and maximum biomass attachment with electrode material (Santoro, *et al.,* 2014). The cost for hydrogen production from MECs is almost ten times higher from advanced abiotic alkaline electrolyzers, and the rate of production is also far below in MECs (Escapa *et al.,* 2016). If we take the example of cathode used in MECs, the cost of cathode and its catalyst comprises of almost 47% of total cost of the reactor (Kundu *et al.,* 2013). It is not feasible to use titanium based cathode for large scale production of hydrogen and other cheaper alternatives should be employed to reduce the cost as much as possible. Characteristics of wastewater used in MECs also influences the amount of hydrogen production. Domestic wastewater for example, has a conductivity of 1 ms/cm which is very less, almost ten times lower than buffered solutions. In most lab experiments phosphate or other buffer solutions are used to increase the buffer capacity and conductivity, which is not a preferable solution to large scale production.

Recent studies have shown that transition metal alloys i.e., Ni, MoS_2, Stainless steel, Carbon Nanotube, graphene, biocathodes etc. are equally efficient in hydrogen production with low cost. But, till now these low-cost cathodes are tested in small scale MECs only. Carbon based materials are proved best anode for MECs because of their low cost, resistant to corrosion and high surface area scalability. Apart from that silicate materials can be used to increase buffer capacity and solution conductivity to help large scale hydrogen production (Lu *et al.*, 2015).

2.9.3 Syntrophic interactions and competitions among microorganisms

The scope for utilising complex organics present in waste water is limited. Simple organic substrates such as volatile fatty acids (VFAs) and alcohols are used by most of the exoelectrogens to extract electrons in MECs for current generation. In addition, they require polymer-degrading bacteria to break down the complex polymers such as protein and cellulose to simple organics (Ren *et al.,* 2007).

Understanding of microbial syntrophic interactions will help in characterisation of microbial interactions which assist in converting waste water into hydrogen.

Syntrophic interaction includes three main route:

1. Interaction between exoelectrogens and fermentative bacteria converting complex substrates into simple organics,
2. Interactions among exoelectogens, homoacetogens and fermentative bacteria,
3. Interaction between methanogens and fermentative bacteria (Lu *et al.,* 2012).

The functioning of microbial community is very crucial for MECs, and understanding microbial syntrophic interaction will help in developing more efficient system for waste to electron conversion (Lee *et al.,* 2010)**.**

2.9.4 Effluent quality

The quality of effluent discharge after microbial electrochemical anaerobic process, does not meet the standard of less than 30 mg/L biochemical oxygen demand (Katuri, *et al.,* 2014). MECs through waste treatment can reduce organic concentration, but to make it a viable process more studies are needed.

2.10 Summary

MEC system is a promising technology for converting organic matter present in wastewater into hydrogen gas with a small energy input. MEC can be a viable technology for sustainable renewable energy production by producing hydrogen from renewable sources such as wastewaters. There are many applications of MECs such as wastewater treatment, hydrogen production and current

generation. But, there can be various factors that can affect the performance of MEC such as MEC configuration, materials used in MEC (anode, cathode, membrane), substrate composition, microbes, applied voltage etc. Various advancements have been done to improve the efficiency of these systems. But it still faces several challenges for scaling up these systems such as cost of materials used in MEC system, lower hydrogen production efficiency, energy cost, and syntrophic ineraction and competition among microorganisms. So, for scaling up there is a need to develop these systems.

CHAPTER III

MATERIALS AND METHODS

The outline of methodology followed in the present study is depicted in Figure 3.1. The study was conducted in Centre for Environmental Science and Technology laboratory no.31

3.1 Wastewater collection and characterization

The dairy industry wastewater was collected from the Verka Milk plant located at Bathinda district (30.2110º N, 74.9455º E), Punjab, India. The collected samples were transported to the laboratory and stored at - 40ºC until further use. The initial physio-chemical characteristics of the wastewater such as pH, alkalinity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), volatile fatty acids (VFA), total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), Total Kjeldahl Nitrogen (TKN), nitrite, nitrate, phosphate were determined as per the protocol described in Standard Methods (APHA, 2012). The inoculum for the reactor was a slurry collected from the biogas plant located at Jassipauwali village, Bathinda.

3.2 Preparation of electrodes

The carbon cloth with a surface area of 6 cm^2 and the graphite sheet with a surface area of 5 cm^2 was used as anode and cathode, respectively. Both the electrodes were coated with iron oxide nanoparticles that were prepared by co-precipitation method (Gini and Yogalakshmi, 2017). The nanoparticle coated anode was immersed in a mixture of volatile fatty acids and slurry (inoculum) for biofilm development. The incubation period for biofilm development is 15-20 days.

3.3 Preparation and characterization of nanoparticles

Iron oxide nanoparticles were prepared previously by Gini and Yogalakshmi, (2017). Nanoparticles were prepared by co-precipitation method in which 0.5 M ferrous chloride and 0.25 M ferric chloride was precipitated by 0.6M NaOH and then the dried cakes of nanoparticles was ground to fine powder. Then nanoparticles were characterized for size, elemental composition and magnetite form by SEM, EDS and FTIR.

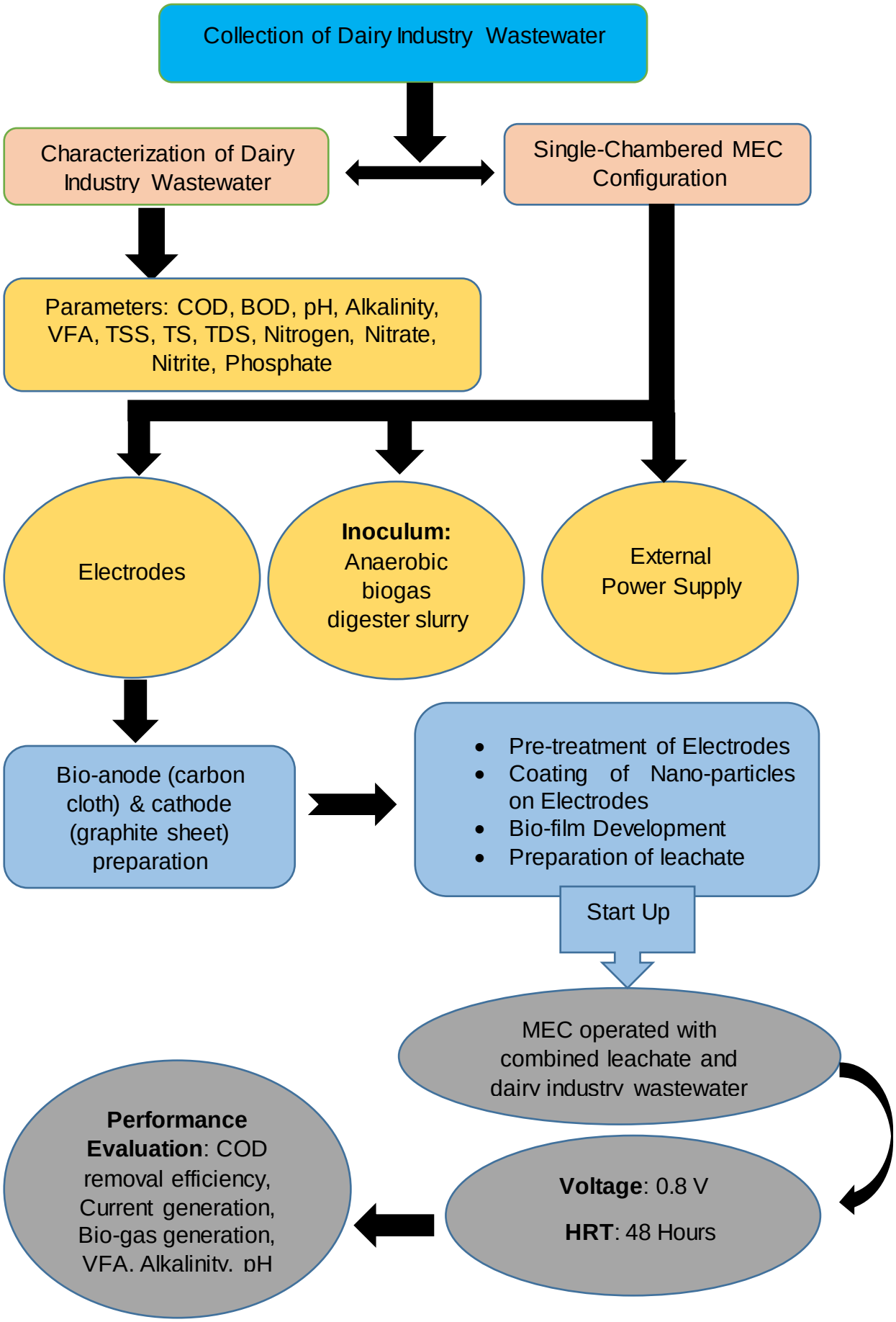


Figure 3.1 Outline of methodology

3.4 Coating of nanoparticles on electrodes

The electrodes with a surface area of 6 cm^2 (anode) and 5 cm^2 (cathode) were immersed in solution containing nanoparticles and methanol and left overnight. Particles engrained over both the electrodes.

3.5 MEC configuration

A single chambered membrane less MEC consisted of a plastic (Plexiglas) cuboid chamber with the dimensions of 7.8cm x 11.3cm x 10cm and working volume of 350 ml. Figure 3.2 depicts the photographic view of the MEC. The anode and the cathode is distanced by 4.5 cm. This would eliminate the risk of short circuit inside the reactor. The electrodes were connected to programmable DC power supply unit of Caddo 4073. To maintain anaerobic condition inside the reactor, it was sealed properly. The gas tight syringe was used to collect the gas generated in the reactor. An inlet and outlet was made in the MEC reactor to add the influent and to decant out the effluent, respectively. Experimental setup of MEC is shown in Figure 3.2.

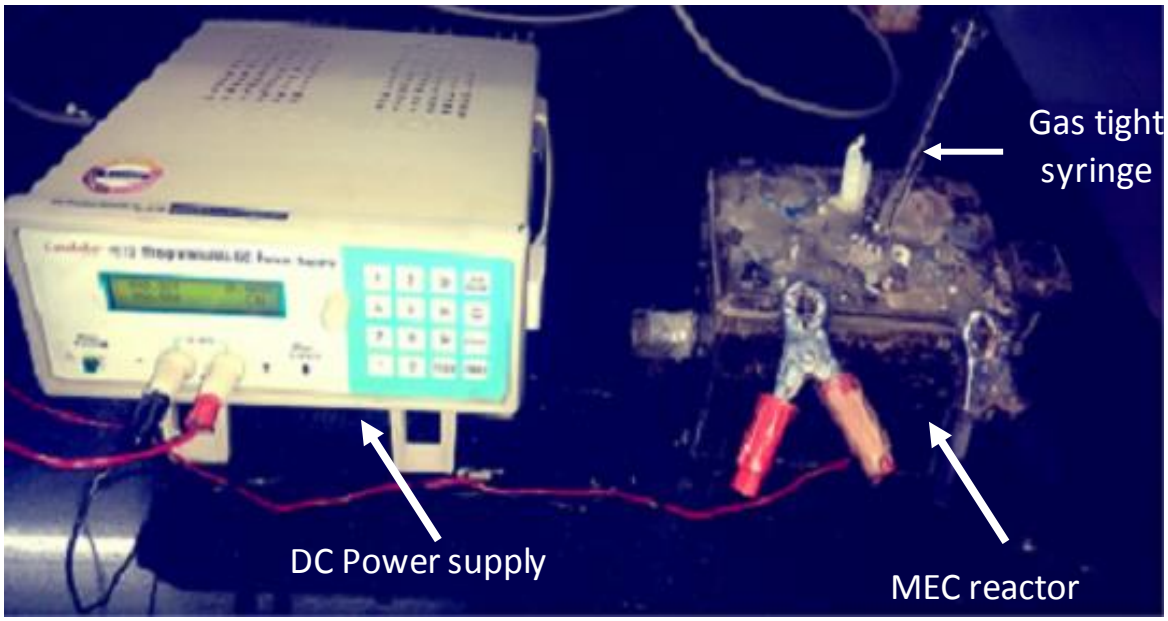


Figure 3.2 Photograph showing the Experimental set up of Microbial Electrolysis cell (MEC)

3.6 MEC start-up and operation with combined leachate and dairy industry wastewater

After the biofilm development on electrodes, they were transferred into the MEC reactor fed with leachate (16%) and dairy industry wastewater (84%). The MEC was operated at an applied voltage 0.8 V for an HRT of 48 hours. The Voltage and HRT were previously optimized using response surface methodology by Gini (2015). The performance of MEC was assessed on the basis of COD removal, current generation and biogas production. At intervals of 24 hours, the effluent sample was collected and analyzed for COD, biogas production and current generation. The stability of the MEC was assessed through pH, alkalinity and VFA which were also monitored every 24 hours. The current generated was measured using multimeter. The cumulative gas generation was recorded at the end of the cycle period.

3.7 Analytical methods

All the analysis was done in triplicates. The physio-chemical parameters were performed according to the Standard Methods for water and waste water examination (APHA, 2012). Table 3.1 summarizes the physio-chemical analysis performed in the present study.

Table 3.1 Analytical Methods

Sr. No.	Parameters	Methods	References
1.	pH	Glass Electrode	Oaklon, PC 2700
2.	Alkalinity	Titration method	APHA 2320 B, 2-27
3.	Volatile Fatty Acids	Distillation method	APHA 5560 C, 5-58
4.	Chemical Oxygen Demand	Open reflux method	APHA 5220 B, 5-15
5.	Biochemical Oxygen Demand	Azide modification	APHA 5210 B, 5-2
6.	Total Solids	Gravimetric method	APHA 2540 B, 2- 56
7.	Total Suspended Solids	Gravimetric method	APHA 2540 D, 2- 58
8.	Total Dissolved Solids	Gravimetric method	APHA 2540 C, 2-57
9.	Total kjeldahl nitrogen	Macro-Kjeldahl method	APHA 4500-Norg B, 4-131
10.	Nitrite	Colorimetric method	4500-NO_2 B, 4-118

11.	Nitrate	Colorimetric method	4500-NO_3 B, 4-120
12.	Phosphate	Stannous Chloride method	APHA 4500-P D, 4147

3.8 Calculations

The current was measured by using multimeter. COD removal efficiency was calculated as

$$\text{COD removal efficiency } (\%) = \frac{\text{COD}_{\text{inf}} - \text{COD}_{\text{eff}}}{\text{COD}_{\text{inf}}} \times 100 \quad \text{.....(3.1)}$$

Where, COD_{inf} is influent COD and COD_{eff} is effluent COD of each day.

Current density was calculated as

$$\text{Current Density (J)} = \frac{I}{A} \quad \text{.....(3.2)}$$

Where I is current in ampere and A is the projected surface area of studied electrodes in cm^2.

Power density was calculated as

$$\text{Power Density} = \frac{V \times I}{A} \quad \text{.....(3.3)}$$

Where V is voltage, I is current in ampere and A is surface area of electrodes.

The hydrogen yield based on COD removal, Y_{H2} (mg-H_2/mg-COD) was calculated as

$$Y_{H2} = \frac{\text{n}_{\text{H2}}\,\text{M}_{\text{H2}}}{\text{v}_{\text{L}}\,\Delta\text{COD}} \quad \text{.....(3.4)}$$

Where, n_{H2} is the moles of hydrogen recovered in the experiment, it can be calculated from the ideal gas law based on the volume of hydrogen recovered, M_{H2} is the molecular weight of hydrogen and v_L is the volume of liquid in the anode chamber. ΔCOD is the change in COD based on the concentrations of influent and effluent for continuous flow test.

Coulombic efficiency is the moles of hydrogen that can be recovered based on the measured current, C_E was calculated as

$$C_E = \frac{8Q}{FV_{an}\Delta COD} \quad \text{.....(3.5)}$$

Where, C_E is columbic efficiency, Q is charge transferred, F is Faraday constant (96485 C/mol-e^-), Van is liquid volume, ∆COD is COD removed and 8 is used to convert COD to moles of electrons.

3.9 Statistical analysis

All the experiments in present study were performed in triplicates to minimize handling error. Data were analysed statistically and results were expressed as Mean ± SEM (Standard error mean).Two ways ANOVA was carried out so as to examine significant difference between the efficiencies. Difference was considered at $P< 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Physiochemical characterization of dairy industry wastewater

The wastewater used in this study was collected from Verka Milk Plant, Bathinda, India and characterized for various parameters. The physiochemical characteristics of dairy industry wastewater are summarized in Table 4.1.

Table 4.1 Physiochemical characteristics of dairy industry wastewater

S. No.	Parameters	Value
1.	Chemical Oxygen Demand (mg/L)	4000
2.	Biochemical Oxygen Demand (mg/L)	1200
3.	pH	7.29
4.	Alkalinity (mg/L)	2200
5.	Volatile Fatty Acids (mg/L)	3072
6.	Total Solids (mg/L)	1900
7.	Total Suspended Solids (mg/L)	585
8.	Total Dissolved Solids (mg/L)	1315
9.	Nitrate (mg/L)	166
10.	Nitrite (mg/L)	107
11.	TKN (mg/L)	33.6
12.	Phosphate (mg/L)	30.2

Dairy wastewaters mainly consists of carbohydrates proteins and fats originating from different dairy products such as milk, butter, yoghurt, ice-cream, various type of desserts and cheese. The characteristics of these wastewaters shows high variation depending on the types of system and methods of operation used (Vidal *et al.,* 2000). The pH and COD of the dairy industry wastewater was 7.29 and 4000 mg/L, respectively. Previous studies on dairy wastewaters reported that pH value of dairy industry wastewater ranged from 6-11 (Demirel *et al.,* 2005). Likewise, the COD also ranged between 2000-6000 mg/L in dairy industry

wastewater. In certain cases, namely the milk processing effluent the COD is reported to be as high as 70000 mg/L (Traversi, *et al.,* 2013). The pH reported in present study showed similarity with reported literature (Gadhe *et al.,* 2015). The high variation in pH could be due to use of alkaline cleaners and sanitizers in the dairy industry (Kasapgil *et al.,* 1994). It is reported in the literature that values of total suspended solids in dairy wastewater ranged between 350-1000 mg/L and in the present study the value of total suspended solids was 585 mg/L. Likewise, Nitrogen and phosphate value ranged between 50-60 mg/L, and 20-50 mg/L, respectively (Ince, 1998). In present study the value of nitrogen, and phosphate was obtained 33.6 mg/L, and 30.2 mg/L, respectively. Suspended solids in dairy wastewaters mainly comes from coagulated milk, cheese curd fines or flavouring ingredients (Brown and Pico, 1979). In industrial dairy wastewaters nitrogen originates from milk protein and can be present in various form organic or inorganic ions (Jimenez *et al.,* 2000). The alkalinity value obtained in this study was higher than the values reported by Demirel, (2003) because the production process involves usage of cleaners during milk production.

4.2 MEC operation with combined leachate and dairy industry wastewater

MEC reactor was operated with combined leachate and dairy industry wastewater for seven continuous cycles and the performance was assessed based on the COD removal, biogas production and current generation. The stability of MEC reactor was investigated through pH, alkalinity and VFA. The results are discussed in detail in following section.

4.2.1 pH

pH is an important parameter in MEC that affects both the current intensity and rate of wastewater treatment. According to the literature reported by Jadhav and Ghangrekar, (2009) the best pH for micro-organism activity in anaerobic conditions is pH 6.5-7.0. Near neutral pH supports anaerobic biological treatment (Metcalf and Eddy, 2003). For the present study as well the pH of combined leachate and dairy wastewater was 7.33. Figure 4.1 depicts the pH profile of MEC operated with combined leachate and dairy industry wastewater. From the figure, it was observed that the pH inside the reactor ranged between 7.32-7.73. According to Lee and Rittmann, (2010), a nearly constant pH in the single chamber MEC

supports that protons produced in the anodic reaction was rapidly neutralized by hydroxyl ions generated in cathodic reaction or were removed by hydrogen production. One of the major goal of MEC is to reduce energy loss and the reported results supports that the energy loss due to pH difference could be minimized in single chamber MEC. The pH 7 is more favourable for hydrogen production too. According to Mohanraj *et al.,* (2014), the optimal initial pH for efficient hydrogen production using *Enterobacter cloacae* was observed to be 7.0. In most studies, the optimal pH in the range of 5.2-7.0 supported hydrogen production (Li and Feng 2007; Li *et al.,* 2008; Jadhav *et al.,* 2009). However, a few contradictory literatures are also reported supporting hydrogen production and pH values the literature presents contradictory results about pH value for hydrogen production. Khanal *et al.,* (2004) and Lee *et al.,* (2002) reported a pH 4.5 and 9.0 for high hydrogen production.

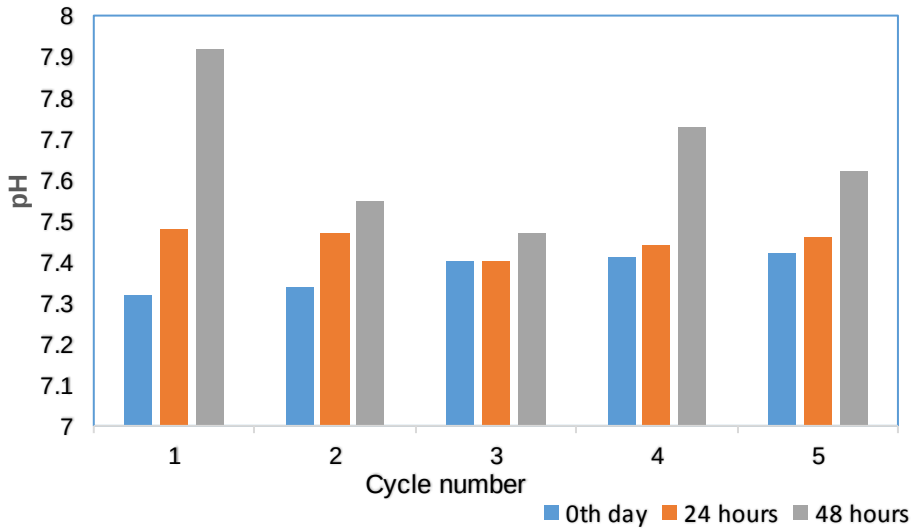


Figure 4.1 pH profile of MEC operated with combined leachate and dairy industry wastewater

4.2.2 COD Removal

Figure 4.2 depicts the effluent COD (mg/L) and the COD removal efficiency of MEC reactor operated with combined leachate and dairy industry wastewater with nanoparticle decorated electrodes (both anode and cathode). The MEC was operated at an OLR of 17.14 gCOD/L/d and HRT of 48 hours. It is clearly evident from the figure that COD removal efficiency increased with time. The decreased

COD of effluent with time indicates the bacterial decomposition of organic matter into VFAs and CO_2 under anaerobic condition. From the figure, it was observed that the initial COD removal efficiency was 82.6% which increased up to 96.5% with magnetic iron oxide nanoparticle coating on the electrodes. The COD removal efficiency of control reactor (MEC operated previously with plain electrodes operated with combined leachate and dairy industry wastewater) was 88% (Nabi, 2016). So, it is clearly evident that nanoparticles coated electrodes showed higher COD removal efficiency than plain electrodes.

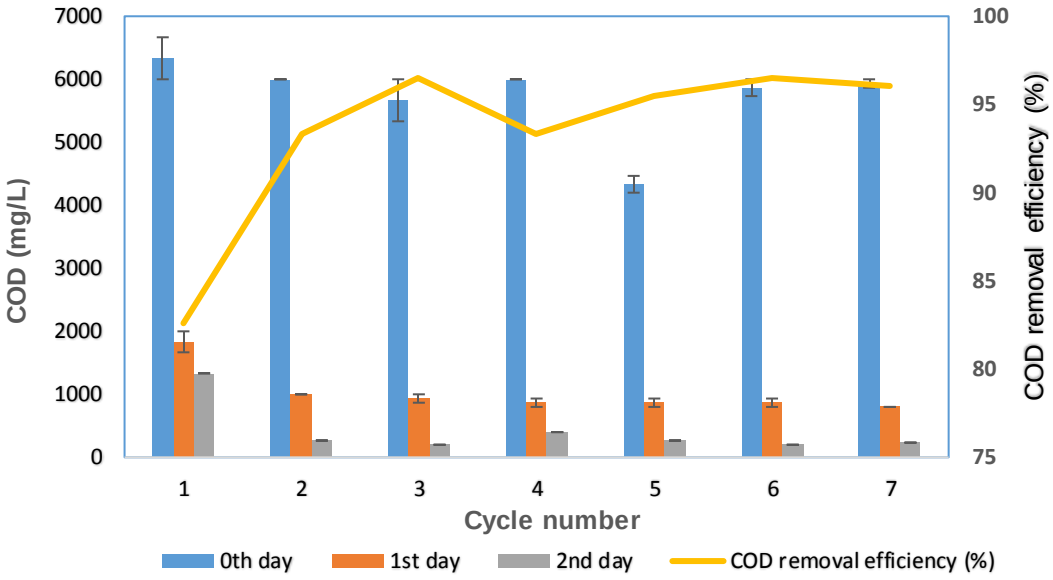


Figure 4.2 COD removal during combined leachate and dairy industry wastewater treatment

The COD removal efficiency achieved in this study is greater than the previous studies. Malik *et al.,* (2014) reported 72.5% COD reduction with distillery wastewater as substrate and iron oxide nanoparticles coated anode (initial substrate concentration of 110 g/L). Gadhe *et al.,* (2015) found 61% COD reduction when dairy wastewater was treated in MEC consisting of hematite nanoparticle coated anode. A maximum COD removal efficiency of 90.5% was achieved with dairy wastewater by using catalyst-less and mediator-less microbial fuel cell (Mansoorian *et al.*, 2016). Kargi and Catalkaya, (2011) achieved 77% COD removal efficiency by treating leachate via electrohydrolysis at 4 V DC voltage.

4.2.3 Alkalinity and VFA profile

Alkalinity and VFA were monitored during the operation cycle of the reactor. The alkalinity and VFA profile is depicted in Figure 4.3 and 4.4, respectively.

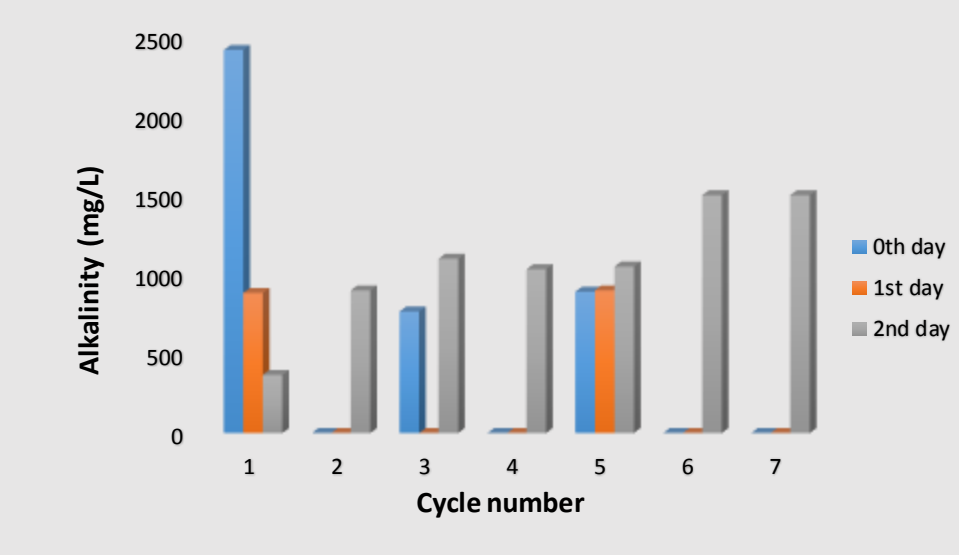


Figure 4.3 Alkalinity profile of MEC operated with combined leachate and dairy industry wastewater

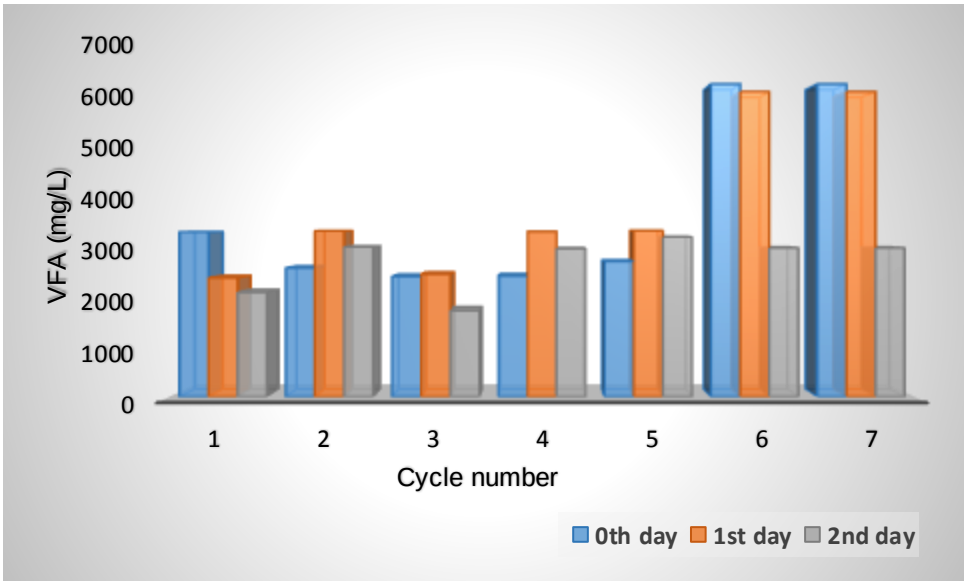


Figure 4.4 VFA profile of MEC operated with combined leachate and dairy industry wastewater

pH indicates the process stability in wastewater with low buffering capacity because high bicarbonates may compensate the pH changes due to the VFA accumulation. In this case, the pH drop will occur only when the process has been severely unbalanced. But alkalinity detects the changes in the buffer capacity of an anaerobic digestion process. So, alkalinity can be a better option for monitoring the digester stability (Malina and Pohland,1992). However, alkalinity and VFAs are inversely proportional to each other. VFA production is always associated with conversion of organic matter into acid intermediates in completely anaerobic condition with the help of acidogens. Acidogens grow relatively faster and are less sensitive to change in pH. So, in present study both were monitored together to have an accurate overview of the digester stability. From the figure, it is clear that during first cycle there was highest alkalinity in the reactor which then decreased with time. The variation in alkalinity and VFA was evident up to 5^{th} cycle of operation and thereafter it showed stability in results. It is clear from the figure that the alkalinity was 0 mg/L on the 0^{th} day which then gradually increased to 1500 mg/L on the 2^{nd} day. The accumulation and consumption of volatile fatty acids might be the possible reason for increase and decrease of alkalinity, respectively. The observations can be well corroborated with the Figure 4.4 that shows VFAs on 0^{th} and 2^{nd} day as 6263 mg/L and 2981 mg/L, respectively. This indicates a healthy acidogenic fermentation of substrate (Krishna *et al.,* 2013). The results of the present study is in good agreement with the study reported by Krishna *et al.,* (2013) who showed sequencing study with anaerobic suspended growth reactor using complex feed and industrial effluent.

4.2.4 Gas generation

Gas chromatographic analysis performed at OLR of 17.14 gCOD/L/d confirmed the composition of gas produced during operation of MEC with combined leachate and dairy industry wastewater. The gas collected from the MEC showed the presence of CO_2, H_2, and N_2 peaks in the gas chromatogram. Figure 4.5 depicts the gas chromatographic peaks.

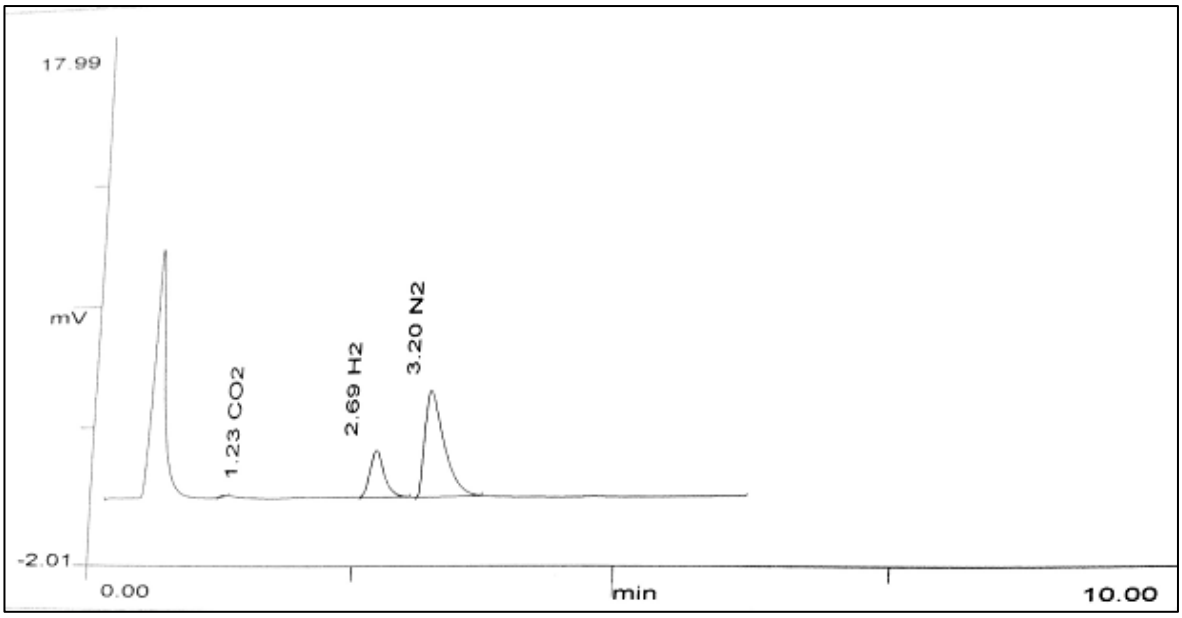


Figure 4.5 Gas Chromatographic analysis showing hydrogen production

Operation of MEC with combined leachate and dairy industry wastewater at an applied voltage of 0.8 V, resulted in the production of 3.192 L/L/d hydrogen. The hydrogen production rate achieved is higher than the control reactor (MEC operated previously with combined dairy industry wastewater and leachate and plain electrodes). The hydrogen generated in the control reactor was around 19 ml/L/d. So, nanoparticles coated electrodes showed higher efficiency in terms of hydrogen production as compare to plain electrodes. The result of this study is comparable with previous studies. Kargi and Catalkaya, (2011) achieved 1277 ml/d hydrogen gas formation with 4 V DC voltage by using landfill leachate as a substrate. Wagner *et al.,* (2009) reported 0.9 L/L/d hydrogen production rate using swine wastewater. Hydrogen production rate of 3.12 L/L/d was reported with sodium acetate as a substrate (Call and Logan, 2008). Hydrogenase, is a key enzyme for hydrogen production that catalyzes the reduction of protons to hydrogen. The hydrogenase enzyme is classified into Fe-Fe and Ni-Fe hydrogenase based on the metal content present at the active site. So, the presence of Fe or Ni metals at active site enhance the bio-hydrogen production. In this study iron oxide nanoparticles are coated on electrodes so this could be a possible reason for enhanced hydrogen production.

4.2.5 Current generation

Figure 4.6 shows the current density profile of MEC operated with combined leachate and dairy industry wastewater. From the figure, it was observed that the MEC operated with Fe nanoparticle decorated electrode showed higher current density than the MEC with plain electrode. The initial current density produced by MEC with plain electrodes was 5.31 A/m^2 which increased up to 7.31 A/m^2 in 24 hours. The maximum current density in 48 hours was 8.61 A/m^2 (Gini and Yogalakshmi, 2017). Likewise, the initial current density with nanoparticle decorated electrodes in MEC was 12.36 A/m^2 and it increased up to 21.6 A/m^2 in 24 hours which is 2.5 times higher than the value achieved by MEC consisting of plain electrodes. The maximum current density achieved with Fe nanoparticles decorated electrodes in MEC was 33.25 A/m^2. The maximum current density achieved by nanoparticles decorated electrodes was 3.86 times higher than generated by plain electrodes with same reactor configuration and operating conditions.

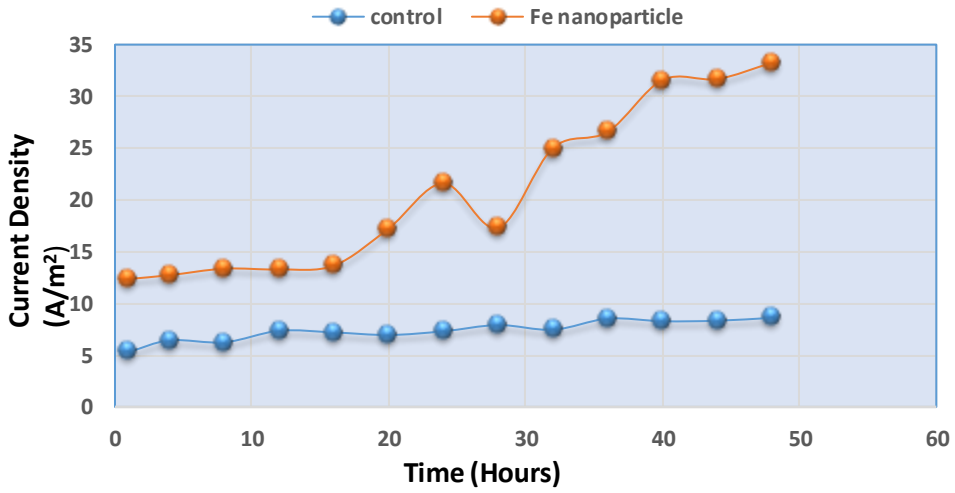


Figure 4.6 Current density during combined leachate and dairy industry wastewater treatment

The result of present study is comparable with previous study (Xu *et al.,* 2012). They showed that the current density achieved by Fe nanoparticles coated anode was 8.25 times higher than generated by control or plain graphite disk. There could be various factor that enhance the current density with addition of

nanoparticles to electrodes such as elemental composition and chemical composition of nanoparticles because these factors determines the conductivity which increase the efficiency of electron transfer from bacteria to electrode and this results in current enhancement (Fan *et al.,* 2011). There may be other possible factors contributing to the observed current generation. Surface area increase by Fe nanoparticles decorated electrodes and Fe corrosion are some of the contributing factors (Xu *et al.,* 2012). But it is also reported that increased surface area is not a major reason for enhancing current density but rough electrode surface that is appropriate for biofilm formation could also be the possible reason for increased current density. There are number of studies for increased current generation by nanoparticles. Depending upon the bacterial behaviour on nanoparticle coated electrodes it is also believed that E.coli cells produce hair like structure on the electrode surface which is similar to pili and that could facilitate the electron transfer between the cells and the electrode. The growth of hair like structures is believed to be stimulated by nanostructures that could play a role in enhancement of current density (Qiao *et al.,* 2008).

4.2.6 Coulombic efficiency

Coulombic efficiency is an established parameter to evaluate how efficient the chemical energy stored in the wastewater was converted into electrical energy or the fraction of COD was converted into electrons (Ivanov *et al.,* 2013). So, it is the ratio of number of electrons transferred to the external circuit to the number of electrons from the oxidation of substrate (Cheng *et al.,* 2006). Figure 4.7 depicts the columbic efficiency obtained in MEC operated with combined leachate and dairy wastewater. In control MEC (consisting of plain electrodes and operated with combined dairy industry wastewater and leachate), 113% coulombic efficiency was achieved (Nabi, 2016). In this study, higher coulombic efficiency (155%) was achieved than previous study. This showed that the maximum chemical energy stored in the combined wastewater was converted into electrical energy. The coulombic efficiency achieved in this study is higher than the previous studies. Call and Logan, (2008) reported the coulombic efficiency in the range of 80-100% for membrane less MEC. Lee *et al.,* (2009) achieved a coulombic efficiency of 161±25% for acetate in a single chamber microbial electrolysis cell. From the figure, it is evident that there was slight decrease in coulombic efficiency with time.

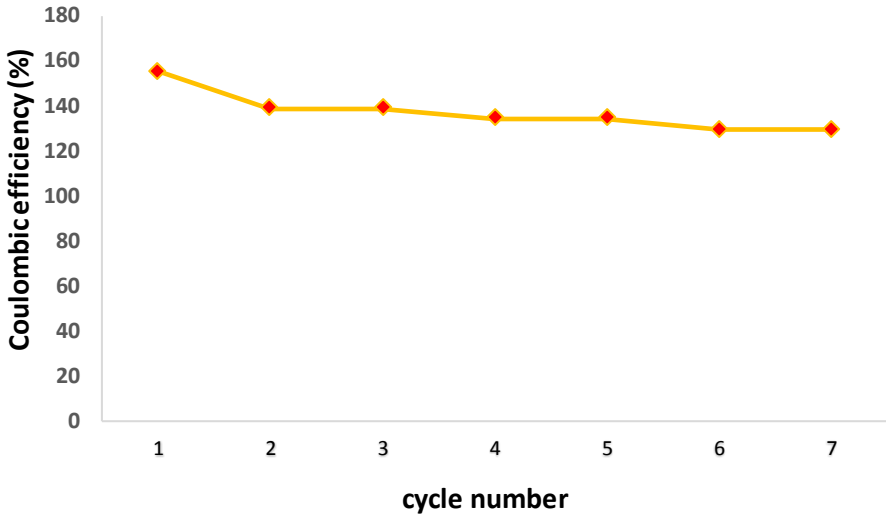


Figure 4.7 Coulombic efficiency during combined leachate and dairy industry wastewater treatment

It is because coulombic efficiency has an inverse proportion with COD removal efficiency. The decrease in C_E showed the inability of bacteria in converting all organic chemicals to electricity (Watanabe, 2008).

CHAPTER V

SUMMARY AND CONCLUSION

5.1 Summary

Microbial electrolysis cells technology was developed for high efficiency biological hydrogen production from waste streams. It possess an ability to convert the organic matter in wastewater to energy in the form of H_2. Globally, every year huge amount of wastewater is generated from industries and other domestic sources. Anaerobic biological wastewater treatment can result in H_2 generation and it is considered to be an ideal methodology to reduce pollution load apart from renewable energy generation.

Leachate a highly toxic but organic rich discharge generated from the landfills or waste dumping and processing sites. High COD, NH_4-N and VFAs. High COD and NH_4-N content and low BOD/COD ratio makes biological treatment of landfill leachate very difficult. Co-treatment of landfill leachate with other wastewater can be an alternative option to unlock the organic richness into energy using MECs. Although MECs have shown better treatment efficiency and biogas production still it's upscaling is limited due to various reasons. The performance of the MEC depends on the electrode, electrode material and the reactions involved within the fuel cell. Recent research has involved nanomaterial in the electrode material to improve the MEC performance. Nanoparticles have unique physical, electrical, and chemical properties which facilitates the study of interactions between bacteria and electrode surfaces. Different types of nanomaterials (carbon nanotubes, metal oxides, titania nanotubes) are being assessed by various researchers to improve the electrode efficiency.

The present study aims to investigate the efficiency magnetic iron nanoparticle coated electrodes in improving the performance of MEC operated with combined leachate and dairy industry wastewater. The performance of the MEC was studied through COD removal, current and biogas generation. The results obtained were compared with previous study (i.e) MEC with simple carbon electrodes (without nanoparticle coated electrode) operated with combined leachate and dairy industry wastewater. The MEC was operated at an OLR of 17.14 gCOD/L/d, applied voltage of 0.8 V and HRT of 2 days. Magnetic iron nanoparticle coated electrode

MEC showed an improved COD removal of 96.5% and current density (33.25 A/m^2) compared to control studies which showed 88% COD removal and 8.61 A/m^2 current density. Hydrogen generation in magnetic iron nanoparticle coated electrode MEC was also high (3.192 L/L/d) as compared to control. This might be due to gas leakage issues or analytical error. Table 5.1 summarizes the comparative results of MEC control and MEC operated with nanoparticle coated electrodes.

Table 5.1 Comparative results of MEC control and MEC operated nanoparticle coated electrodes

S. No.	Parameters	MEC with nanoparticle coated electrodes	MEC control
1.	COD removal efficiency	96.5%	88%
2.	Current density	33.25 A/m^2	8.61 A/m^2
3.	Hydrogen gas production	3.192 L/L/d	19 ml/L/d
4.	Coulombic efficiency	155%	113%

5.2 Conclusion

The major findings of the present study include higher efficiency was achieved by using membraneless single chamber MEC reactor with nanoparticle coated electrodes than previously reported studies.

- Magnetic iron nanoparticle decorated electrodes improved the performance of MEC through COD removal and current generation
- A COD removal efficiency of 96.5% was achieved at an applied voltage of 0.8 V and HRT of 48 hours in MEC operated with combined leachate and dairy industry wastewater.
- Magnetic iron nanoparticle coated electrodes (carbon cloth bio-anode and graphite sheet cathode) showed higher current density and coulombic efficiency of 33.25 A/m^2 and 155%, respectively.
- During the process, the Hydrogen production rate of 3.192 L/L/d was achieved at the OLR of 17.14 gCOD/L/d.
- The stability parameters such as pH, alkalinity and VFA were maintained and did not affect the process much.

- ❖ The MEC with decorated electrodes showed better efficiency than control MEC which showed a COD removal, current density and coulombic efficiency of 88%, 8.61 A/m^2 and 113%, respectively.

5.3 Scope for further studies

Some recommendations for further studies in MEC are given below: -

- ➢ Although the voltage required for MEC operation is very low than that of water electrolysis process but the energy consumption is still high, especially for long term operation in rural or remote area where electricity distribution is difficult to reach. So, there is a need to find out the way for reduction of electric energy cost or development of alternative renewable power sources (e.g. solar power) for successful application of MECs.
- ➢ There is a need to develop application oriented reactor design to lower the cost of construction as well as energy losses.
- ➢ There are many different application scopes of MECs that could be further expanded such as biosensor and recalcitrant pollutants removal.
- ➢ The spectrum of pollutants treated with MECs could be even expanded, while the treatment capacity of MECs needs to be further improved.
- ➢ The integration of MECs with existing separation, convention and treatment processes (e.g. anaerobic digestion) can be helpful for overcoming drawback and bringing benefits to each other, and thereby boosting the waste conversion and energy production.
- ➢ A detailed study of micro-organisms that function as exoelectrogens is essential. This will provide the most useful strains that will generate high power.

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