Enhanced Bioenergy Production Using Wastewater in Hybrid - Microbial Electrolysis Cell (MEC)

By

Muhammad Muddasar

Reg. No.: 00000318393

Session 2019-21

Supervised by

Dr. Rabia Liaquat

A Thesis Submitted to U.S.-Pakistan Center for Advanced Studies in Energy in partial fulfillment of the requirement for the degree of

MASTER of SCIENCE in

ENERGY SYSTEMS ENGINEERING

U.S.-Pakistan Center for Advanced Studies in Energy (USPCAS-E)

National University of Sciences and Technology (NUST)

H-12, Islamabad 44000, Pakistan

August 2021

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS/MPhil thesis written by Mr. **Muhammad Muddasar** (Registration No. 00000318393), of U.S.-Pakistan Center for Advanced Studies in Energy has been vetted by undersigned, found complete in all respects as per NUST Statues/Regulations, is within similarity indices limit and accepted as partial fulfillment for award of MS/MPhil degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

Signature: _____

Name of Supervisor: Dr. Rabia Liaquat

Date: _____

Signature (HoD): _____

Date: _____

Signature (Dean/Principal): _____

Date: _____

CERTIFICATE

This is to certify that the work in this thesis has been carried out by Mr. **Muhammad Muddasar** and completed under my supervision in Biofuels Lab, U.S.-Pakistan Center for Advanced Studies in Energy (USPCAS-E), National University of Sciences and Technology, H-12, Islamabad, Pakistan.

Supervisor:

_____

Dr. Rabia Liaquat
USPCAS-E
NUST, Islamabad

GEC member # 1

_____

Dr. Naseem Iqbal
USPCAS-E
NUST, Islamabad

GEC member # 2:

_____

Dr. Nadia Shahzad
USPCAS-E
NUST, Islamabad

GEC member # 3:

_____

Dr. Mustafa Anwar
USPCAS-E
NUST, Islamabad

HoD-ESE:

_____

Dr. Rabia Liaquat
USPCAS-E
NUST, Islamabad

Dean/Principal:

_____

Dr. Adeel Waqas
USPCAS-E
NUST Islamabad

ACKNOWLEDGEMENT

I would like to thank Allah Almighty without His blessing this work would not have been possible. This dissertation is whole heartedly dedicated to my parents, who have been a source of continuous inspiration to me and provided me with the strength and vision to achieve my goals.

I would like to thank my supervisor, for her brilliant supervision, guidance, and encouragement he gave me throughout my research and thesis phase. I have been lucky to find a supervisor that helped me and cared so much about my progress and answered my questions and queries as soon as possible.

I would also like to thank Department of Energy Systems Engineering, USPCAS-E, NUST, all lab engineers, lab technicians, all GEC members, ICT Endowment Fund, and IsDB scholarship program for their support throughout this degree program.

DEDICATION

I would like to dedicate this thesis to my loving parents, my inspirations in life who taught me how to fight against the struggles of life, my father who supported me through every thick and thin of life, my mother who taught me to be patient and work hard until you achieve your goals, my teachers whom guidance helped me to achieve this status in my life and my friends who were always there for me.

ABSTRACT

Conventional fossil fuel resources have been using these days for meeting world's growing energy demands. The use of coal, oil, and natural gas results in greenhouse gases emissions as well as environmental pollution. Clean energy resources are of much interest these days to meet energy demands and reduce environmental pollutions. The Microbial Electrolysis Cell (MEC) is a novel technology for wastewater treatment to produce bioenergy. Typically, an assimilated biofilm is required by the MEC bio-anode to break down the organic content, but biofilm assimilation is a time-consuming process. This study used an unassimilated nickel-foam anode in a single-chamber MEC and reported successful bioenergy production at the end of the first cycle. Synthetic Dairy Manure Wastewater (SDMW) was used as a substrate as well as an inoculum in this solar-powered tubular MEC.

The effects of the exposed surface area of the bio-anode on bioenergy production were also evaluated, using rate limited bio-anode - MEC and fully exposed bio-anode - MEC separation techniques. The former technique achieves a maximum methane production rate of 30.35 ± 0.03 ml/l, 14.2% more than that achieved by the later mentioned technique (26.4 ± 0.05 ml/l). Hydrogen production was approximately 800 ± 5 mm^3 in both experimentations. The maximum generated current in the rate limited bio-anode – MEC was 35.5 mA. Scanning Electron Microscope (SEM) images confirmed the formation of rod-shaped along with round-shaped microbial communities on the anode surface and, interestingly, round-shaped bacteria were also grown on the cathode surface. The production of bioenergy via an unassimilated bio-anode after 13 days of operation, in conjunction with the formation of a microbial community, was a significant success in this area and has opened up many research opportunities for producing instant bioenergy from organic waste.

Keywords: Microbial electrolysis cell, Hydrogen production, methane production, Bioenergy production

TABLE OF CONTENTS

ABSTRACT vi

TABLE OF CONTENTS vii

LIST OF FIGURES x

LIST OF TABLES xiii

LIST OF ABBREVIATIONS xiv

LIST OF PUBLICATIONS xv

Chapter 1 1

1 Introduction 1

1.1 Background 1

1.2 Hydrogen – Fuel of Future 2

1.3 Conventional Techniques for Hydrogen Production 4

1.3.1 Steam Reforming 4

1.3.2 Partial Oxidation 4

1.3.3 Coal Gasification 5

1.4 Waste to Hydrogen – Innovative Technologies 6

1.4.1 Photo-fermentation 6

1.4.2 Dark Fermentation (DF) 7

1.4.3 Microbial Electrolysis Cell (MEC) 8

1.5 Problem Statement 9

1.6 Objectives 10

1.7 Scope of research 10

1.8 Thesis Structure 11

Summary 12

References 13

Chapter 2 17
2 Literature Review 17
2.1 Microbial Electrolysis Cell 17
2.2 Reactor Configuration 18
2.2.1 Double Chamber MEC 18
2.2.2 Single Chamber MEC 20
2.3 Electrode Materials 21
2.3.1 Cathode materials 21
2.3.2 Anode materials 22
2.4 Substrates 22
2.4.1 Non-fermentable organics 23
2.4.1.1 Sodium Acetate 23
2.4.1.2 Glycerol 23
2.4.1.3 Glucose 24
2.4.2 Fermentable organic substrates 28
2.4.3 Domestic wastewater 28
2.4.4 Sugar industry wastewaters 30
2.4.5 Industrial wastewaters 31
2.5 Hybrid MECs 31
2.5.1 Anaerobic Digestion (AD)-MEC hybrid system 31
2.5.2 Microbial Fuel Cell (MFC)-MEC hybrid system 32
2.5.3 Solar Powered MEC - hybrid system 33
2.5.4 Microbial Desalination Cell (MDC)-MEC hybrid system 34
Summary 36
References 37

Chapter 3 45
3 Materials and Methodology 45
3.1 Designing of the MEC 45
3.2 Construction of the MEC 47
3.3 Configuration of the MEC system 50
3.4 Characteristics of the wastewater: 51
3.5 Characteristics of the solar cell: 52
3.6 MEC start-up and operation: 53
3.7 Investigations and Calculations: 54
Summary: 56
References 57
Chapter 4 58
4 Results and Discussion 58
4.1 Polycrystalline solar cell – MEC mechanism 58
4.2 Bioenergy production from the unassimilated anode 59
4.3 Current generation from the unassimilated anode 63
4.4 Biofilm assimilation at electrodes 64
Summary 71
References 72
Chapter 5 76
5 Conclusion and Recommendations 76
5.1 Conclusion 76
5.2 Recommendation 77
Appendix A 78

LIST OF FIGURES

Figure 1-1 : 2020 and 2030 energy consumption 1

Figure 1-2 : Hydrogen energy production methods and major uses of hydrogen gas 3

Figure 1-3 : Thermodynamic representation of the partial oxidation and steam reforming of methane 5

Figure 1-4 : Schematic representations of Photo fermentation and Dark-fermentation 7

Figure 1-5 : Biological ways for producing biohydrogen (H_2) 8

Figure 2-1 : Microbial Electrolysis Cell (MEC) - schematic diagram..... 17

Figure 2-2 : Double Chamber Microbial Electrolysis Cell 19

Figure 2-3 : Single Chamber Microbial Electrolysis Cell 20

Figure 2-4 : Schematic diagram of the Microbial Electrolysis Cell (MEC) - Anaerobic Digestion (AD)..... 32

Figure 2-5 : Microbial Electrolysis Cell (MEC) - Microbial Fuel Cell (MFC) Hybrid System..... 33

Figure 2-6 : (a) Schematic diagram of self-sustained Hybrid system - Microbial Electrolysis Cell (MEC) - Dye Synthesized Solar Cell (DSSC). (b) Experimental setup of Microbial Electrolysis Cell (MEC) - Dye Synthesized Solar Cell (DSSC) Hybrid system 34

Figure 2-7 : Microbial Desalination Cell (MDC)- Microbial Fuel Cell (MEC) Hybrid System..... 35

Figure 3-1 : Electrodes shape and placement (previous study) 46

Figure 3-2 : (a) Schematic and (b) Experimental representation of the MEC Design..... 46

Figure 3-3 : Ni-foam with 300g/m^2 surface density - anode 47

Figure 3-4 : Stainless steel mesh grade 304L - cathode..... 48

Figure 3-5 : Rate limited bio-anode - MEC (left) and fully exposed bio-anode - MEC (right) 49

Figure 3-6 : Schematic representation of the complete MEC system 50

Figure 3-7 : Polycrystalline silicon solar cell 52

Figure 3-8 : Set-up of complete lab-scale MEC system 53

Figure 3-9 : (A) Gas chromatography - GC-2010 (B) Portable Multi-parameter Professional Waterproof EC/TDS/Resistivity/Salinity Meter (C) Data acquisition system and (D) Scanning electron microscope (SEM) 55

Figure 4-1 : Solar - MEC system electron transfer mechanism representing Z scheme of photosynthesis 58

Figure 4-2 : Hydrogen production using rate limited bio-anode - Microbial Electrolysis Cell 60

Figure 4-3 : : Methane production rate using rate limited bio-anode - Microbial Electrolysis Cell 61

Figure 4-4: Bioenergy production Trends using fully exposed Microbial Electrolysis Cell (above) hydrogen production (below) Methane Production rate 62

Figure 4-5: Current produced in Rate limited bio-anode - MEC (left) and fully exposed bio-anode - MEC (right) 64

Figure 4-6 Scanning Electron Microscopy (SEM) images of Ni-foam bio-anode: (A, B, C) Ni-foam images before use with 200 um, 50 um and 20 um magnification respectively; (D, E, F) Ni-foam images after use in MEC with 200 um, 50 um and 20 um magnification respectively for comparison of morphological changes; (G, H, I) Images of biofilm formed on Ni-foam surface and shape of the microbial community formed with 20 um, 10 um and 2um. (Red box depicts the area from where magnified SEM image was taken in next frame) (Round or circular microbial community was marked with yellow circles and rod-like microbial community was marked with green circles) 66

Figure 4-7 : EDS results of Ni-foam anode (a)before use and (b) after use 67

Figure 4-8 Elemental compositions (weight %) of Ni-foam anode (a) before and (b) after experimentation 68

Figure 4-9: Scanning Electron Microscopy (SEM) images of Stainless Steel Mesh 304L cathode with 20um magnification (A) before utilization and (B) after use (Round or circular microbial community was marked with yellow circles and debris-free SSM wire was marked with red rectangular box) 68

Figure 4-10 : EDS results of Stainless steel mesh 304L cathode (a) before use (b) after use. 69

Figure 4-11: Elemental compositions (weight %) of Stainless-steel mesh 304L (a) before and (b) after experimentation 70

LIST OF TABLES

Table 2-1: Reported MECs fed with acetate as a substrate 25

Table 2-2 : Published MECs fed with glycerol as a substrate 26

Table 2-3 : Reported MECs fed with glucose as a substrate 27

Table 2-4 : Various reported MECs fed with fermentable organic substrate 29

Table 3-1 : Dairy wastewater characteristics 51

LIST OF ABBREVIATIONS

GHG	Greenhouse Gases
DF	Dark Fermentation
SDG	Sustainable Development Goal
PEM	Proton Exchange Membrane
CEM	Cation Exchange Membrane
HPR	Hydrogen Production Rate
C.E	Columbic Efficiency
COD	Chemical Oxygen Demand
GDE	Gas Diffusion Electrode
AEM	Anion Exchange Membrane
AD	Anaerobic Digestion
ABR	Anaerobic Baffled Reactor
DSSC	Dye-Sensitized Solar Cell
MDC	Microbial Desalination Cell
HER	Hydrogen Evolution Reaction
BES	Bio-Electrochemical Systems
MEC	Microbial Electrolysis Cell
HPR	Hydrogen Production Rate
COD	Chemical Oxygen Demand
MFC	Microbial Fuel Cell
SSM	Stainless Steel Mesh
DMWS	Dairy Manure Wastewater Slurry
SDMW	Synthetic Dairy Manure Wastewater
SEM	Scanning Electron Microscope
EDS	Energy-Dispersive X-Ray Spectrometry

LIST OF PUBLICATIONS

1. **"Evaluating the use of unassimilated bio-anode with different exposed surface areas for bioenergy production using solar-powered microbial electrolysis cell."** International Journal of Energy Research (IF 5.164, W Category, Q1) by Wiley. https://doi.org/10.1002/er.7091
2. **“Performance efficiency comparison of Microbial Electrolysis Cells (MECs) for sustainable production of biohydrogen – A comprehensive review”** International Journal of Energy Research (I.F 5.164, W Category, Q1) by Wiley. **(Under review)**

Chapter 1

Introduction

1.1 Background

World energy consumption forecast depicts a sharp rise in the energy demand trend, with the annual consumption of energy projected to reach around 16.5 billion tons and increased by 15 % before 2030 [1]. 21st century has not yet seen any substantial decrease in conventional fossil fuel demands. Therefore, natural gas, coal and oil remains the main resources to meet energy demands like, fuel production, electricity generation, heating, and energy for power plants. Figure 1-1 depicts the energy consumption (million tons) of conventional fossil fuels (coal, oil, and natural gas), nuclear energy, hydro energy, biomass and biofuels, wind and solar energy in the year 2020 and compares it with forecasted energy consumption rate in the year 2030 [2].

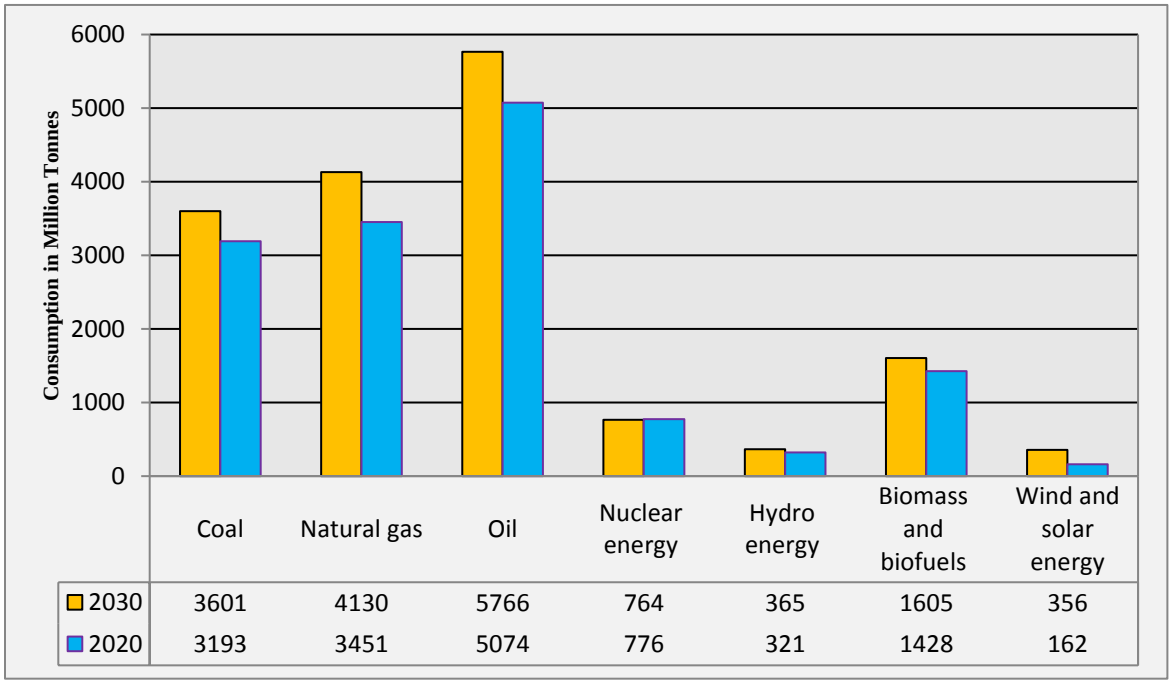

Figure 1-1 : 2020 and 2030 energy consumption [2]

Energy generation at such a fast pace using conventional fossil fuels will ultimately deplete most of the natural available energy resources and results in future energy security crisis. In addition to this, the combustion of fossil fuel produces Greenhouse gases (GHGs) that are primarily accountable for global climate change and greenhouse effect [3][4]. The world's most used energy resource is oil, and the transportation sector is the major contributor for this high oil consumption. GHG emissions from the transportation have increased at much rapid pace than any other energy consuming sector and reported to be increased by 84% since 1970. The emissions from transportation sector could reach around 12 Gt CO_2/year by 2050 without implementation of proper environmental adaptation strategies. According to a recent study, 1 GWh energy generation using coal produces 889 tons CO_2 followed by oil and natural gas that is about 735 and 502 tons CO_2 respectively. Energy security, GHG emissions, global warming, and climate change are some of the major concerns caused by rapid consumption of conventional fossil fuels, especially oil. Therefore, the most crucial preventive action in the immediate future is to lessen the energy consumption rate as well as to invest in alternative, carbon free, renewable energy resources to mitigate such environmental concerns and to fulfill the necessities of living in a sustainable society [5,6].

1.2 Hydrogen – Fuel of Future

Hydrogen (H_2) is considered as a viable option for clean energy production because combustion of hydrogen ends up with useful energy and water only. Hydrogen as an energy sources is gaining more importance due to its odorless, lightest, colorless and non-carcinogenic properties. It is the most promising, cost-efficient solution as a clean secondary energy fuel for the future economy as it is an environmental friendly, sustainable, non-toxic fuel, has the highest energy content per weight and has nearly zero pollutant emissions and water as the only end product [7,8]. Hydrogen can be used as fuel in power generation systems using fuel cells, in transportation sector and in turbines or internal combustion engines to produce electricity. Various hydrogen production methods (Figure 1-2), both renewable and non-renewable, have been developed so far i-e natural gas reforming, auto-thermal reforming, partial oxidation, pyrolysis of organic matter, coal gasification, biomass conversion, biomass gasification, biomass reforming, dark

fermentation, thermochemical water splitting, plasma arc decomposition, photo-electrolysis, metal acid reaction and water electrolysis using renewable energy resources (wind, solar, tidal etc.), but all have certain limitations [9–12].

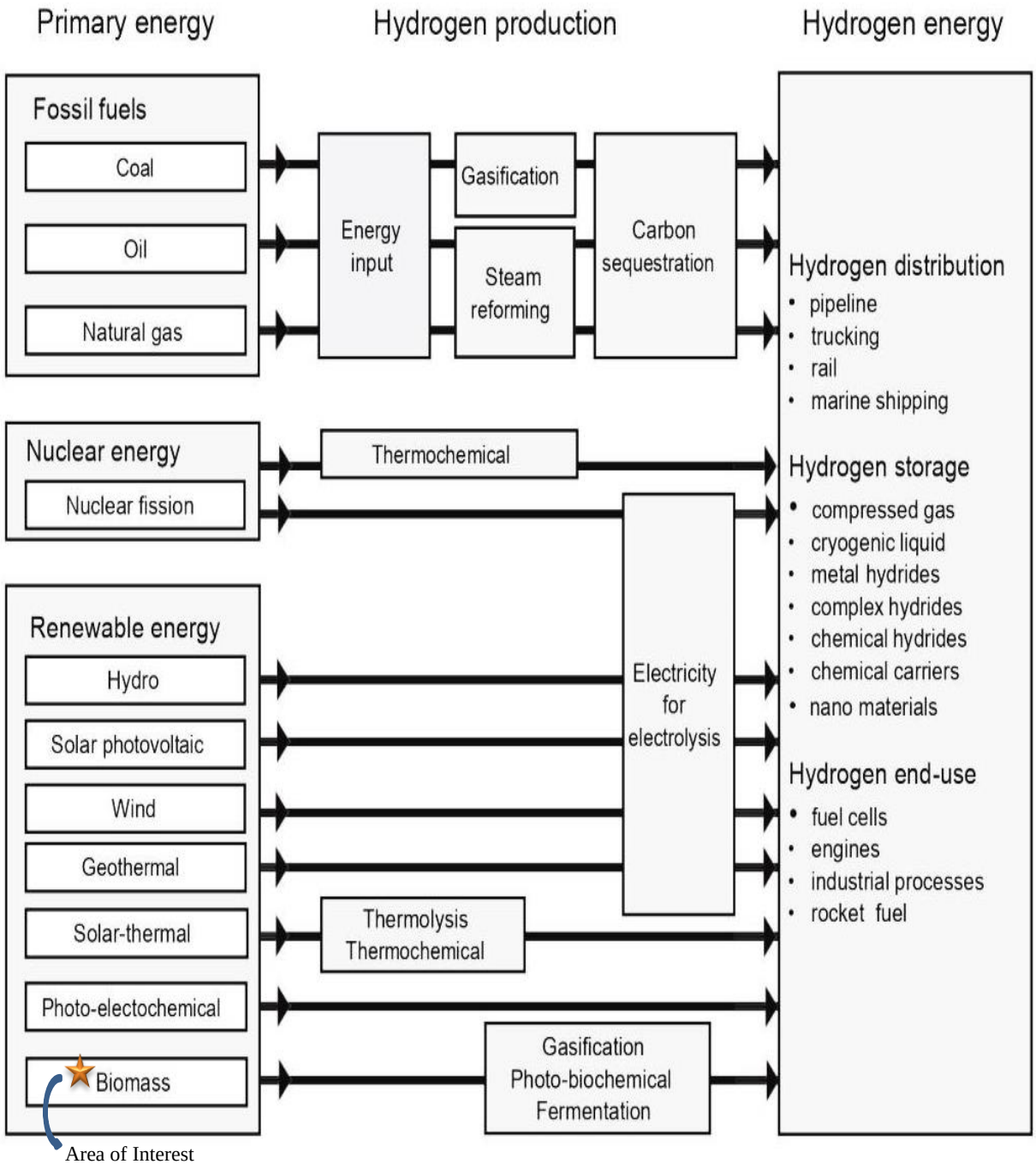

Figure 1-2 : Hydrogen energy production methods and major uses of hydrogen gas [13]

1.3 Conventional Techniques for Hydrogen Production

1.3.1 Steam Reforming

Steam reforming is the most developed, highly efficient and least expensive industrial process for hydrogen production by the catalytic conversion of natural gas to syngas (H_2 + CO). The first step is heating of the gas using steam at 700–1100 °C in the presence of a nickel catalyst. This reaction is endothermic in nature and splits CH_4 into H_2 and carbon monoxide (CO) [14].

$$CH_4 + H_2O \rightarrow CO + 3\,H_2$$

The byproduct of this reaction, CO, goes through a water gas shift reaction to convert this byproduct to useful hydrogen energy. This step involves passing of CO with steam at 360 °C over the iron oxide or other oxides.

$$CO + H_2O \rightarrow CO_2 + H_2$$

The downside to this process is that its byproducts are major atmospheric release of CO_2, CO and other greenhouse gases. The major drawback of this process is that it requires an external heating source and also byproducts like CO_2, CO and other greenhouse gases are associated with this process [10].

1.3.2 Partial Oxidation

Partial oxidation of methane or other hydrocarbons is an energy efficient technology similar to reforming with conversion efficiency up to 70%. Partial oxidation involves combustion of methane by supplying limited amount of air or oxygen (less than that required for complete oxidation) to produce H_2 rich syngas. This process is less expensive than that of stream reforming as it does not requires any catalyst but high temperature of this process and complex handling makes this process inappropriate for industrial scale production [15,16].

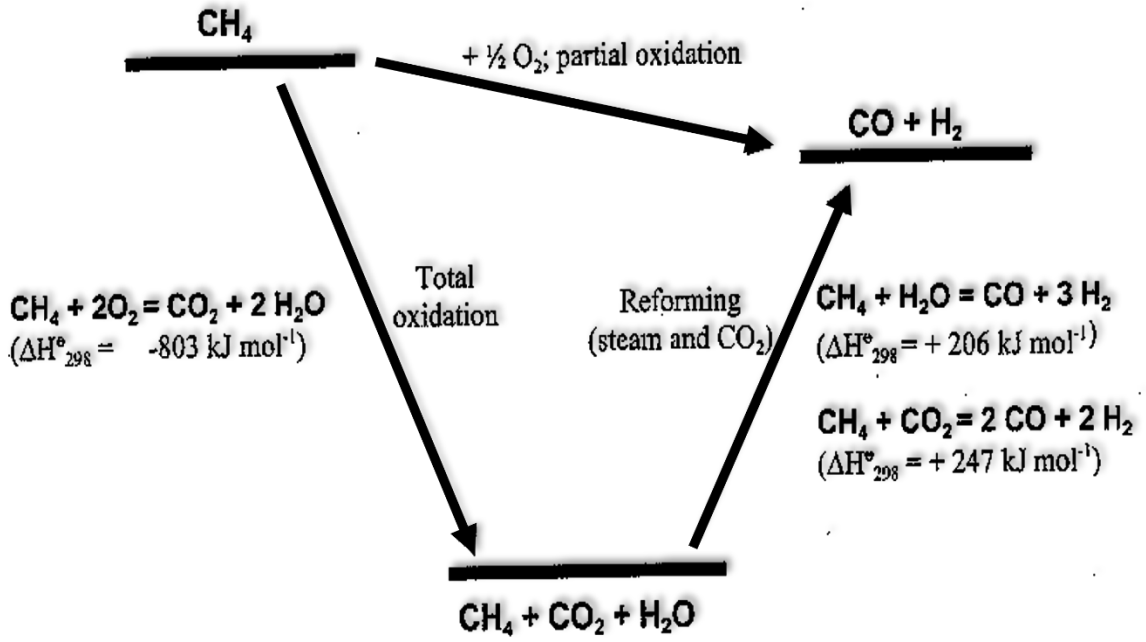

Figure 1-3 : Thermodynamic representation of the partial oxidation and steam reforming of methane [15]

1.3.3 Coal Gasification

Coal gasification is an economical and technically practical option to produce hydrogen in the large-scale plants because the raw material for this process is cheap and abundant in nature. The hydrogen production cost by this process is slightly higher than that of steam reforming due to its reactor setup and operational costs [16,17].

Nevertheless, it is difficult to validate that the use of hydrogen merely can overcome the climatic issues. The interesting point to be noted here is that hydrogen is a secondary energy source like electricity and is derived from primary energy resources. The method selected for production of hydrogen is of critical concern which defines whether to get environmental benefits and energy security by using hydrogen or not. Hydrogen production using coal as primary energy will ultimately results in same difficulties of energy security, GHG emissions and climate change but if hydrogen is produced using renewable energy resources (wind, solar, hydro, biomass etc.), it reduces GHG emissions, minimizes climate change problem and contributes to the energy security for future generation [18].

Therefore, carbon neutral, environmental friendly and sustainable hydrogen production methods are of keen interest [10].

1.4 Waste to Hydrogen – Innovative Technologies

Hydrogen production from organic wastes, wastewaters, and biomass proves to be one of the most efficient and sustainable hydrogen production methods. The major biomass resources are forest residues, agricultural crops and resides, animal residues and wastewaters, domestic wastewaters and industrial effluents. Organic wastes from all these activities can act as the electron donors, react with the produced proton, and used for fermentative biohydrogen production. In spite of the great potential of extracting renewable and sustainable energy out of the organic wastes and agricultural residues, we haven't achieved the maximum yield due to lack of mature technologies. So as to make biomass energy affordable and practical in the near future, renewable energy researchers have to purpose innovative and cost-effective techniques to capture precious energy out of these organic substrates. To cope with this, innovative and emerging technologies such as Microbial Electrolysis Cell (MEC), Dark Fermentation (DF), Photo-fermentation or a combination of these technologies could be a good decision to obtain the biohydrogen energy from biomass [12,18,19].

1.4.1 Photo-fermentation

Photo-fermentation process is a very confident method to produce hydrogen owing to the sufficient hydrogen production rate, use of renewable energy for fermentation, the mild reaction conditions, and utilization of organic waste. Photosynthetic bacteria (nitrogenase enzymes) play a vital part in conversion of substrate to hydrogen during photo-fermentation process. Therefore, the selection of photosynthetic bacteria is a critical step as it has direct effect on the conversion efficiency. Treatment of organics wastes and excellent conversion of organic waste to H_2 and CO_2 are some of the major advantages of this process. The disadvantages of this process include low light conversion efficiencies, the high energy demand by nitrogenase enzymes and need of anaerobic conditions for large areas [12,19,20].

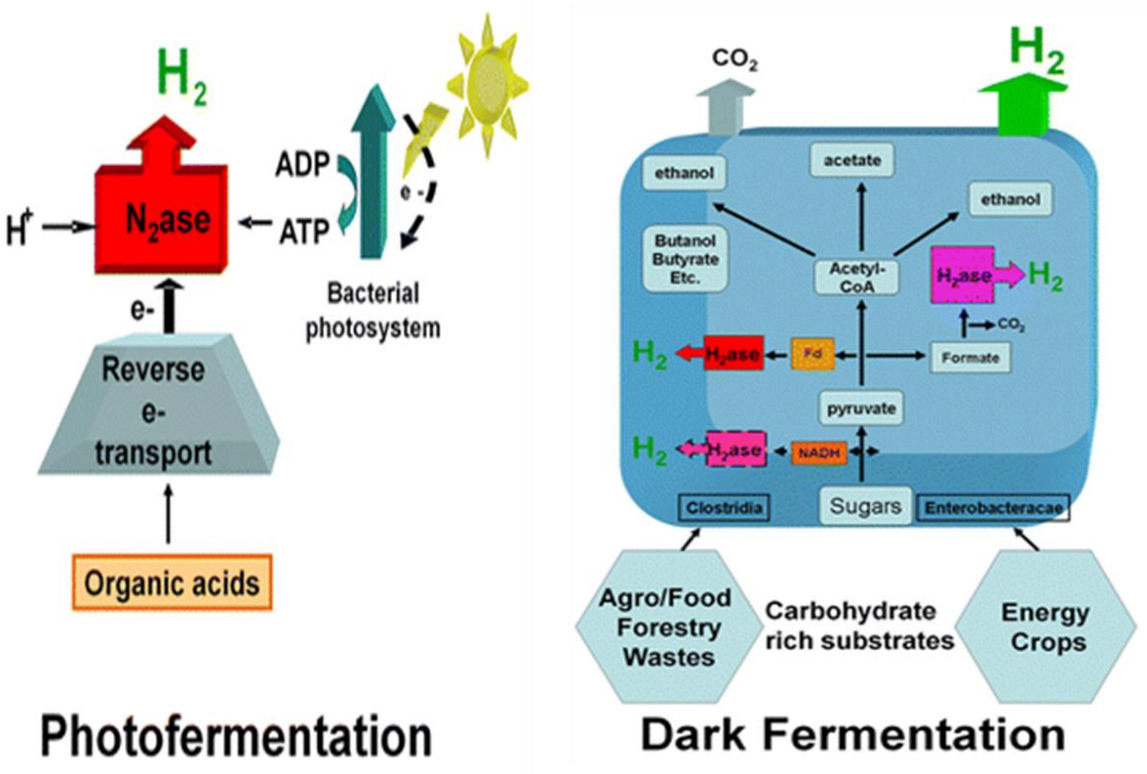

Figure 1-4 : Schematic representations of Photo fermentation and Dark-fermentation [19]

1.4.2 Dark Fermentation (DF)

Dark fermentation is a unique fermentative conversion method to extract stored biochemical energy from organic wastes in absence of light and convert it into other useful forms of energy. This conversion technique is less expensive as compared to photo-fermentation as the DF process does not require solar input processing. This technology can be used for wastewater treatment as well as various organic substrates can be used for bio-hydrogen production in DF process but comparative low H_2 yield, low COD removal rate and lack of control on this technology makes it hard to adapt. Low COD removal means the effluent from DF still have capability to produce bio-hydrogen. Therefore, solution for this problem is of great interest [20–24].

Figure 1-5 depicts the biochemical reactions undergoing in various biological processes for bio hydrogen production using organic waste as substrate.

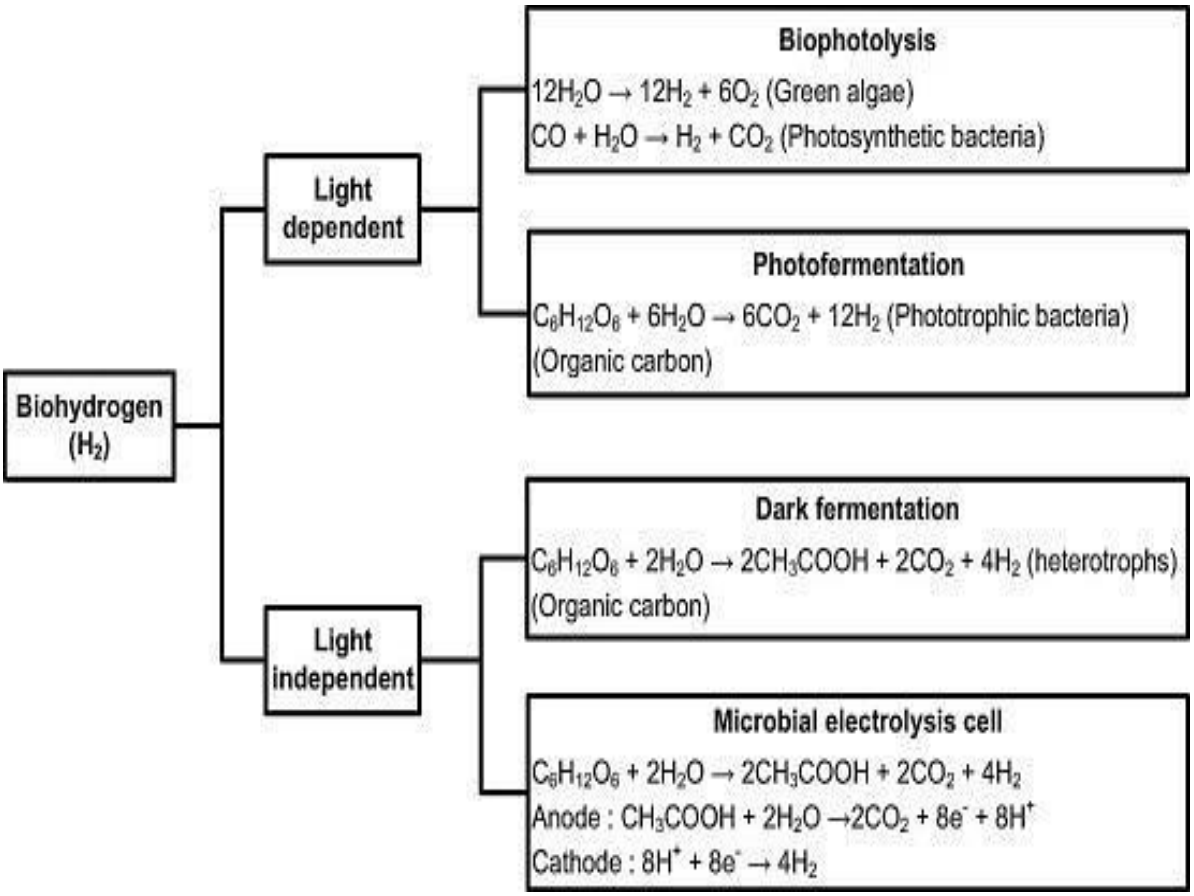

Figure 1-5 : Biological ways for producing biohydrogen (H_2) [20]

1.4.3 Microbial Electrolysis Cell (MEC)

MEC is an innovative BES technology, which produced hydrogen by decomposition of organic wastes (Liquid) or wastewaters in anodic compartment in a sustainable and renewable manner. Various organic substrates, wastewaters as well as effluent from industrial process can be used as substrate in MEC; therefore, the problems associated with DF effluent can also be solved using MEC. This technique of biohydrogen generation requires small external voltage (0.3-0.9), which is approximately half of that required for electrolysis of water [25–27].

MEC is an innovative technique for the wastewater treatment and has significant leads over other hydrogen generation technologies. (1) The output by the electrolysis of waste organic matter is hydrogen gas which makes it a value-added technology (2) The hydrogen recovery from MEC is in the range of 67%-91%, while its value is only 20% in

dark fermentation. (3) Water electrolysis has ten times less maximum energy yield as compared to MECs. Moreover, MECs require small external voltage (0.3-0.9), which is approximately half of that required for electrolysis of water [28,29]. (4) The microbial organisms present in MEC inoculum and anode can breakdown various available substrates, ranging from industrial and lignocellulosic waste as well as different wastewaters [29–31]. Despite its many advantages, MECs are not still matured enough to take over the other hydrogen production technologies because of various problems discussed in section 1.5.

1.5 Problem Statement

MEC has great potential to be the most prominent technology for bioenergy production (hydrogen and methane) as it can exploit organic byproducts and wastewaters to produce bioenergy, but the main problem associated with MEC is that most of the researchers utilized the conventional power supply to run MEC which indirectly affects the environment. Sustainable Development Goal (SDG) 7 focuses on a clean and sustainable energy supply to meet the worldwide energy demand [32]. Numerous alternative energy resources are available but solar energy is pondered as the most suitable choice for fulfilling the energy needs because the enormous energy content of sunlight (1000 W/m^2) is available on the earth daily [33,34]. Therefore, solar cells were suggested as a replacement for conventional DC power sources because they can deliver adequate power for MEC operation.

All the researchers have utilized the pre-assimilated bio-anode in MEC for hydrogen evolution. The time needed for the assimilation of biofilm at bio-anode surface is a time-consuming process. The assimilation of microorganisms on bio-anode surface was usually done by using anode in the Microbial Fuel Cell (MFC) for at least two months. Biofilm assimilation can also be done by using anode in MEC until a stable amount of hydrogen was produced for at least 5 batch cycles. The time needed for the assimilation of biofilm for bioenergy production should be reduced or such material should be used that produces bioenergy without assimilation in order to commercialize this technology. If a breakthrough in the bioenergy production rate could be achieved, then it will provide a

promising solution for the production of biofuels especially hydrogen with low input energy.

1.6 Objectives

Taking into account all the recent advances of MEC, this research will use the most suitable materials in MEC for enhancing bioenergy production using wastewater. The objectives of this research are as follows:

- Use of unassimilated Ni-foam bio-anode to evaluate its effect on bioenergy production.
- Enhancing the bioenergy production rate using dairy manure wastewater both as inoculum and substrate.
- Prevent the rate limitation due to bioanode using co-axial configuration of anode to increase surface area.
- Using a renewable energy resource (solar cells) to supply the required power to MEC.

1.7 Scope of research

In this research, an unassimilated Ni-foam was used as anode in solar-powered single –chamber tubular MEC and the effects of exposed surface of unassimilated anode was examined using two different separation techniques. The substrate utilized in this study was synthetic dairy manure wastewater due to its abundance in country, easy availability, and presence of microbial community in it. Gas chromatography, Scanning Electron Microscopy and Electron Dispersive X-Ray Spectroscopy was performed to evaluate the bioenergy production rate of methane and hydrogen, growth of microbial community on bio-anode and variations in elemental composition before and after the experiment.

1.8 Thesis Structure

Chapter 1

- Provides an overview about world energy consumption, followed by the discussion on hdrogen- future fuel.
- Various hydrogen production methods have been discussed in detail. The objectives of this research and the scope of the ressearch was also discussed.

Chapter 2

- The comprehensive literature review was presented in this chapter.
- MEC reactor configuration, electrode materials, substrates utilized and hybrid MEC systems were discussed in detail.

Chapter 3

- This chapter covered the complete flow of research. The discussion started from the designing of the MEC, followed by its construction and configuration.
- At the end of this chapter, the startup of MEC followed by analysis and calculatons was discussed.

Chapter 4

- All the results obatined using lab-sclae MEC was plotted in this chapter.
- Discussion on the parameters and reasons that could be responsible for these results have been ellaborated.

Chapter 5

- This chapter concludes the complete research methodology, obatined objectives, the results obtained by this research and a thorough discussion on obtained results.
- Recommendations for upcoming researchers have also suggested.

Summary

This chapter discussed the background of the proposed work and enlightened how hydrogen gas could be a competitive alternative as a clean energy source. Various conventional hydrogen production techniques i.e., steam reforming, partial oxidation, and coal gasification, were discussed in detail and the advantages and disadvantages of each technique was presented in this chapter. Furthermore, innovative waste to hydrogen production techniques that are Dark Fermentation, Photo-fermentation, and Microbial Electrolysis Cell (MEC), were compared and it was suggested that MEC was novel and competent technology to effectively produce bioenergy. Finally, the reason behind selection of this research, the objectives of this study and the scope of the proposed work was discussed.

References

[1] Khare V, Khare C, Nema S, Baredar P. Chapter 1 - Introduction to Energy Sources. In: Khare V, Khare C, Nema S, Baredar PBT-TES, editors., Elsevier; 2019, p. 1–39. https://doi.org/https://doi.org/10.1016/B978-0-12-814881-5.00001-6.

[2] Ralph S, Roberto S, Felix C, Xochitl C-N, Marcio D. Transport. Clim. Chang. 2014 Mitig. Clim. Chang. Contrib. Work. Gr. III to Fifth Assess. Rep. Intergovern-mental Panel Clim. Chang., Cambridge University Press; 2014.

[3] Velders GJM, Andersen SO, Daniel JS, Fahey DW, McFarland M. The importance of the Montreal Protocol in protecting climate. Proc Natl Acad Sci 2007;104:4814 LP – 4819. https://doi.org/10.1073/pnas.0610328104.

[4] Avtar R, Tripathi S, Aggarwal AK, Kumar P. Population–Urbanization–Energy Nexus: A Review. Resour 2019;8. https://doi.org/10.3390/resources8030136.

[5] Statistics M of. Energy Statistics, 2019 of India. 2019.

[6] AEDB. "Alternative Energy Development Board." 2015 Report 2015.

[7] Escapa A, Manuel M-F, Morán A, Gómez X, Guiot SR, Tartakovsky B. Hydrogen Production from Glycerol in a Membraneless Microbial Electrolysis Cell. Energy & Fuels 2009;23:4612–8. https://doi.org/10.1021/ef900357y.

[8] Khan MZ, Nizami DA-S, Rehan M, Ouda O, Sultana S, Ismail I, et al. Microbial electrolysis cells for hydrogen production and urban wastewater treatment: A case study of Saudi Arabia. Appl Energy 2017;185:410–420. https://doi.org/10.1016/j.apenergy.2016.11.005.

[9] Abe JO, Popoola API, Ajenifuja E, Popoola OM. Hydrogen energy, economy and storage: Review and recommendation. Int J Hydrogen Energy 2019;44:15072–86. https://doi.org/https://doi.org/10.1016/j.ijhydene.2019.04.068.

[10] Dincer I, Acar C. Review and evaluation of hydrogen production methods for better sustainability. Int J Hydrogen Energy 2015;40:11094–111. https://doi.org/https://doi.org/10.1016/j.ijhydene.2014.12.035.

[11] Acar C, Dincer I. Comparative assessment of hydrogen production methods from renewable and non-renewable sources. Int J Hydrogen Energy 2013;39:1–12. https://doi.org/10.1016/j.ijhydene.2013.10.060.

[12] Bičáková O, Straka P. The resources and methods of hydrogen production

2014;7:175–88.

[13] Chapter 1 Why Hydrogen Energy? Hydrog. Energy Challenges Prospect., The Royal Society of Chemistry; 2008, p. 1–33. https://doi.org/10.1039/9781847558022-00001.

[14] Zanthoff HW. steam reforming, 2020. https://doi.org/10.1002/9783527809080.cataz15706.

[15] York A, Xiao T, Green M. Brief Overview of the Partial Oxidation of Methane to Synthesis Gas. Top Catal 2003;22:345–58. https://doi.org/10.1023/A:1023552709642.

[16] Basu P. Chapter 11 - Biomass Combustion and Cofiring. In: Basu Pyrolysis and Torrefaction (Third Edition) PBT-BG, editor., Academic Press; 2018, p. 393–413. https://doi.org/https://doi.org/10.1016/B978-0-12-812992-0.00011-X.

[17] Speight J. Overview of Gasification 2020:35–65. https://doi.org/10.1002/9781118773970.ch2.

[18] Ball M, Wietschel M. The future of hydrogen – opportunities and challenges. Int J Hydrogen Energy 2009;34:615–27. https://doi.org/https://doi.org/10.1016/j.ijhydene.2008.11.014.

[19] Dincer I. Green methods for hydrogen production. Int J Hydrogen Energy 2012;37:1954–71. https://doi.org/https://doi.org/10.1016/j.ijhydene.2011.03.173.

[20] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudie R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Appl Energy 2015;144:73–95. https://doi.org/https://doi.org/10.1016/j.apenergy.2015.01.045.

[21] Kadier A, Jain P, Lai B, Kalil MS, Kondaveeti S, Alabbosh KFS, et al. Biorefinery perspectives of microbial electrolysis cells (MECs) for hydrogen and valuable chemicals production through wastewater treatment. Biofuel Res J 2020;7:1128–42. https://doi.org/10.18331/BRJ2020.7.1.5.

[22] Tang JH. A Review of Suitable Substrates for Hydrogen Production in Microbial Electrolysis Cells. IOP Conf Ser Earth Environ Sci 2021;621:12145. https://doi.org/10.1088/1755-1315/621/1/012145.

[23] Hasibar B, Ergal İ, Abreu Dias S, Bochmann G, Rittmann SK-MR, Fuchs W.

Competing acetate consumption and production inside a microbial electrolysis cell. J Environ Chem Eng 2020;8:103847. https://doi.org/https://doi.org/10.1016/j.jece.2020.103847.

[24] Palomo-Briones R, Razo-Flores E, Bernet N, Trably E. Dark-fermentative biohydrogen pathways and microbial networks in continuous stirred tank reactors: Novel insights on their control. Appl Energy 2017;198:77–87. https://doi.org/10.1016/j.apenergy.2017.04.051.

[25] Cardeña R, Cercado B, Buitrón G. Chapter 7 - Microbial Electrolysis Cell for Biohydrogen Production. In: Pandey A, Mohan SV, Chang J-S, Hallenbeck PC, Larroche CBT-B (Second E, editors. Biomass, Biofuels, Biochem., Elsevier; 2019, p. 159–85. https://doi.org/https://doi.org/10.1016/B978-0-444-64203-5.00007-1.

[26] Varanasi JL, Veerubhotla R, Pandit S, Das D. Chapter 5.7 - Biohydrogen Production Using Microbial Electrolysis Cell: Recent Advances and Future Prospects. In: Mohan SV, Varjani S, Pandey ABT-MET, editors. Biomass, Biofuels Biochem., Elsevier; 2019, p. 843–69. https://doi.org/https://doi.org/10.1016/B978-0-444-64052-9.00035-2.

[27] Xiang L, Dai L, Guo K, Wen Z, Ci S, Li J. Microbial electrolysis cells for hydrogen production. Chinese J Chem Phys 2020;33:263–84. https://doi.org/10.1063/1674-0068/cjcp2005075.

[28] Jeon Y, Kim S. Persistent Hydrogen Production by the Photo-Assisted Microbial Electrolysis Cell Using a p-Type Polyaniline Nanofiber Cathode. ChemSusChem 2016;9:3276–9. https://doi.org/https://doi.org/10.1002/cssc.201600906.

[29] Kadier A, Al-Shorgani N, Sonawane J, Singh A, Kalil M, Abu Hasan H, et al. Microbial Electrolysis Cell (MEC): An Innovative Waste to Bioenergy and Value-Added By-product Technology, 2020, p. 95–128. https://doi.org/10.1002/9783527343829.ch4.

[30] Zhang Y, Angelidaki I. Microbial electrolysis cells turning to be versatile technology: Recent advances and future challenges. Water Res 2014; p. 11-25. https://doi.org/10.1016/j.watres.2014.02.031.

[31] Katuri K, Ali M, Saikaly P. The role of microbial electrolysis cell in urban wastewater treatment: integration options, challenges, and prospects. Curr Opin

Biotechnol 2019;57:101–10. https://doi.org/10.1016/j.copbio.2019.03.007.

[32] Seyedsayamdost E. Sustainable Development Goals, 2020, p. 251–2. https://doi.org/10.4324/9780367816681-102.

[33] Chae K-J, Choi M-J, Kim K-Y, Ajayi F, Chang I-S, Kim I. A Solar-Powered Microbial Electrolysis Cell With a Platinum Catalyst-Free Cathode To Produce Hydrogen. Environ Sci Technol 2009;43:9525–30. https://doi.org/10.1021/es9022317.

[34] von Friedeburg C. Solar Energy for a Cleaner World. Sci Res 2017. https://doi.org/10.14293/S2199-1006.1.SOR-PHYS.EJWPJW.v1.

Chapter 2

Literature Review

2.1 Microbial Electrolysis Cell

Microbial Electrolysis cell (MEC) is a novel BES technology, related to Microbial Fuel Cell (MFC), which interrelate the exoelectrogens metabolism with electrochemistry to produce hydrogen in sustainable and renewable manners from organic wastes (Liquid) or wastewaters. The perception of MEC was first presented in 2005 by two different laboratories [1]. A conventional MEC consists of a microbial anode, separator (Ion exchange membrane), a hydrogen evolution cathode, and H-shape double chamber configuration, even though researchers have also developed single chamber MECs by eliminating the need for a separator. Figure 2-1 shows a schematic representation of an MEC consists of bio-anode and cathode to bio-hydrogen.

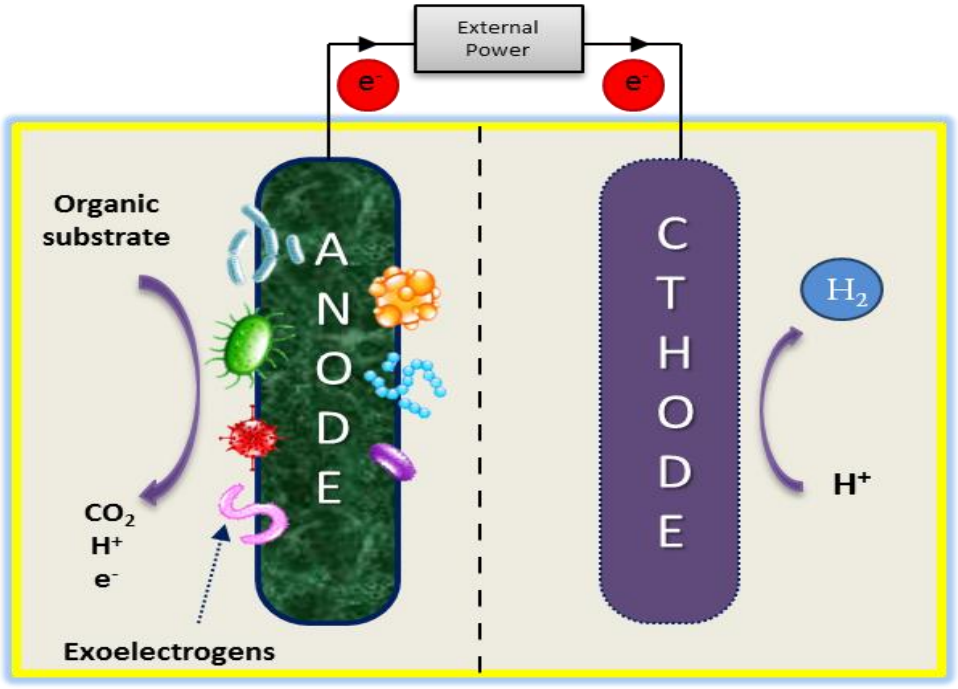

Figure 2-1 : Microbial Electrolysis Cell (MEC) - schematic diagram

The anode of MECs has biofilm consisting of electrochemically active bacteria that oxidizes the organic matter present in wastewater and generates protons and electrons. Directional migration of these charged particles towards cathode is supported by small external voltage (0.2-0.8V), where they combine to produce hydrogen [2]. MEC has significant leads over other hydrogen generation technologies. MECs have engaged much attention in the researcher's community, as proved by the fact that the base critical review article on MEC has more than 750 citations till now [3]. More than 10 comprehensive review articles have been published on MECs over the last decade, which shows the massive research work that has been carried out to enhance sustainable hydrogen production. These review articles contain updated information about suitable substrates used in MECs [4,5], biocatalysts used in MECs [6], anode and cathode materials utilized in MECs for hydrogen production [7,8], potential applications, advances and challenges of the MECs, biocathodes utilized till now and future prospects about them [2], available MEC reactor configuration and design for production of biohydrogen [9], separators utilized in MEC and the use of MEC in wastewater treatment systems [10].

2.2 Reactor Configuration

MEC can be divided into two reactor configurations:

1. Double Chamber MEC
2. Single Chamber MEC

Both MEC have more or less same working principle with only difference between these two is presence of ions exchange membrane between anode and cathode in double chamber MEC.

2.2.1 Double Chamber MEC

A simple double chamber MEC has H-shaped reactor setup and ions exchange membrane is placed in a tube which connects anode and cathode chambers. Numerous membranes, such as proton exchange membrane (PEM), cation exchange membrane (CEM), and charge-mosaic membranes (CMM), were utilized till now to separate both

compartments. Membrane is a vital component of double chamber MEC and is used to avoid diffusion of generated hydrogen from cathode to anode [9][11].

The anode of MEC operates in a fully anaerobic atmosphere and has microbial community present on its surface to oxidize the organic substrates. A gas collection bag or syringe was attached on top of cathodic compartment to collect produced gas. Considering acetate as substrate in double chamber MEC, following reaction are responsible for hydrogen production:

$$\text{Anode: } C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+$$

$$\text{Cathode: } 8H^+ + 8e^- \rightarrow 4H_2$$

Particularly, this configuration has various energy losses due to many factors, such as Ohmic loss, activation loss, and concentration loss [1][12]. Figure 2-2 demonstrates the schematic representation of double chamber membraneless MEC.

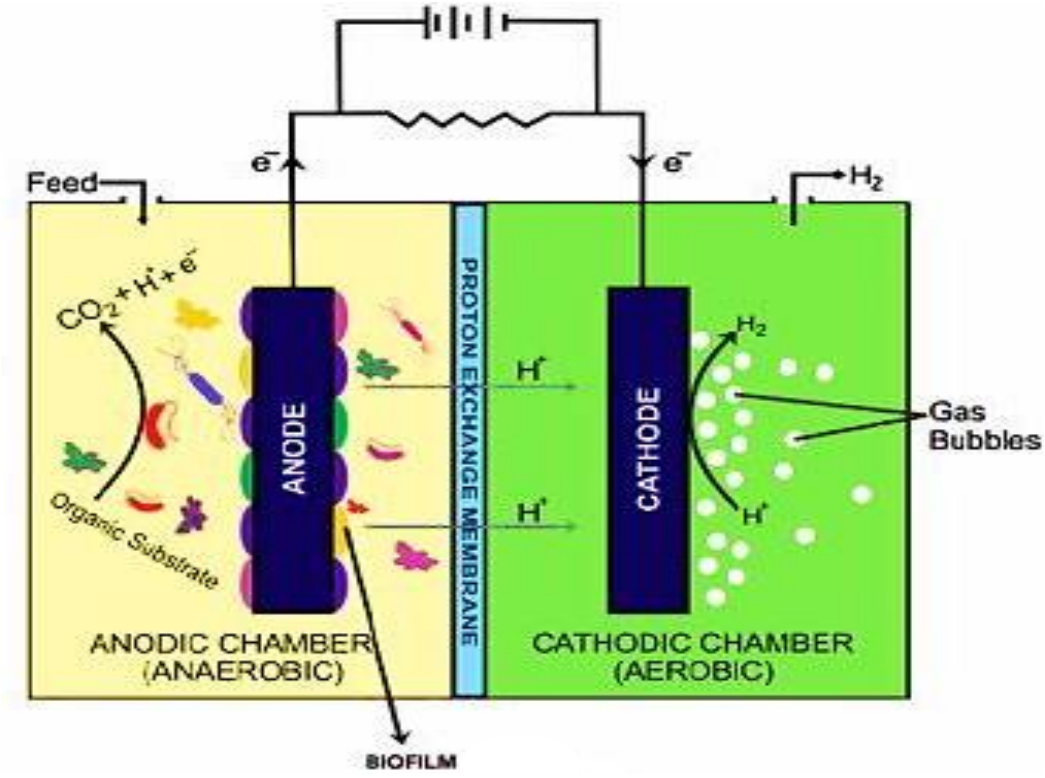

Figure 2-2 : Double Chamber Microbial Electrolysis Cell [13]

2.2.2 Single Chamber MEC

Single-chamber MEC has been designed to simplify the reactor configuration. This design was first proposed and experimented in 2008 in order to overcome the potential ohmic losses and to enhance bio-hydrogen production rate [14]. In a single-chamber MEC, both anode and cathode are present in same anaerobic compartment and the assimilated biofilm at the anode surface break-down the organic wastewater substrate by producing free-moving protons and electrons. The electrons move from the outer circuit towards cathode and the protons can directly diffuse to the cathode to produce hydrogen [15].

High hydrogen production by decrease in internal resistance can be achieved in single chamber MEC with less system cost. The drawback with single chamber MEC is the easy diffusion of hydrogen to the anode resulting in methane formation and severe energy loss because of interruption in the anode reaction and consumption of produced hydrogen by methanogenic microorganisms [16]. Figure 2-3 demonstrates the schematic representation of single chamber membraneless MEC.

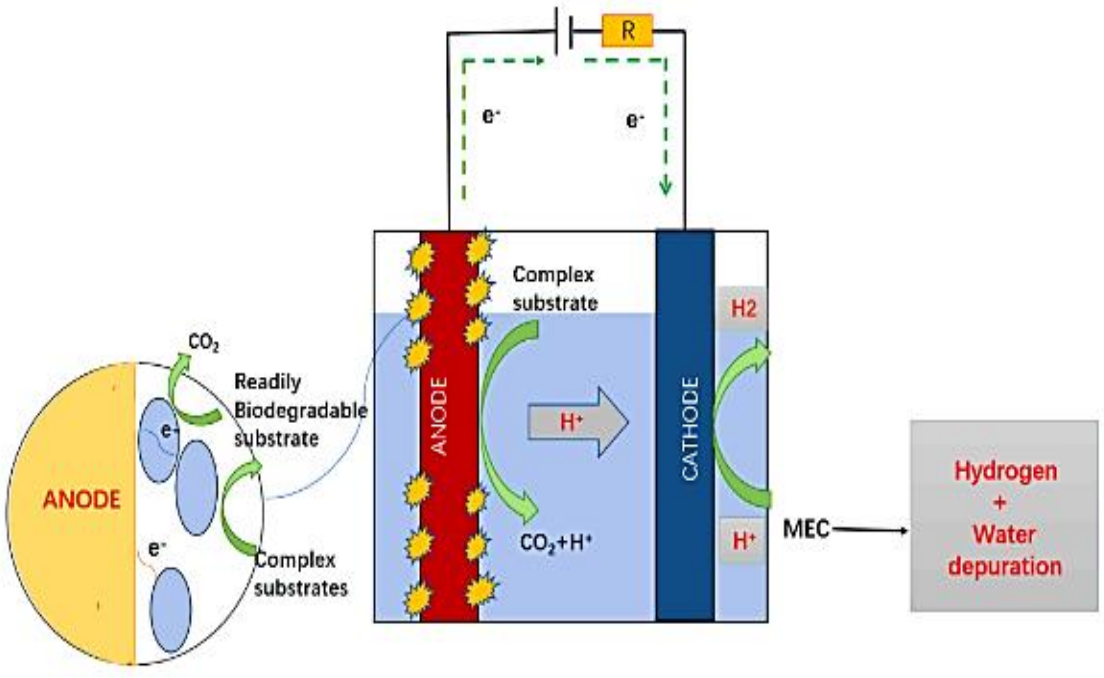

Figure 2-3 : Single Chamber Microbial Electrolysis Cell [12]

2.3 Electrode Materials

Carbonaceous and metallic materials are usually used for anode and cathode, respectively. Both electrode materials must undertake electro-catalysis for the breakdown of organic substrate at the anode and bio-hydrogen production reaction at the cathode. The most critical parameter in microbial electrolysis cells is biofilm development [17].

2.3.1 Cathode materials

The breakdown of organic substrate takes place at the anode surface which releases electrons and protons. The generated electrons flow through an external circuit toward the cathode, where they react with the protons that migrate from the anolyte to the catholyte crossing the membrane to produce bio-hydrogen [18].

Various materials can be used as cathode in MEC, including carbonaceous materials, stainless-steel meshes, nickel-foam, and titanium. Carbon-based cathodes have slow Hydrogen Evolution Reaction (HER) rate due to their high over-potential [19]. HER rate depends on the strength of hydrogen-metal bond, and therefore of the electrode material. Various catalysts like platinum can be used with cathode to reduce this problem but platinum is very rare and expensive material. Moreover, platinum may have negative effect on MEC performance after getting contaminated by other compound i.e. sulfides and cyanides [20].

The most satisfactory substitutes of platinum for fast HER are transition metal compounds of first row of periodic table because of their moderate catalytic activity, abundance, and stability [21]. The stainless steel and nickel-based materials, among other materials, are the extensively reported cathode materials till now because these materials have low cost, are plentiful in nature, excellent catalytic action for HER and have stable electrochemical property [12,18]. A large number of comparative experiments and researches (Table 2-1, Table 2-2, Table 2-3, Table 2-4) discovered that the use of transition metal elements could significantly enhance the HER performance of the cathode in MEC.

2.3.2 Anode materials

An ideal material for anode should possess large surface area, excellent biocompatibility to facilitate bacterial attachment growth, decent corrosion resistance, and high electronic conductivity. Usually carbonaceous materials such as carbon mesh, carbon cloth, carbon paper, vitrified carbon material, graphite felt, graphite rod, graphite fiber brush, and activated carbon granules were used as anode in MEC. The cost, handling of material, availability of materials, and stability should be taken into consideration before selecting it as anode in MEC. Every carbonaceous material has its own downsides depending upon its chemical properties [18,22].

Carbon cloth is a durable and flexible choice for MEC but it is comparatively more expensive than carbon paper. Carbon paper is cheap and thin material but due to its low strength, it does not allow biofilm formation. Carbon felt can take over this biofilm formation issue, but large electrical resistance of this material limits its use. Graphite materials have superior stability and electric conductivity compared to the ordinary carbon-based materials but is more expensive. The graphite fiber brush is considered as the most favorable anode material in future because of its easy availability, low electrical resistance and high surface area [12,17,23].

Aside from graphitic and carbonaceous anodes, there are some highly conductive, corrosion-resistant, highly porous materials, which can also be used as anodes in MECs. Nickel foam, stainless steel meshes, and titanium are some of these materials, which form anode by joining with graphite fiber or carbon cloth. These prospective materials must be pretreated at high temperature with ammonia to reduce the start-up time of the reaction and enhancing the overall hydrogen production efficiency of the system [18]. A list of extensively used anode materials is presented in Table 2-1, Table 2-2, Table 2-3, Table 2-4.

2.4 Substrates

Researchers utilized various substrates including glucose, cellulose, acetic acid, and different organic wastewaters to produce hydrogen using MECs.

2.4.1 Non-fermentable organics

2.4.1.1 Sodium Acetate

Acetate (Sodium Acetate) has been reported as the most commonly used substrate for MEC (Table 2-1) as it is obtained as a by-product from dark fermentation. Rozendal et al. reported one of the first performance efficiencies of the MEC, fed with acetate and reported the maximum HPR of 0.02 m^3-H_2 /m^3-d in MEC reactor having working volume of 6.6 L and applied voltage 500 mV [24]. The best performance efficiency of MEC fed with acetate was reported by Rivera et al. in 2015, He stated the use of 0.55 V applied voltage to get a maximum HPR of 81 mL-H_2/L-d in a double chamber configuration [25]. Jeon et al. used P-type Polyaniline Nano-fibers (PANInfs) cathode material in three different MECs using acetate as substrate and reported the maximum HPR of 1.78 m^3-H2/m^3-d and C.E of 98% at an applied voltage of 0.8 V [26]. The latest study reported the maximum HPR of 7.06 ± 0.24 L-H_2/L-d by applying 1 V voltage for tubular configuration of MEC. The hydrogen recovery efficiency was more than 98% according to that study [27]. Recent research conducted by Hesibar et al. in 2020 to find the most competitive MEC for hydrogen production reported the best HPR of 0.31 ± 0.08 mmol-H^2/L-d at 0.8 V applied potential and the concentration of acetate was 6 g/L in a single chamber MEC (Table 2-1) having working volume 100mL [28]. Acetate is the most commonly used substrate till now as it is required for anode colonization before using any other organic wastewater as substrate.

2.4.1.2 Glycerol

Glycerol, a widely used commodity chemical by pharmaceutical industries, is a non-fermentable carbon source. Glycerol is mainly obtained as a by-product during the biodiesel purification techniques. It is being overproduced as 10L of glycerol is being produced for every 100L of biodiesel purification. Initially, researchers believed that glycerol can only be used in MECs with a mediator to produce hydrogen (Table 2-2). Initial studies reported very low HPR through MEC fed with Glycerol, an experimental study carried out in 2007 reported the utilization of glycerol containing wastewater as a substrate in double chamber MEC having platinum mesh cathode. The reported maximum HPR for

0.5 V applied voltage was only 0.77 mol H_2/ mol-glycerol [29]. Another study reported the use of continuous flow series plates MEC configuration with working volume 200 mL and cathode made-up of E-TEK gas diffusion electrode with platinum (Pt) load of 0.5 mg cm^{-2} to produce hydrogen from glycerol without any mediator, the maximum HPR of 0.6 L-H_2/L-d was obtained by that experimental setup at an applied voltage of 1.0 V [30]. The latest research utilized crude glycerol in an alkaline MEC having a single chamber cubical configuration (Table 2-2). Graphite fiber with Polytetrafluoroethylene (PTFE) diffusion layer and 5 mg Pt/cm^2 was used as cathode and the maximum HPR of 0.46 L-H_2/L-d and C.E of 55% was achieved for 0.8 V applied potential [31].

2.4.1.3 **Glucose**

Hydrogen production using glucose substrate is also a critical point of research during the last decade (Table 2-3). Selembo et al. reported the maximum HPR of 1.87 ± 0.30 m^3 H_2/ m^3/d and the C.E 105 ± 10% at applied voltage 0.9 V. He used a single chamber membrane-less microbial electrolysis cell with working of 28 mL, fed with glucose to produce hydrogen [32]. Research published in 2012, reported the maximum HPR of 0.37 ± 0.04 m^3-H_2/ m^3-d using glucose as a substrate in single-chamber MEC at 4 ^{0}C temperature and applied voltage 0.8 V [33]. Zhang et al. published a research paper in which he utilized double anode configuration, single chamber membrane-less reactor, and methane inhibitor to enhance HPR using glucose as substrate. His research work reported a maximum HPR of 2.39 ± 0.3 m^3H_2/m^3/d at an applied voltage of 0.8V [34]. A recent publication reported the use of glucose in single-chamber MEC (Table 2-3) and compared it with various other substrates. He reported that glucose was not that much effective compared with other substrates like sodium acetate and glucose produce only 9.12% hydrogen [5].

Table 2-1: Reported MECs fed with acetate as a substrate

Reactor Design	Volume (mL)	Anode	Cathode	Applied Voltage (V)	C.E (%)	Max. HPR ($m^3H_2/m^3/d$)	Ref.
Double Chamber	3300 × 2	Graphite felt (dia. 240mm)	Titanium mesh with 0.5 mg/cm^2 Pt catalyst	0.5	53±3.5	0.02	[24]
Single chamber	48	Brush made of graphite fibers	Brush made of stainless steel-304L	0.6	78±5	1.7 ± 0.1	[35]
Double Chamber	35	Plain carbon paper	Carbon Paper with Pt	0.35	92.0 ± 1.8	7.86 ± 0.31 mL/L/d	[36]
				.54	93.2 ± 0.01	10.95 ± 0.64 mL/L/d	
				.80	98.0 ± 1.9	14.54 ± 0.12 mL/L/d	
Double Chamber	40	Graphite felt – compressed	Ni-foam (1360 kg m^{-3})	1.0	90	50	[37]
Double chamber	120	Graphite brush – Heat treated	Carbon cloth with 30% PTFE and 0.5 mg/cm^2 Pt catalyst	0.7	--	0.08 mmol/L/d	[38]
Double Chamber	Anode = 550 Cathode = 225	Carbon fibers attached on stainless steel plate	Stainless steel mesh (304L Grade)	1.0	91	0.53	[39]
Single Chamber	3.4 7.3 13.3	--	P-type Polyaniline Nanofibers (PANInfs)	0.4 0.6 0.8	61 91.5 98.2	0.18 1.02 1.78	[26]
Tubular Double Chamber	1000	316L Stainless steel fiber felt	Pt coated titanium mesh tube	1.0	98	7.06 ± 0.24 L/L/d	[27]
Single Chamber	100	Carbon cloth	Carbon cloth with 0.5 mg/cm^2 Pt catalyst	0.8	--	0.31 ± 0.08 mmol/L/d	[28]

Table 2-2 : Published MECs fed with glycerol as a substrate

Reactor Design	Volume (mL)	Anode	Cathode	Applied Voltage (V)	C.E (%)	Max. HPR ($m^3H_2/m^3/d$)	Ref.
Double Chamber	300	Carbon cloth	Platinum Mesh	0.5	--	0.77 mol H_2/mol-glycerol	[29]
Continuous flow- series plates	210	Graphite felt	0.5 mg/cm^2 Pt catalyst loaded E-TEK gas diffusion electrode	1	--	0.6 L/L/d	[30]
Single Chamber	28.27	Graphite brush – Ammonia treated	Wet-proofed carbon cloth with a platinum catalyst	0.5	99 ± 10	0.80 ± 0.08	[32]
				0.9	104 ± 7	2.01 ± 0.41	
Double Chamber	25	Graphite brush (Heat treated)	stainless steel mesh and carbon cloth	0.6	--	0.015 ± 0.003	[40]
				0.8		0.029 ± 0.0008	
				1.0		0.050 ± 0.0008	
Double Chamber	28	graphite fiber brush	0.5 mg/cm^2 Pt catalyst coated graphite fiber	0.8	35	0.021	[15]
Cube – MEC – Single Chamber	400	Graphite brush	Graphite Fiber with PTFE diffusion layer and 5 mg Pt/cm^2 Pt	0.8	55	0.46 L/L/d	[31]

Table 2-3 : Reported MECs fed with glucose as a substrate

Reactor Design	Volume (mL)	Anode	Cathode	Applied Voltage (V)	C.E (%)	Max. HPR ($m^3H_2/m^3/d$)	Ref.
Single Chamber	28.27	Graphite brush – Ammonia treated	Wet-proofed carbon cloth with a platinum catalyst	0.5	127 ± 23	0.83 ± 0.18	[32]
				0.9	105 ± 10	1.87 ± 0.30	
Single Chamber	26	graphite brush	carbon cloth with 0.5 mg/cm^2 Pt	0.6 (4^{0}C)	82 ± 13	0.25 ± 0.03	[33]
				0.8 (4^{0}C)	74 ± 8	0.37 ± 0.04	
				0.6 (25^{0}C)	59 ± 6	1.01 ± 0.05	
Single Chamber Membrane-less	64	Square graphite felts -2 pieces	carbon cloth with 0.5 mg Pt/cm^2	0.5	70	1.65 ± 0.11	[34]
				0.8	75	2.39 ± 0.3	
				0.9	75	2.24 ± 0.2	
Single Chamber Membrane-less	500	Carbon felt	Platinum	1.0	--	9.12 % (Hydrogen Recovery)	[5]

2.4.2 Fermentable organic substrates

Lignocellulosic biomass is the most plentiful substrate available for the production of bio-ethanol and other biofuels. This raw material consists of cellulose, hemicellulose, and a binding agent (lignin). Virgin biomass, Waste biomass, and energy crops are the three major classifications of lignocellulosic biomass. It is an auspicious feedstock for profitable and low-cost energy production due to its easy availability and renewability. Only few studies reported the use of lignocellulosic biomass as substrate in various MECs. Maximum HPR using cellulose as substrate was validated by Cheng et al. in a dual-chamber MEC at hydrogen yields (63%). It was estimated that producing 1kg of H_2 would take 7.5 kg of cellulose at a 100% yield [41].

Another study stated the production of hydrogen using cellulose effluent and reported the maximum HPR of 1.01. ± 0.16 L-H_2/L-day and 220% energy efficiency by comparing energy from hydrogen produced during the process with required electricity [42]. However, this feedstock cannot be directly utilized in MECs as microbes can't digest lignin (high-molecular weight compounds) readily to produce hydrogen.

2.4.3 Domestic wastewater

Ditzig et al. accomplished a major breakthrough in 2007 as he reported the production of hydrogen using domestic wastewater as a substrate in MEC () and founded Bio-electrochemically Assisted Microbial Reactors (BEAMRs). He used a carbon electrode with graphite granules in an anode compartment and achieved a maximum HPR of 0.0125 mg-H_2/mg-Chemical Oxygen Demand (COD) at applied voltage 0.41 V and C.E of 26 % [43]. Many researchers are working on BEAMRs to produce sustainable hydrogen using domestic wastewater as it can help to mitigate water pollution as well as in the production of cost-effective fuels. A study reported the use of single chamber MEC with continuous flow operation mode, inoculated with 100 mL domestic wastewater and an applied voltage of 1V, to produce hydrogen at a rate of 0.462 L-H_2/L-d [44].

Table 2-4 : Various reported MECs fed with fermentable organic substrate

Reactor Design	Volume (mL)	Anode	Cathode	Applied Voltage (V)	C.E (%)	Hydrogen Recovery (%)	Ref.
Double Chamber	192 or 256	nonwet-proofed carbon paper	0.5 mg/cm^2 Pt catalyst loaded carbon paper	0.23 0.32 0.41 0.50 0.59	9.6 10.4 12.0 16.8 26.4	2.8 3.3 1.8 10.5 37.5	[43]
Continuous flow Single Chamber	90	Graphite felt 5mm thick	Ni-based gas diffusion electrode (GDE) with a Ni load of 0.4 mg/cm^2	1 - Constant	65 59 57 55 54 38	-- 45 44 42 43 45	[44]
6 Cassettes	120L	sheet of carbon felt	stainless steel wire wool - grade 1	1.2	55	0.015 L-H_2/L/day	[45]
Tubular MEC	1000	Graphite felt	EDCORE/ Pt	0.9	98.5 ± 1.0	0.69 ± 0.05 m^3-H_2/m^3-d	[46]
			AMI-7100CR/Pt		86.9 ± 5.0	0.80 ± 0.03 m^3-H_2/m^3-d	
			Acrylic framed AEM wrapped by carbon cloth/Pt		73.5 ± 6.8	0.92 ± 0.03 m^3-H_2/m^3-d	
Double Chamber	250	Graphite	Ni-plate	1.0	45.1	0.817	[47]
			Stainless steel-mesh		54.5	1.329	
			Ni-foam		59.1	1.594	
Double chamber - box	250	Plain graphite plate	Nickel Foam/ NiO.rGO	1.0	54.67	4.38	[48]
			Nickel Foam/ CO_3O_4.rGO		56.64	3.66	
Dual Chamber	250	Plain graphite	$NiMoO_4$/Nickel foam	1.0	58.72	4.28 ± 0.13	[49]

Heidrich et al. published research in 2013, stated the maximum HPR of 0.015 L-H_2/L/day and C.E 55% was achieved using raw domestic wastewater in a cassette-like reactor configuration having a working volume of 120 L and applied voltage 1.2 V [45]. The latest research reported the tubular MEC configuration fed with domestic wastewater substrate and membrane electrode assembly to produce hydrogen at the rate of 0.92 ± 0.03 m^3-H_2/m^3/d at an applied voltage of 0.9V with C.E of 73.5%. This study utilized 3 different material combinations but acrylic framed anion exchange membrane draped by carbon cloth/Pt exhibited the maximum HPR [46].

2.4.4 Sugar industry wastewaters

Sugar industry wastewater gets much attention during the previous 2 years as more than 10 research papers have been published on the title "hydrogen production using sugar industry effluent". Research utilized double chamber MEC and investigated three unique materials as cathode (Nickel foam, Nickel Plate, and stainless steel) to check the most suitable material for maximum hydrogen production using sugar industry effluent. Nickel foam showed the maximum HPR of 1.59 mmol/L-D using sugar refinery effluent at an applied voltage of 1.0 V [47]. Metal oxide/graphene nanocomposite catalysts had also been used for the biohydrogen production in MEC fed with sugar refinery. NiO.rGO and CO_3O_4.rGO nanocomposites were synthesized and coated on Nickel cathode. A study reported superior performance of the cathode with nanocomposites and at an applied voltage of 1.0V displayed the maximum HPR of 4.38 ± 0.11 mmol/L/D, cathodic hydrogen recovery of 20.8%, and C.E of 65.6% [48]. The latest paper reported the enhanced hydrogen production using the nickel molybdate nanocatalyst, those synthesized nanocatalysts were applied at nickel foam cathode surface used in MEC to produce hydrogen. Anode utilized in this study was graphite felt and the substrate used was sugar industry effluent having COD 4200 ± 20 mg/L. The MEC with the novel catalyst performed exceptionally well and achieved the maximum HPR of 0.12 ± 0.01 L-H_2/L/D with 58.2% C.E [49].

2.4.5 Industrial wastewaters

Researchers are trying to make MECs more efficient and sustainable in nature therefore lots of research have been done on MECs using industrial wastewaters as their substrates. A study reported hydrogen production at the rate of 0.74 m^3-H_2/ m^3/d using potato industry wastewater in a single chamber membraneless MEC reactor having a working volume of 28 mL. Graphite fiber brush with ammonia treatment was used as an anode, wet proofed carbon cloth with Pt catalyst was used as cathode and the voltage of 0.9V was supplied through DC power supply. Dairy manure was also utilized in this study but reported that MEC fed with dairy manure didn't produce current [22]. Tenca et al. utilized two different industrial wastewaters; methanol-rich industrial wastewater and wastewater from a food processing industry. Wastewater from the food processing industry was rich in complex carbohydrates and had much less COD removal rate than industrial wastewater (85%). Three different cathodes were utilized but only cathode containing Pt produce a significant amount of biogas using industrial wastewater with C.E 10 ± 2 % [50]. A recent study performs experimental analysis using three different substrates, counting vegetable waste, rice waste, and mixed substrate, in a dual-chamber MEC and reported the maximum HPR of 2.46 mmol/L/D at an applied voltage of 0.8 V with 4.67 g/L concentration of the substrate at the start of experimental analysis. These outcomes recommend that mixed substrates may be more appropriate for hydrogen production in MEC [51].

2.5 Hybrid MECs

MECs solely can produce biohydrogen through organic wastes but are still not capable enough to take over the markets, therefore, integration of MECs with other technologies is the most prominent option to make these systems efficient and market competitive.

2.5.1 Anaerobic Digestion (AD)-MEC hybrid system

Waste organic matters break down in a sequence of processes to yield biogas in a conventional AD process. AD process occurs in the absence of oxygen, which is a major requirement for the growth of methanogenic bacteria [52]. MEC-AD hybrid systems

(Figure 2-4) have gained much importance during the last five years because this integrated system promotes the degradation of organic matter [53] and enriches the exoelectrogen species and hydrogenotrophic methanogenic species on anode and cathode surfaces respectively, thus enhancing the biogas yield [54]. A recent publication reported the 1.4-fold enhanced biogas which is approximately 0.18-0.2 mL-CH_4/mL-d methane production by utilizing AD-MEC integrated system [55]. This integrated system faces numerous problems such as low efficient large-scale operation and biofilm development at electrode surfaces [56].

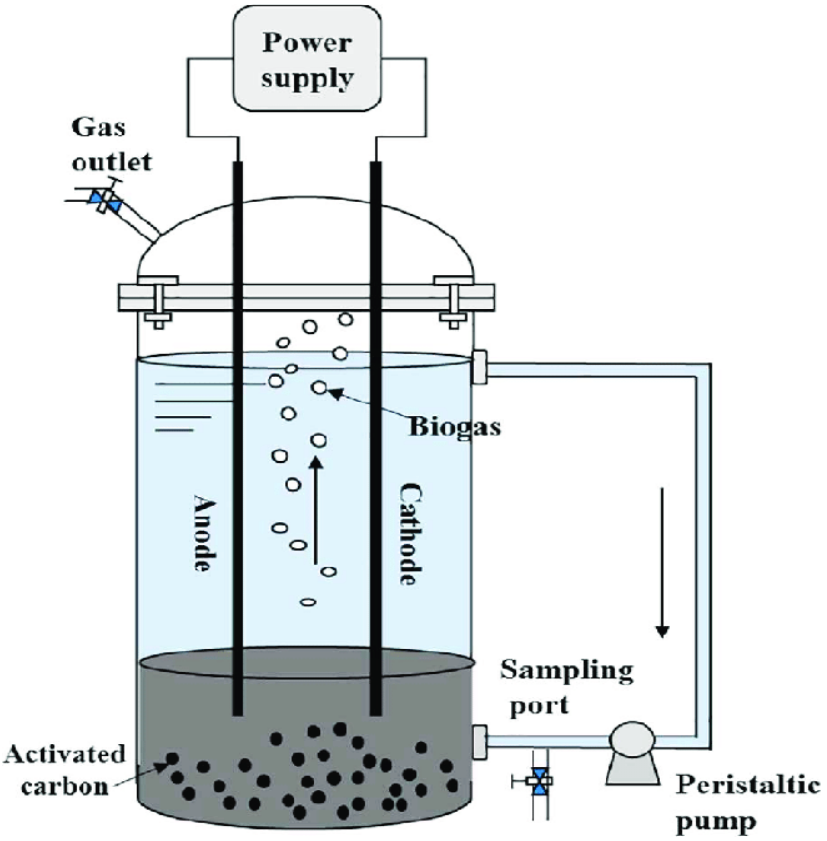

Figure 2-4 : Schematic diagram of the Microbial Electrolysis Cell (MEC) - Anaerobic Digestion (AD) [57]

2.5.2 Microbial Fuel Cell (MFC)-MEC hybrid system

MFC-MEC hybrid systems (Figure 2-5) have many advantages but the major advantage is the solution to the energy requirement problem for biohydrogen production. The First MFC-MEC hybrid system was experimented by Sun et al. in 2008, he reported the power supply through MFC, and hydrogen production was from the degradation of acetate in MEC [58]. MFC-MEC hybrid system may also be used for CO_2 reduction or

carbon capture as reported in a study published in 2012. This study focused on the production of formic acid in MEC powered by MFC [59]. Liu et al. published a study in which he reported fecal water treatment as well as electric power generation using Anaerobic Baffled Reactor (ABR)-MFC-MEC integrated system. The maximum COD removal rate was 1.35 kg COD/m^3/day, and 0.42 V output voltage was achieved by the combined system [60]. However, here are three problems associated with MFC-MEC hybrid systems that need to be solved: cathodic pH in both the MFC and MEC has a slight effect, substrate concentration has an impact on the system efficiency, and continuous operation needs to be further investigated.

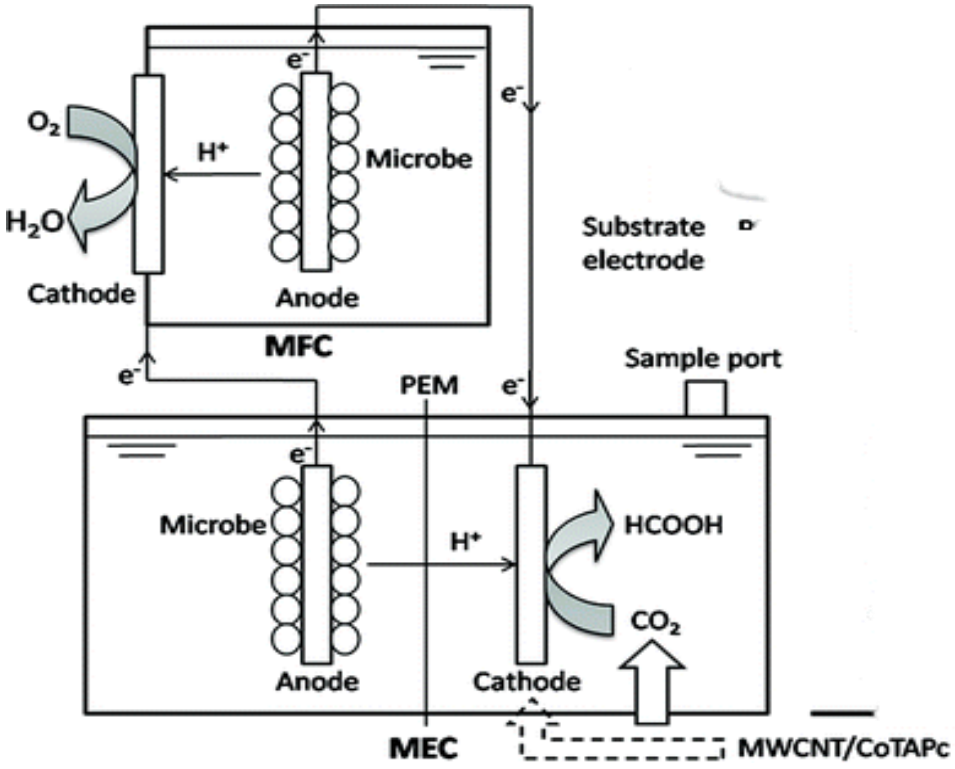

Figure 2-5 : Microbial Electrolysis Cell (MEC) - Microbial Fuel Cell (MFC) Hybrid System [59]

2.5.3 Solar Powered MEC - hybrid system

Solar energy is a great source of renewable energy. Solar cells can be used to provide the required power using sunlight to the MEC for hydrogen production. The solar cell-MEC hybrid system (Figure 2-6) is a very promising technology as it can be used to convert sunlight into valuable fuels like methane, hydrogen, and ethanol. The first such

system was reported in 2009, a dual-chamber H-shaped MEC was used for hydrogen production, and the external power of 0.6V, required for MEC, was supplied with Dye-sensitized solar cell (DSSC). The reported hybrid system utilized Pt coated titanium plate cathode to produce 400mmol H_2 and the cathode recovery efficiency and C.E was reported to be 78% and 40% respectively [61]. In the same year, another DSSC-MEC hybrid system (Figure 2-6) was introduced and the cathode used in MEC was Pt free, made up of carbon nanopowder coated electrode. The hydrogen produced by Pt free cathode MEC at an applied voltage of 0.7 V was almost of the same amount as reported by Pt cathode MEC [62]. No significant work has been made till now in this field and future work is needed to make efficient solar-powered MEC systems.

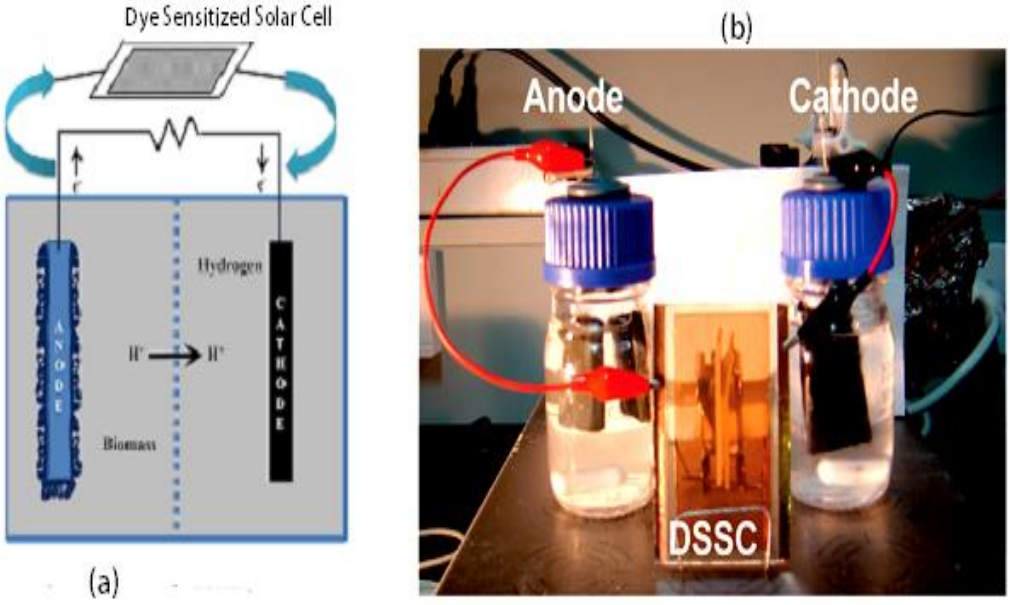

Figure 2-6 : (a) Schematic diagram of self-sustained Hybrid system - Microbial Electrolysis Cell (MEC) - Dye Synthesized Solar Cell (DSSC). (b) Experimental setup of Microbial Electrolysis Cell (MEC) - Dye Synthesized Solar Cell (DSSC) Hybrid system [62][63]

2.5.4 Microbial Desalination Cell (MDC)-MEC hybrid system

MDC-MEC hybrid systems are of unique importance because they boost the energy recovery, effective nitrogen removal, and desalination performance of the system. The first practical demonstration of the MDC-MEC hybrid system (Figure 2-7) was performed by Mehanna et al. in 2010. This study reported the maximum HPR of 0.16 m^3-H_2/m^3/d and 68 % conductivity was reduced in a single cycle at 0.55 V applied voltage [64]. Another study

reported the use of a MDC-MEC hybrid system for simultaneous salinity and organic matter removal. This study stated a higher salt removal rate of about 84%, C.E approximately equal to 100%, and the maximum HPR of 3.6 m^3-H_2/m^3/d, at an applied voltage of 1.2 V [65]. The latest publication by Li et al. found that the MDC-MEC hybrid system achieved a positive energy balance of 0.0267 KW h/m^3. He also reported a 63.7% salinity removal rate within 48 hours of the operation at the concentration of 5 g/L [66]. This hybrid system faces a large deviation in pH value during its operation between anode and cathode chamber and still needs improvements to get commercialized.

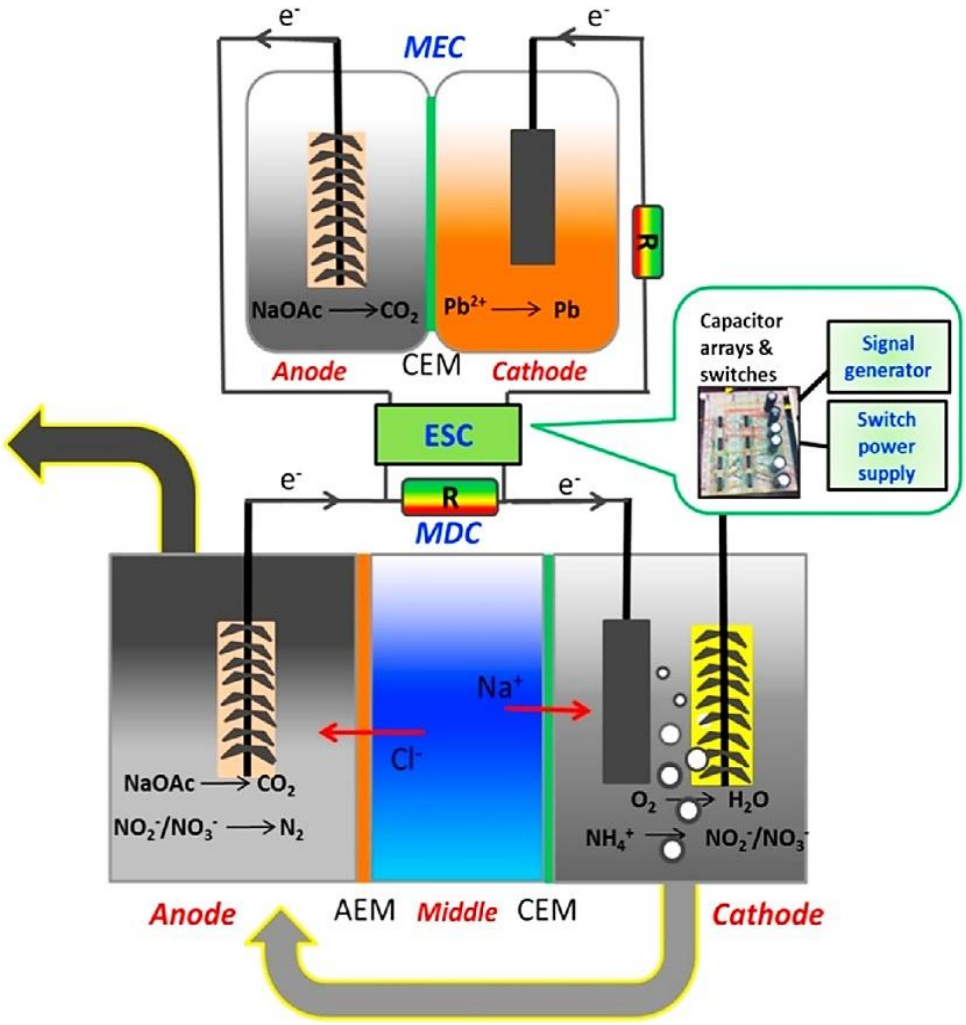

Figure 2-7 : Microbial Desalination Cell (MDC)- Microbial Fuel Cell (MEC) Hybrid System[66]

Summary

This chapter compiles most of the previous published work on Microbial Electrolysis Cell (MEC). Firstly, the history of the MEC along with its basic components, its importance and advantages were reported. Afterwards, the chapter emphases mainly on the MEC reactor configuration, the experimented electrode materials in MEC and the substrates used in MEC for bio-hydrogen production.

Double chamber MEC and single chamber MEC are two basic configurations of the reactor. Double chamber MEC produces pure hydrogen in the cathode compartment but is an expensive system and has energy losses due to the presence of membrane between anode and cathode. Single chamber MEC has been designed to simply the reactor configuration, reduce system cost and to overcome energy losses by directly exposing both electrodes eliminating the use of membrane. The downside of single chamber MEC is conversion of produced hydrogen to methane due to the presence of methanogens.

Metallic materials are usually utilized as cathode in MEC due to their fast Hydrogen Evolution Reaction (HER) rate. Anode of MEC is usually made up of carbonaceous materials i.e., carbon paper, carbon felt, graphite felt etc. but nickel foam, stainless steel and titanium can also be used as anode. Another section of this chapter discussed the details of most reported substrates in MEC i.e., non-fermentable organic substrates (acetate, glycerol, and glucose), fermentable organic substrates, and variety of organic wastewaters (domestic, industrial and sugar industry effluent).

Lastly, the integration of MECs with other technologies have been discussed in detail, such as, Anaerobic Digestion (AD)-MEC hybrid system, Microbial Fuel Cell (MFC)-MEC hybrid system, Solar Powered MEC - hybrid system, and Microbial Desalination Cell (MDC)-MEC hybrid system. These are the most prominent options to make MEC systems self-sustainable as well as to compete in the market.

References

[1] Liu H, Grot S, Logan BE. Electrochemically Assisted Microbial Production of Hydrogen from Acetate. Environ Sci Technol 2005;39:4317–20. https://doi.org/10.1021/es050244p.

[2] Hua T, Li S, Li F, Zhou Q, Ondon BS. Microbial electrolysis cell as an emerging versatile technology: a review on its potential application, advance and challenge. J Chem Technol Biotechnol 2019;94:1697–711. https://doi.org/https://doi.org/10.1002/jctb.5898.

[3] Logan BE, Call D, Cheng S, Hamelers HVM, Sleutels THJA, Jeremiasse AW, et al. Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter. Environ Sci Technol 2008;42:8630–40. https://doi.org/10.1021/es801553z.

[4] Tang JH. A Review of Suitable Substrates for Hydrogen Production in Microbial Electrolysis Cells. IOP Conf Ser Earth Environ Sci 2021;621:12145. https://doi.org/10.1088/1755-1315/621/1/012145.

[5] Shao Q, Li J, Yang S, Sun H. Effects of different substrates on microbial electrolysis cell (MEC) anodic membrane: biodiversity and hydrogen production performance. Water Sci Technol 2019;79:1123–33. https://doi.org/10.2166/wst.2019.107.

[6] Hasany M, Mardanpour MM, Yaghmaei S. Biocatalysts in microbial electrolysis cells: A review. Int J Hydrogen Energy 2016;41:1477–93. https://doi.org/https://doi.org/10.1016/j.ijhydene.2015.10.097.

[7] Kundu A, Sahu JN, Redzwan G, Hashim MA. An overview of cathode material and catalysts suitable for generating hydrogen in microbial electrolysis cell. Int J Hydrogen Energy 2013;38:1745–57. https://doi.org/https://doi.org/10.1016/j.ijhydene.2012.11.031.

[8] Zheng J, Li J, Xu K, Long X, Sun H, Yang S. Effects of Different Substrates on MEC Anode Film Formation and Extracellular Polymer. IOP Conf Ser Earth Environ Sci 2020;555:12124. https://doi.org/10.1088/1755-1315/555/1/012124.

[9] Kadier A, Simayi Y, Abdeshahian P, Azman NF, Chandrasekhar K, Kalil MS. A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alexandria Eng J 2016;55:427–43. https://doi.org/https://doi.org/10.1016/j.aej.2015.10.008.

[10] Katuri K, Ali M, Saikaly P. The role of microbial electrolysis cell in urban wastewater treatment: integration options, challenges, and prospects. Curr Opin Biotechnol 2019;57:101–10. https://doi.org/10.1016/j.copbio.2019.03.007.

[11] Rozendal RA, Sleutels T, Hamelers HVM, Buisman C. Effect of the type of ion exchange membrane on performance, ion transport, and pH in biocatalyzed electrolysis of wastewater. Water Sci Technol 2008;57:1757–62. https://doi.org/10.2166/wst.2008.043.

[12] Xiang L, Dai L, Guo K, Wen Z, Ci S, Li J. Microbial electrolysis cells for hydrogen production. Chinese J Chem Phys 2020;33:263–84. https://doi.org/10.1063/1674-0068/cjcp2005075.

[13] Khan MZ, Nizami DA-S, Rehan M, Ouda O, Sultana S, Ismail I, et al. Microbial electrolysis cells for hydrogen production and urban wastewater treatment: A case study of Saudi Arabia. Appl Energy 2017;185:410–420. https://doi.org/10.1016/j.apenergy.2016.11.005.

[14] Call D, Logan B. Hydrogen Production in a Single Chamber Microbial Electrolysis Cell Lacking a Membrane. Environ Sci Technol 2008;42:3401–6. https://doi.org/10.1021/es8001822.

[15] Montpart N, Rago L, Baeza JA, Guisasola A. Hydrogen production in single chamber microbial electrolysis cells with different complex substrates. Water Res 2015;68:601–15. https://doi.org/https://doi.org/10.1016/j.watres.2014.10.026.

[16] Zhang Y, Angelidaki I. Microbial electrolysis cells turning to be versatile technology: Recent advances and future challenges. Water Res 2014;Just accep. https://doi.org/10.1016/j.watres.2014.02.031.

[17] Varanasi JL, Veerubhotla R, Pandit S, Das D. Chapter 5.7 - Biohydrogen Production Using Microbial Electrolysis Cell: Recent Advances and Future Prospects. In: Mohan SV, Varjani S, Pandey ABT-MET, editors. Biomass, Biofuels Biochem., Elsevier; 2019, p. 843–69. https://doi.org/https://doi.org/10.1016/B978-0-444-64052-9.00035-2.

[18] Cardeña R, Cercado B, Buitrón G. Chapter 7 - Microbial Electrolysis Cell for Biohydrogen Production. In: Pandey A, Mohan SV, Chang J-S, Hallenbeck PC, Larroche CBT-B (Second E, editors. Biomass, Biofuels, Biochem., Elsevier; 2019,

p. 159–85. https://doi.org/https://doi.org/10.1016/B978-0-444-64203-5.00007-1.

[19] Jafary T, Daud WRW, Ghasemi M, Kim BH, Md Jahim J, Ismail M, et al. Biocathode in microbial electrolysis cell; present status and future prospects. Renew Sustain Energy Rev 2015;47:23–33. https://doi.org/https://doi.org/10.1016/j.rser.2015.03.003.

[20] Kadier A, Simayi Y, Kalil MS, Abdeshahian P, Hamid AA. A review of the substrates used in microbial electrolysis cells (MECs) for producing sustainable and clean hydrogen gas. Renew Energy 2014;71:466–72. https://doi.org/https://doi.org/10.1016/j.renene.2014.05.052.

[21] Lu S-Y, Jin M, Zhang Y, Niu Y-B, Gao J-C, Li CM. Chemically Exfoliating Biomass into a Graphene-like Porous Active Carbon with Rational Pore Structure, Good Conductivity, and Large Surface Area for High-Performance Supercapacitors. Adv Energy Mater 2018;8:1702545. https://doi.org/https://doi.org/10.1002/aenm.201702545.

[22] Kiely PD, Cusick R, Call DF, Selembo PA, Regan JM, Logan BE. Anode microbial communities produced by changing from microbial fuel cell to microbial electrolysis cell operation using two different wastewaters. Bioresour Technol 2011;102:388–94. https://doi.org/https://doi.org/10.1016/j.biortech.2010.05.019.

[23] Sun M, Zhang F, Tong Z-H, Sheng G-P, Chen Y-Z, Zhao Y, et al. A gold-sputtered carbon paper as an anode for improved electricity generation from a microbial fuel cell inoculated with Shewanella oneidensis MR-1. Biosens & Bioelectron 2010;26:338–43. https://doi.org/10.1016/j.bios.2010.08.010.

[24] Rozendal RA, Hamelers HVM, Euverink GJW, Metz SJ, Buisman CJN. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int J Hydrogen Energy 2006;31:1632–40. https://doi.org/https://doi.org/10.1016/j.ijhydene.2005.12.006.

[25] Rivera I, Buitrón G, Bakonyi P, Nemestóthy N, Bélafi-Bakó K. Hydrogen production in a microbial electrolysis cell fed with a dark fermentation effluent. J Appl Electrochem 2015;45:1223–9. https://doi.org/10.1007/s10800-015-0864-6.

[26] Jeon Y, Kim S. Persistent Hydrogen Production by the Photo-Assisted Microbial Electrolysis Cell Using a p-Type Polyaniline Nanofiber Cathode. ChemSusChem

2016;9:3276–9. https://doi.org/https://doi.org/10.1002/cssc.201600906.

[27] Guo K, Prévoteau A, Rabaey K. A novel tubular microbial electrolysis cell for high rate hydrogen production. J Power Sources 2017;356:484–90. https://doi.org/https://doi.org/10.1016/j.jpowsour.2017.03.029.

[28] Hasibar B, Ergal İ, Abreu Dias S, Bochmann G, Rittmann SK-MR, Fuchs W. Competing acetate consumption and production inside a microbial electrolysis cell. J Environ Chem Eng 2020;8:103847. https://doi.org/https://doi.org/10.1016/j.jece.2020.103847.

[29] Sakai S, Yagishita T. Microbial production of hydrogen and ethanol from glycerol-containing wastes discharged from a biodiesel fuel production plant in a bioelectrochemical reactor with thionine. Biotechnol Bioeng 2007;98:340–8. https://doi.org/https://doi.org/10.1002/bit.21427.

[30] Escapa A, Manuel M-F, Morán A, Gómez X, Guiot SR, Tartakovsky B. Hydrogen Production from Glycerol in a Membraneless Microbial Electrolysis Cell. Energy & Fuels 2009;23:4612–8. https://doi.org/10.1021/ef900357y.

[31] Badia-Fabregat M, Rago L, Baeza JA, Guisasola A. Hydrogen production from crude glycerol in an alkaline microbial electrolysis cell. Int J Hydrogen Energy 2019;44:17204–13. https://doi.org/https://doi.org/10.1016/j.ijhydene.2019.03.193.

[32] Selembo PA, Perez JM, Lloyd WA, Logan BE. High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. Int J Hydrogen Energy 2009;34:5373–81. https://doi.org/https://doi.org/10.1016/j.ijhydene.2009.05.002.

[33] Lu L, Xing D, Ren N, Logan BE. Syntrophic interactions drive the hydrogen production from glucose at low temperature in microbial electrolysis cells. Bioresour Technol 2012;124:68–76. https://doi.org/https://doi.org/10.1016/j.biortech.2012.08.040.

[34] Zhang J, Bai Y, Fan Y, Hou H. Improved bio-hydrogen production from glucose by adding a specific methane inhibitor to microbial electrolysis cells with a double anode arrangement. J Biosci Bioeng 2016;122:488–93. https://doi.org/https://doi.org/10.1016/j.jbiosc.2016.03.016.

[35] Call DF, Merrill MD, Logan BE. High Surface Area Stainless Steel Brushes as

Cathodes in Microbial Electrolysis Cells. Environ Sci Technol 2009;43:2179–83. https://doi.org/10.1021/es803074x.

[36] Sun M, Sheng G-P, Mu Z-X, Liu X-W, Chen Y-Z, Wang H-L, et al. Manipulating the hydrogen production from acetate in a microbial electrolysis cell–microbial fuel cell-coupled system. J Power Sources 2009;191:338–43. https://doi.org/https://doi.org/10.1016/j.jpowsour.2009.01.087.

[37] Jeremiasse AW, Hamelers HVM, Saakes M, Buisman CJN. Ni foam cathode enables high volumetric H2 production in a microbial electrolysis cell. Int J Hydrogen Energy 2010;35:12716–23. https://doi.org/https://doi.org/10.1016/j.ijhydene.2010.08.131.

[38] Pisciotta JM, Zaybak Z, Call DF, Nam J-Y, Logan BE. Enrichment of Microbial Electrolysis Cell Biocathodes from Sediment Microbial Fuel Cell Bioanodes. Appl Environ Microbiol 2012;78:5212 LP – 5219. https://doi.org/10.1128/AEM.00480-12.

[39] Yang N, Hafez H, Nakhla G. Impact of volatile fatty acids on microbial electrolysis cell performance. Bioresour Technol 2015;193:449–55. https://doi.org/10.1016/j.biortech.2015.06.124.

[40] Chookaew T, Prasertsan P, Ren ZJ. Two-stage conversion of crude glycerol to energy using dark fermentation linked with microbial fuel cell or microbial electrolysis cell. N Biotechnol 2014;31:179–84. https://doi.org/https://doi.org/10.1016/j.nbt.2013.12.004.

[41] Cheng S, Logan B. Sustainable and efficient biohydrogen production via electrohydrogenesis. Proc Natl Acad Sci U S A 2007;104:18871–3. https://doi.org/10.1073/pnas.0706379104.

[42] Lalaurette E, Thammannagowda S, Mohagheghi A, Maness P-C, Logan B. Hydrogen production from cellulose in a two-stage process combining fermentation and electrohydrogenesis. Int J Hydrogen Energy 2009;34:6201–10. https://doi.org/10.1016/j.ijhydene.2009.05.112.

[43] Ditzig J, Liu H, Logan B. Production of hydrogen from domestic wastewater using a bioelectrochemically assisted microbial reactor (BEAMR). Int J Hydrogen Energy 2007;32:2296–304. https://doi.org/10.1016/j.ijhydene.2007.02.035.

[44] Escapa A, Gil-Carrera L, García V, Morán A. Performance of a continuous flow microbial electrolysis cell (MEC) fed with domestic wastewater. Bioresour Technol 2012;117:55–62. https://doi.org/10.1016/j.biortech.2012.04.060.

[45] Heidrich ES, Dolfing J, Scott K, Edwards SR, Jones C, Curtis TP. Production of hydrogen from domestic wastewater in a pilot-scale microbial electrolysis cell. Appl Microbiol Biotechnol 2013;97:6979–89. https://doi.org/10.1007/s00253-012-4456-7.

[46] Jwa E, Yun Y-M, Kim H, Jeong N, Park S-C, Nam J-Y. Domestic wastewater treatment in a tubular microbial electrolysis cell with a membrane electrode assembly. Int J Hydrogen Energy 2019;44:652–60. https://doi.org/https://doi.org/10.1016/j.ijhydene.2018.11.036.

[47] Jayabalan T, Matheswaran M, Naina Mohammed S. Biohydrogen production from sugar industry effluents using nickel based electrode materials in microbial electrolysis cell. Int J Hydrogen Energy 2019;44:17381–8. https://doi.org/https://doi.org/10.1016/j.ijhydene.2018.09.219.

[48] Jayabalan T, Matheswaran M, Preethi V, Naina Mohamed S. Enhancing biohydrogen production from sugar industry wastewater using metal oxide/graphene nanocomposite catalysts in microbial electrolysis cell. Int J Hydrogen Energy 2020;45:7647–55. https://doi.org/https://doi.org/10.1016/j.ijhydene.2019.09.068.

[49] Jayabalan T, Matheswaran M, Radhakrishnan TK, Naina Mohamed S. Influence of Nickel molybdate nanocatalyst for enhancing biohydrogen production in microbial electrolysis cell utilizing sugar industrial effluent. Bioresour Technol 2021;320:124284. https://doi.org/https://doi.org/10.1016/j.biortech.2020.124284.

[50] Tenca A, Cusick R, Schievano A, Oberti R, Logan B. Evaluation of low cost cathode materials for treatment of industrial and food processing wastewater using microbial electrolysis cells. Int J Hydrogen Energy 2013;38:1859–1865. https://doi.org/10.1016/j.ijhydene.2012.11.103.

[51] Zhang L, Wang Y-Z, Zhao T, Xu T. Hydrogen production from simultaneous saccharification and fermentation of lignocellulosic materials in a dual-chamber microbial electrolysis cell. Int J Hydrogen Energy 2019;44:30024–30. https://doi.org/https://doi.org/10.1016/j.ijhydene.2019.09.191.

[52] Kim M, Li D, Choi O, Sang B-I, Chiang PC, Kim H. Effects of supplement additives on anaerobic biogas production. Korean J Chem Eng 2017;34:2678–85. https://doi.org/10.1007/s11814-017-0175-1.

[53] Yu Z, Leng X, Zhao S, Ji J, Zhou T, Khan A, et al. A review on the applications of microbial electrolysis cells in anaerobic digestion. Bioresour Technol 2018;255:340–8. https://doi.org/https://doi.org/10.1016/j.biortech.2018.02.003.

[54] Dou Z, Dykstra C, Pavlostathis S. Bioelectrochemically assisted anaerobic digestion system for biogas upgrading and enhanced methane production. Sci Total Environ 2018;633:1012–21. https://doi.org/10.1016/j.scitotenv.2018.03.255.

[55] Xu X-J, Wang W-Q, Chen C, Xie P, Liu W-Z, Zhou X, et al. The effect of PBS on methane production in combined MEC-AD system fed with alkaline pretreated sewage sludge. Renew Energy 2020;152:229–36. https://doi.org/https://doi.org/10.1016/j.renene.2020.01.052.

[56] Zhang Q, Hu J, Lee D-J. Biogas from anaerobic digestion processes: Research updates. Renew Energy 2016;98:108–19. https://doi.org/https://doi.org/10.1016/j.renene.2016.02.029.

[57] Xu S, Zhang Y, Liwen L, Liu H. Startup performance of microbial electrolysis cell assisted anaerobic digester (MEC-AD) with pre-acclimated activated carbon. Bioresour Technol Reports 2018;5. https://doi.org/10.1016/j.biteb.2018.12.007.

[58] Sun M, Sheng G-P, Zhang L, Xia C-R, Mu Z-X, Liu X-W, et al. An MEC-MFC-Coupled System for Biohydrogen Production from Acetate. Environ Sci Technol 2008;42:8095–100. https://doi.org/10.1021/es801513c.

[59] Zhao H, Zhang Y, Zhao B, Chang Y, Li Z. Electrochemical Reduction of Carbon Dioxide in an MFC–MEC System with a Layer-by-Layer Self-Assembly Carbon Nanotube/Cobalt Phthalocyanine Modified Electrode. Environ Sci Technol 2012;46:5198–204. https://doi.org/10.1021/es300186f.

[60] Liu H, Leng F, Guan Y, Yao Y, Li Y, Xu S. Simultaneous Pollutant Removal and Electricity Generation in a Combined ABR-MFC-MEC System Treating Fecal Wastewater. Water, Air, Soil Pollut 2017;228:179. https://doi.org/10.1007/s11270-017-3342-4.

[61] Ajayi FF, Kim K-Y, Chae K-J, Choi M-J, Kim S-Y, Chang I-S, et al. Study of

hydrogen production in light assisted microbial electrolysis cell operated with dye sensitized solar cell. Int J Hydrogen Energy 2009;34:9297–304. https://doi.org/https://doi.org/10.1016/j.ijhydene.2009.09.096.

[62] Chae K-J, Choi M-J, Kim K-Y, Ajayi F, Chang I-S, Kim I. A Solar-Powered Microbial Electrolysis Cell With a Platinum Catalyst-Free Cathode To Produce Hydrogen. Environ Sci Technol 2009;43:9525–30. https://doi.org/10.1021/es9022317.

[63] Kadier A, Jain P, Lai B, Kalil MS, Kondaveeti S, Alabbosh KFS, et al. Biorefinery perspectives of microbial electrolysis cells (MECs) for hydrogen and valuable chemicals production through wastewater treatment. Biofuel Res J 2020;7:1128–42. https://doi.org/10.18331/BRJ2020.7.1.5.

[64] Mehanna M, Kiely PD, Call DF, Logan BE. Microbial Electrodialysis Cell for Simultaneous Water Desalination and Hydrogen Gas Production. Environ Sci Technol 2010;44:9578–83. https://doi.org/10.1021/es1025646.

[65] Kim Y, Logan BE. Simultaneous removal of organic matter and salt ions from saline wastewater in bioelectrochemical systems. Desalination 2013;308:115–21. https://doi.org/https://doi.org/10.1016/j.desal.2012.07.031.

[66] Li Y, Styczynski J, Huang Y, Xu Z, McCutcheon J, Li B. Energy-positive wastewater treatment and desalination in an integrated microbial desalination cell (MDC)-microbial electrolysis cell (MEC). J Power Sources 2017;356:529–38. https://doi.org/https://doi.org/10.1016/j.jpowsour.2017.01.069.

Chapter 3

Materials and Methodology

3.1 Designing of the MEC

Design of the MEC was one of the most complicated problem that must be solved prior to experimentation. The designing of the MEC was divided into two categories:

- Designing of the reactor chamber
- Designing of the electrodes

Two types of the MEC reactor were generally in use to produce bioenergy. The first possible design was H-shaped, double chamber MEC, and was first introduced in 2005 [1]. This chamber consists of separate anode and cathode compartments and has mostly used where purity of the hydrogen gas is the first priority. The disadvantage of this design is the use of membrane, which makes this design very expensive. Single chamber, tubular or rectangular MEC was the second possibility. This study selected the single-chamber, tubular reactor design for bioenergy production. The reasons for the selection of this design were the easy fabrication of single chamber MEC, the cost effective way to produce hydrogen as no membrane was required, and high substrate utilization efficiency [2,3].

The designing of the electrodes for MEC was referred as the placement and orientation of the electrodes during the MEC operation. Mostly, both electrodes were placed at corners of the single-chamber MEC. The electrodes were placed at 1.5 cm apart using plastic screws or J-cloth. Electrodes were cut in rectangular shapes and approximately had same surface area (Figure 3-1) [4]. The placement distance of both electrodes and the surface area of the anode with respect to cathode were two key parameters for designing the effective MEC.

Figure 3-1 : Electrodes shape and placement (previous study) [4]

This research focused on using tubular shape reactor bottle and the anode and cathode, utilized in this research, were placed in a coaxial configuration (Figure 3-2) to increase the surface area of the anode, to improve the hydrogen production efficiency of MEC, and to avoid rate limitation due to bio anode [5].

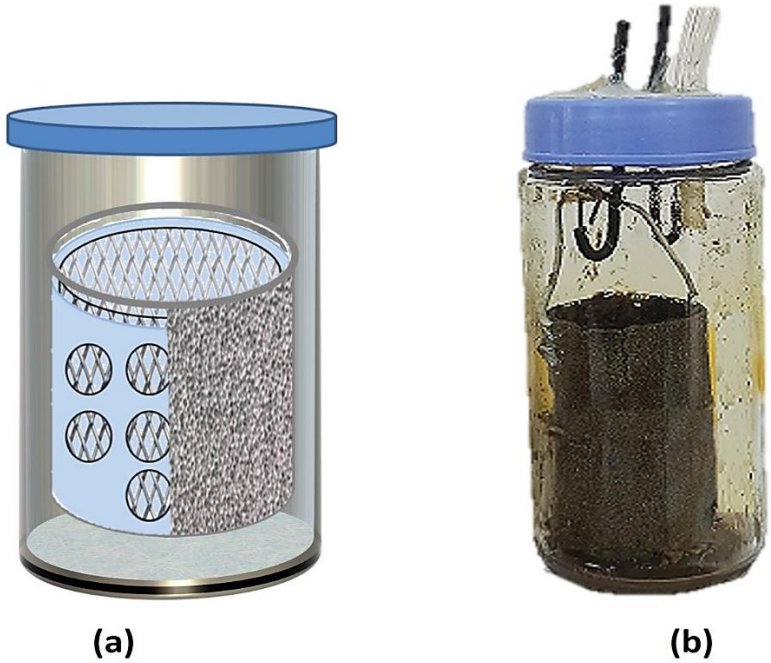

Figure 3-2 : (a) Schematic and (b) Experimental representation of the MEC Design

3.2 Construction of the MEC

Various reactor working volume have been reported for bio-energy production. Most of the researchers employed a reactor having 27 ml working volume to examine the performance of the MEC. This study utilized a single-chamber MEC reactor was constructed from a soda-lime glass bottle with a working volume of 200 ml (total volume ≈ 250 ml, 50 ml headspace, 52 mm diameter, and 106 mm height) (Figure 3-2-b).

The selected reactor chamber was completely sealed with silicon glue to prevent the infiltration of oxygen into the reactor and to maintain an anaerobic environment. The coaxially arrangement of electrodes was selected. The anode was placed in outer circle having diameter nearly equals to 4.7 cm. There is a 0.3 cm separation (two types) between both electrodes. The cathode utilized in this research was placed inside the separation and have diameter of 3.5 cm.

The Ni-foam (Figure 3-3), having a thickness of 2 mm and surface density of 300 g/m^2 (Anping Longyi Mesh Manufacture Co., Ltd., China) was used as the anode in MEC to produce biohydrogen. The selection of Ni-foam as anode was because of its large surface area, excellent biocompatibility to facilitate bacterial attachment growth, decent corrosion resistance, and high electronic conductivity. Anode material was immersed in 500 ml of deionized water for 2 h, then rinsed with deionized water, and set on aluminum foil for drying prior to the use in MEC.

Figure 3-3 : Ni-foam with 300g/m^2 surface density - anode

The Stainless-Steel Mesh (SSM) grade 304L (Anping Longyi Mesh Manufacture Co., Ltd., China) was used as cathode in the MEC system for bioenergy production (Figure 3-4). SSM mesh is finely flat electrode, therefore, it allows very close electrode spacing. Otherwise, if graphite or carbon felt was utilized, it would not allow close electrode spacing as it may cause short circuit. Moreover, the SSM mesh could also produce higher surface areas because of various pores as compared to that of flat sheet electrode [6]. The utilized cathode was of 100 mesh size and had the filter accuracy of 150 microns. SSM cathode was smoothed with SiC sandpaper, rinsed with deionized water ultrasonically, washed with acetone, and then again washed in deionized water and placed in an incubator for drying overnight (>12 h) prior to the use.

Figure 3-4 : Stainless steel mesh grade 304L - cathode

Both electrodes were maintained at a distance of 0.3 cm apart using two different separation techniques shown in Figure 3-5.

- Rate limited bio-anode – MEC
- Fully exposed bio-anode - MEC

The rate limited bio-anode - MEC separation technique was utilized to investigate the effects of limited exposed surface area on the bioenergy production. In rate limited bio-anode - MEC separation technique, both electrodes were separated by utilizing an acrylic

pipe having thickness 0.3 cm. There was total 21 holes present on that acrylic pipe. Each hole had a diameter of 0.8 cm making total exposed surface area to be 10.56 cm^2).

The fully exposed bio-anode – MEC was utilized to compare the results of rate limited bio-anode – MEC, discuss the impacts of fully expose bio-anode on the MEC performance efficiency, and to suggest to most compatible separation technique. This technique utilized a plastic mesh having thickness of 0.3 cm and 20 mesh size (20 number of openings per square inch), making total exposed surface area to be approximately 63.12 cm^2.

Figure 3-5 : Rate limited bio-anode - MEC (left) and fully exposed bio-anode - MEC (right)

A 99.9% pure copper wire was used as a current collector for both electrodes. Copper wire was soldered with Ni-foam anode to make a rigid connection. It was wounded tightly with SSM cathode as the SSM could not be soldered with copper wire. There are four openings on the reactor covering lid which were used for the anode, cathode, gas collection, and nitrogen purging. Figure 3-2 (b) represented the fully assembled single-chamber, tubular MEC.

3.3 Configuration of the MEC system

Hereafter, the fabricated cell was ready for the auxiliary connections to complete the MEC system for bioenergy production (Figure 3-6). All the supplementary connections were made using a silver coated copper wire. Allegator clips were used in all wiring connections as they could make a firm connection. The voltage required for the MEC system operation was supplied using a polycrystalline silicon solar cell. The supplied voltage was controlled by using a potentiometer.

The current collector of the bio-anode, made up of Ni-foam, was connected to the positive terminal of the power supply and the negative terminal led to a highly precise external resistance of 10 Ω and the cathode. A potentiometer is placed between the positive terminal of the solar cell and anode's current collector to control the supplied voltage from the source. A data acquisition system was connected across the 10 Ω external resistance to record the voltage. The produced gas from the MEC system was collected in a graduated cylinder, held in inverted position with the help of stand.

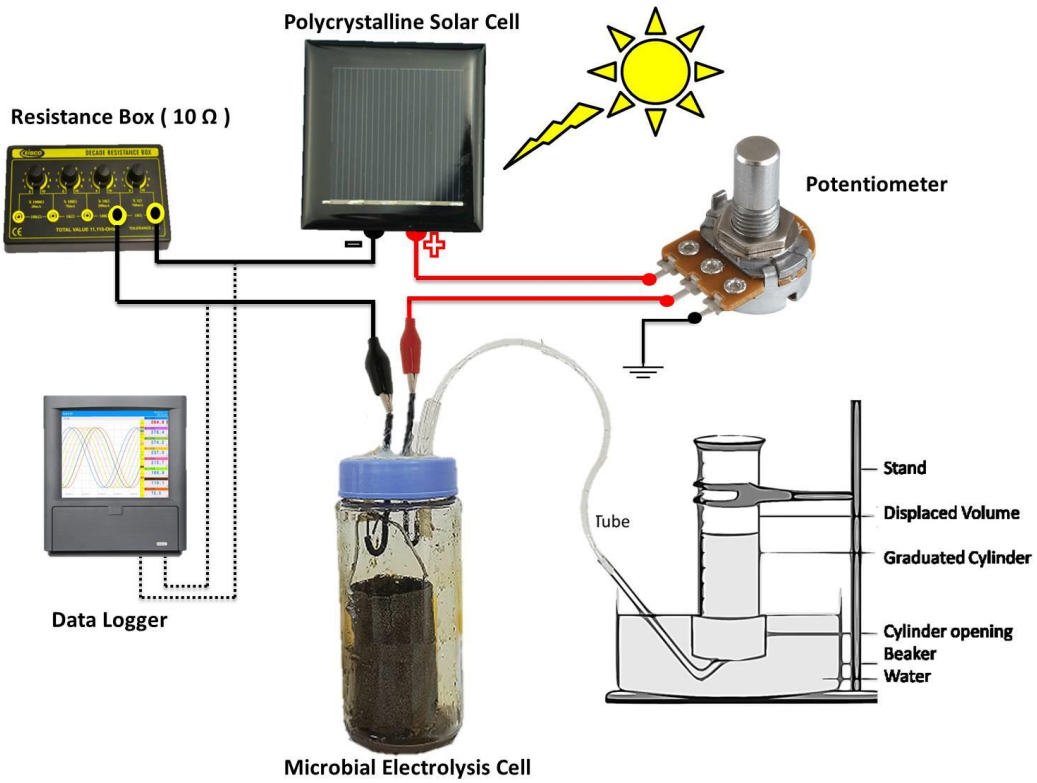

Figure 3-6 : Schematic representation of the complete MEC system

3.4 Characteristics of the wastewater:

The Dairy manure wastewater slurry (DMWS) was obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan and stored at 4 ^{o}C. The DMWS sample was rigorously examined and categorized in the laboratory prior to its use in MEC. The DMWS was used as a microorganism source in unassimilated chamber, which would help to break down the organic content. The microorganisms present in the stored DMWS got hibernated at 4 ^{o}C. These microorganisms got revived in an incubator working at 37 ^{o}C for 24 hours before use in the MEC.

Hereafter, various additives such as, 1 g/L of sodium acetate (electron donor), 25 mL vitamin and mineral solution, 0.31 g/L ammonium chloride, 0.13 g/L potassium chloride, were added to DMWS. The pH of the prepared solution was adjusted close to 10.5 using buffer solution. The buffer solution was made up of 0.1 M $NaHCO_3$ soultion with 2.10 g NaOH added into it. The final solution obtained after adding all these additives was Synthetic Dairy Manure Wastewater (SDMW). Characteristics of raw DMWS and SDMW were summarized in Table 3-1.

Table 3-1 : Dairy wastewater characteristics

Parameters	Synthetic dairy manure wastewater	Dairy manure wastewater slurry
pH	10.52	8.67
Salinity (PSU)	5.73	2.35
Conductivity (mS)	10.14	4.41
Resistivity (Ω)	98.6	227
TDS (g/L)	5.075	2.210

3.5 Characteristics of the solar cell:

The polycrystalline silicon solar cell was imported from China (Solarshining Co., Ltd, China). The total surface area of the solar cell was 54 × 54 mm. This solar cell exhibited an open-circuit voltage (V_{oc}) of 1.6 V, a short circuit photocurrent (I_{sc}) of 4.05 mA/cm^2, the solar conversion rate of 16% and a fill factor (FF) of 0.73 under standard operating conditions (1000 W/m^2, 1.5 A.M). The purchased silicon solar cell had no connections, but there was place for positive and negative connection was marked. Therefore, copper wires were soldered at the given points using a solder rod and wire. The other ends of the wires were attached with allegator clips for easy connections.

The silicon solar cell was illuminated using an artificial light source (100 W Daylight LED, Eco Smart). The light produced using the 100 W lamp has irradiance of about 834 W/m^2. This intensity of emitted irradiance in the specific lab area was measured using a solar meter (DS-05A solar meter, Daystar Inc.) The silicon solar cell was tested in smart grids lab, prior to its use to the MEC system. The solar cell, placed at 1 feet distance from the light source, was lightened up using a 100 W lamp. A digital multimeter was used to check the voltage generated by the solar cell at these radiations. A potentiometer was also utilized to check and control the produced voltage from the solar cell.

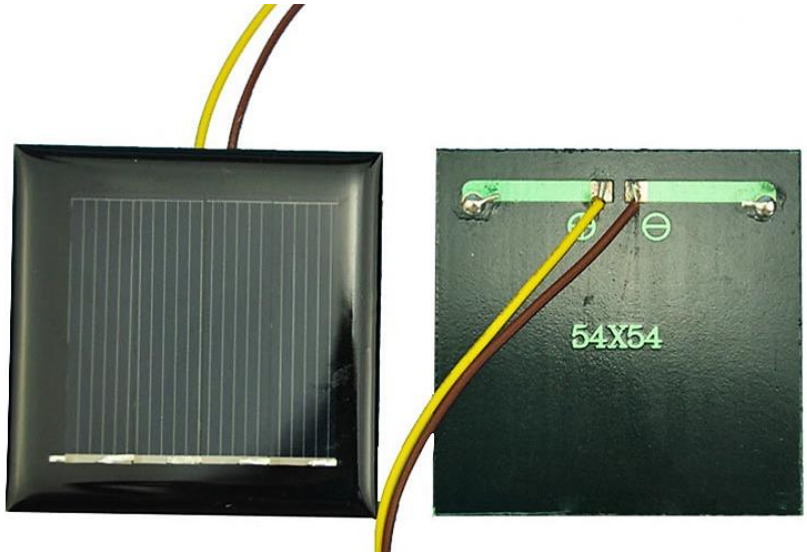

Figure 3-7 : Polycrystalline silicon solar cell

3.6 MEC start-up and operation:

One all the auxiliary connections were made with MEC chamber, the system was ready for the first cycle (Figure 3-8). The first MEC utilized a fresh Ni-foam anode to examine the effect of unassimilated bio-anode on the bioenergy production of the MEC. The MEC system was operated in fed batch mode and started up with SSM 304 L cathode. The MEC was fed with a 250 ml of SDMW. After injecting the fresh SDMW in the reactor, the reactor was flushed with nitrogen gas (purity > 99.9%) for 15 min to make anaerobic environment in MEC.

The produced gas from the MEC chamber was collected and measured from an inverted 100 ml graduated cylinder by the water displacement method. Firstly, the tub was filled with water and placed near the MEC system. Then the 100 ml cylinder was completely filled with water and its opening was covered with cardboard. The cylinder was carefully inverted into the tub to avoid air pockets in it. A stand was utilized to hold the inverted cylinder in position.

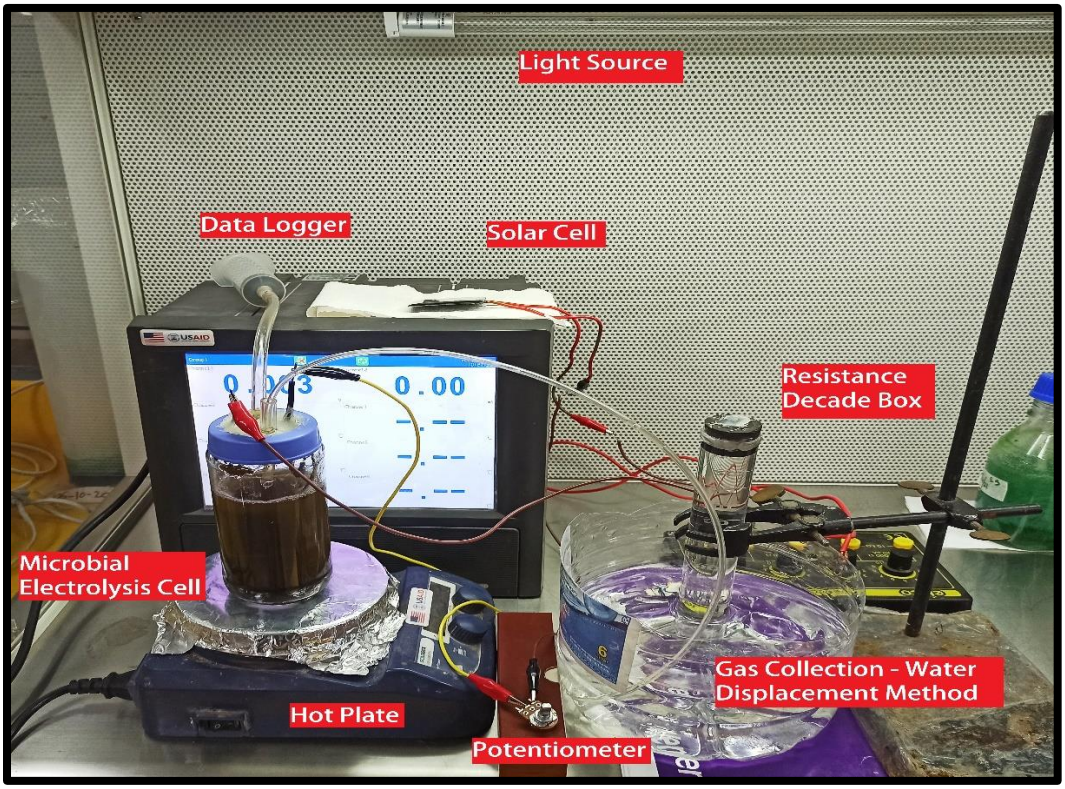

Figure 3-8 : Set-up of complete lab-scale MEC system

The dairy manure could have the capability to produce methane in presence of anaerobic environment. Therefore, a control system was also utilized control to check the production of methane solely from the DMWS. For this purpose, a sealed anaerobic glass chamber, filled with 200 ml DMWS, placed in an incubator at 37 °C and 120 rpm, was considered as a control system. The produced gas was daily collected from the inverted glass cylinder and examined for gas compositional testing. All the MEC experiments were conducted at a constant supplied voltage of 0.6 V, controlled temperature (31 °C) with standard atmospheric pressure (14.7 psi). All the reported data have a percentage error of ± 5%.

3.7 Investigations and Calculations:

The collected gas sample was taken out by syringe and needle from the top of the inverted cylinder, which was sealed by a silicon lid. The extent of various produced gases (H_2, CH_4, CO_2, etc.) in the sample were examined using gas chromatography (GC-2010 Pro, SHIMADZU Japan) equipped with TCD column (RT-MS5A, 30 m × 0.32 mm ID, 30 μm) and nitrogen as a carrier gas. The voltage across the resistance was recorded to measure the current (mA) using a data acquisition system (Hangzhou *Pangu Automation* System Co., Ltd) with a recording frequency of 30 minutes. The resistivity, electrical conductivity (EC), salinity, total dissolved solids (TDS), and pH were assessed by a portable Multi-parameter Professional Waterproof EC/TDS/Resistivity/Salinity Meter (HI 98192 - HANNA Instruments, Italy).

The morphology of each electrode and the pattern of the assimilated biofilm at the anode surface were tested and validated using a Scanning electron microscope (SEM) (VEGA 3, TESCAN, Czech Republic) before and after the experiment. Both utilized electrodes were taken out of the MEC reactor and dried before the SEM analysis [7]. Some elemental analysis should be performed to check the effect of the grown microbial community on the electrodes. The harsh environment due to the use of SDMW could also have impact on the elemental composition of the electrodes. Therefore, quantitative composition and elemental analysis of electrodes were determined using Energy-dispersive X-ray spectrometry (EDS) (Oxford Instruments, UK).

In all the experiments conducted, the maximum volumetric bioenergy production rates (Q_{H2}, Q_{CH4}) are desirable to be evaluated to examine the performance of unassimilated bio-anodes in MECs. The "Q" expresses the volumetric production rates of hydrogen (Q_{H2}) and methane (Q_{CH4}) and can be calculated by dividing the collected volume of a specific gas (m^3) per chamber volume (m^3) per day.

Figure 3-9 : (A) Gas chromatography - GC-2010 (B) Portable Multi-parameter Professional Waterproof EC/TDS/Resistivity/Salinity Meter (C) Data acquisition system and (D) Scanning electron microscope (SEM)

Summary:

This chapter discusses about the various stages for the set-up of a full lab-scale MEC system. Firstly, the designing of the MEC chamber was considered and it was classified into two categories. The single chamber MEC design with co-axial orientation of the electrodes was selected for optimum operation. The MEC was constructed in a tubular glass bottle and the Ni-foam was selected as anode and the stainless-steel mesh 304L was selected as cathode material. Two electrode separation techniques were utilized to check the performance of MEC, based on the exposed electrode's surface area. All the auxiliary connections, made with MEC to make a full lab-scale system, was discussed.

The characteristics of Synthetic Dairy Manure Wastewater (SDMW) as well as the characteristics of the polycrystalline silicon solar cell was discussed in detail. The start-up of the MEC system, injection of SDMW, nitrogen purging, and the collection of produced gas was reviewed. Finally, the analysis of the produced gas using gas chromatography and the examination of the electrodes was performed using scanning electron microscopy (SEM) and Energy-dispersive X-ray spectrometry (EDS).

References

[1] Liu H, Grot S, Logan BE. Electrochemically Assisted Microbial Production of Hydrogen from Acetate. Environ Sci Technol 2005;39:4317–20. https://doi.org/10.1021/es050244p.

[2] Hassanein A, Witarsa F, Lansing S, Qiu L, Liang Y. Bio-Electrochemical Enhancement of Hydrogen and Methane Production in a Combined Anaerobic Digester (AD) and Microbial Electrolysis Cell (MEC) from Dairy Manure. Sustain 2020;12. https://doi.org/10.3390/su12208491.

[3] Montpart N, Rago L, Baeza JA, Guisasola A. Hydrogen production in single chamber microbial electrolysis cells with different complex substrates. Water Res 2015;68:601–15. https://doi.org/https://doi.org/10.1016/j.watres.2014.10.026.

[4] Kadier A, Simayi Y, Abdeshahian P, Azman NF, Chandrasekhar K, Kalil MS. A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alexandria Eng J 2016;55:427–43. https://doi.org/https://doi.org/10.1016/j.aej.2015.10.008.

[5] Rousseau R, Etcheverry L, Roubaud E, Basseguy R, Delia M-L, Bergel A. Microbial electrolysis cell (MEC): Strengths, weaknesses and research needs from electrochemical engineering standpoint. Appl Energy 2020;257. https://doi.org/10.1016/j.apenergy.2019.113938.

[6] Zhang Y, Merrill MD, Logan BE. The use and optimization of stainless steel mesh cathodes in microbial electrolysis cells. Int J Hydrogen Energy 2010;35:12020–8. https://doi.org/https://doi.org/10.1016/j.ijhydene.2010.08.064.

[7] Gomes LC, Mergulhão FJ. SEM Analysis of Surface Impact on Biofilm Antibiotic Treatment. Scanning 2017;2017:2960194. https://doi.org/10.1155/2017/2960194.

Chapter 4

Results and Discussion

4.1 Polycrystalline solar cell – MEC mechanism

The spontaneous electron transfer process, the energy band diagram of the polycrystalline silicon solar cell, and the redox potential of various other stages of the Hybrid Solar – MEC system were shown in (Figure 4-1). The essential applied potential, needed by the MEC for directional migration of charges for hydrogen production, was provided by the solar-powered MEC. The MEC and solar cell jointly work in a way similar to the Z-scheme in the natural photosynthesis process [1–3]. P-doped and N-doped silicon semiconductors were joined together to form a P-N junction, where the N-doped silicon material shifted the Fermi level nearer to the conduction band and the P-doped silicon material shifted the Fermi level closer to the valence band [4,5]. The excess electrons present in the N-type region, transfer towards the P-type region to fill the excessive present hole in that region. The transfer of the majority carriers from both regions created a potential barrier.

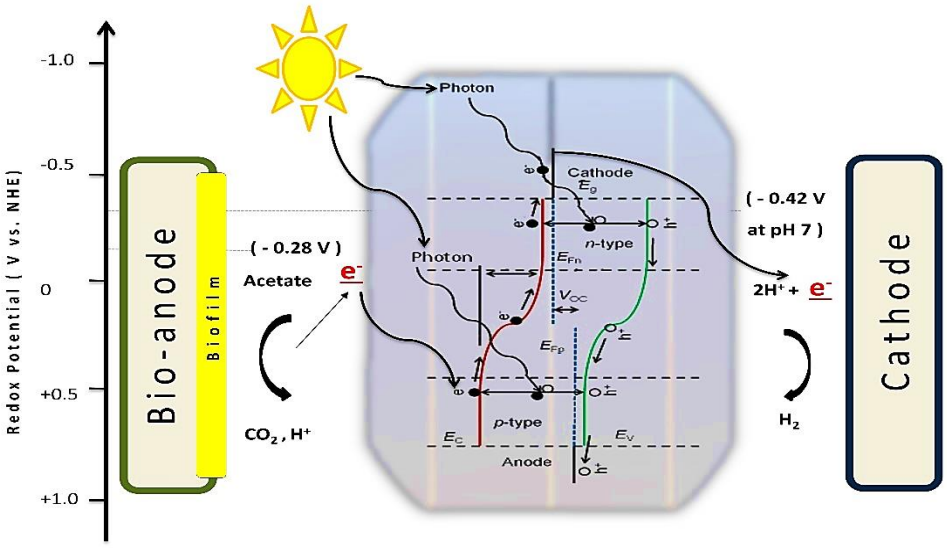

Figure 4-1 : Solar - MEC system electron transfer mechanism representing Z scheme of photosynthesis

In this system, sunlight is absorbed by electron-hole pairs and excites electrons to move towards the conduction band, leaving behind holes that move in similar manners but in opposite directions towards the valance band of the polycrystalline silicon. Electrons present in the conduction band move toward the cathode of the MEC through an external circuit and react with H^+ to produce hydrogen. This difference between energy levels results in an electromotive force that can generate current in a closed circuit (by connecting it with MEC). In the meantime, the exoelectrogenic microorganisms like *Geobacter sulfurreducens,* present in the bio-anode of MEC, oxidize the organic substrate available and produce H^+ ions and electrons [6,7]. The generated electrons were moved towards the p-type region of the solar cell to recombine with the hole and make electron-hole pair, making the circuit complete. The cycle continues as long as the solar energy lightens the polycrystalline silicon solar cell, and the organic substrate is oxidized.

4.2 Bioenergy production from the unassimilated anode

The fresh Ni-foam anode was placed in the MEC without prior exposure to the biological environment. Ni-foam was selected as the anode because of its large surface area, excellent biocompatibility to facilitate bacterial attachment growth, decent corrosion resistance, and high electronic conductivity. The control produced a negligible quantity of methane by the end of the 13^{th} day. Therefore, it was not further investigated. Figure 4-2 and Figure 4-3 depicts the trend of total hydrogen production (mm^3) as well as variations in the methane production rate per day using a rate limited bio-anode - MEC repectively.

Hydrogen production linearly increased for the first four days of operation and the maximum production was 139.1 ± 0.05 mm^3 by the end of day 4. This linearly increasing hydrogen production could probably be due to the presence of exoelectrogenic bacteria in the SDMW, such as *Pseudomonas* and *Geobacter sulfurreducens* [8–10]. These bacteria promoted the breakdown of organic content into electrons and protons and transferred the produced electrons toward the cathode, where they recombined with H^+ ions to produce hydrogen. When the measured current dropped below 0.1 mA, we concluded that all the substrate was utilized. This separation technique took 13 days for complete utilization of the substrate. It was observed that the performance of existing microbial species in synthetic wastewater reached its maximum by the end of day 4 and then experienced an

exponential decrease in the hydrogen production, resulted in a 76.28 % decrease in production by the end of day 13 as compared to that of maximum production. This decline in hydrogen production rate could be an indication that most of the organic content has been utilized or it may be due to the scavenging effect of inevitably existing acetoclastic and hydrogenotrophic methanogens [11].

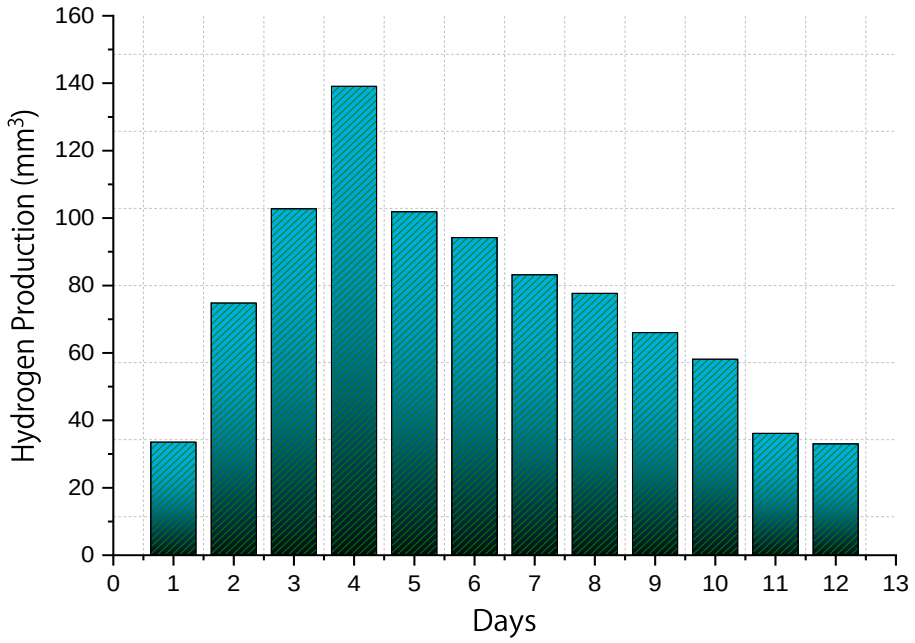

Figure 4-2 : Hydrogen production using rate limited bio-anode - Microbial Electrolysis Cell

These methanogens, especially hydrogenotrophic, were responsible for CH_4 production in acetate-fed MEC working under alkaline conditions, as they converted the produced hydrogen gas into methane [12]. Figure 4-3 shows that there was very low methane production rate (ml/l) was recorded till the end of the 7th day, which was collectively less than 10 % of the maximum methane production rate. As soon as the retention time of synthetic wastewater exceeded the one-week duration, an exponential increase in methane production rate was noted and the maximum methane production rate was 30.35 ± 0.03 ml/l by the end of the 13th day. About 67 % of the total produced gas was methane on the 13th day and the total produced methane was about 32.13 ± 0.15 ml. This rapid increase in the methane production rate after the first week was likely due to the dominant growth of hydrogenotrophic methanogens with time in an alkaline environment [13]. These methanogens formed microbial communities on the bio-anode and cathode

surfaces during the cycle time. Consequently, converted the produced hydrogen gas into methane.

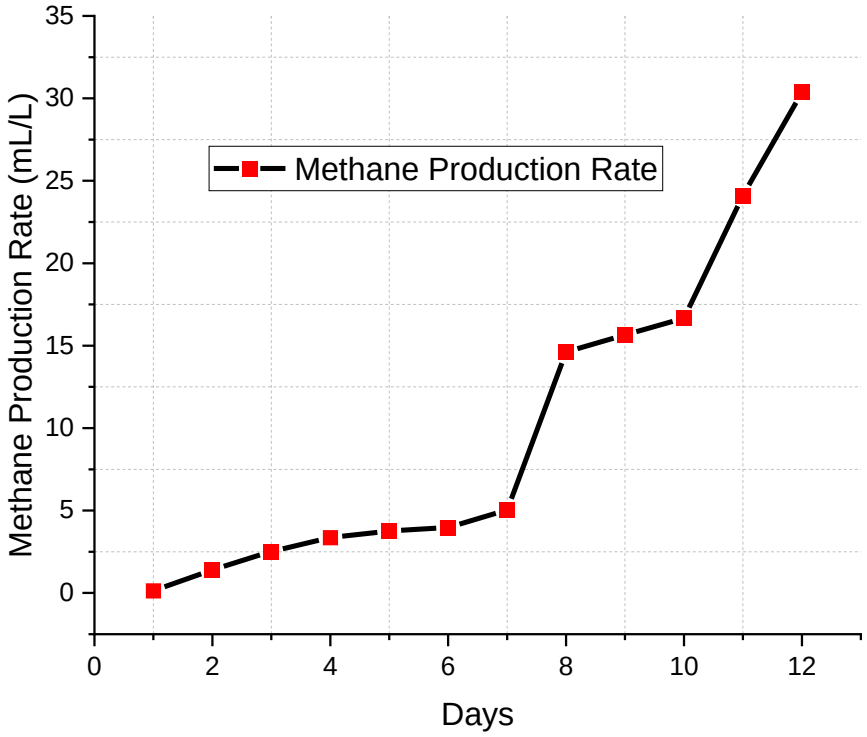

Figure 4-3 : : Methane production rate using rate limited bio-anode - Microbial Electrolysis Cell

Figure 4-4 presented the bioenergy production rate and trends, recorded using the fully exposed bio-anode – MEC. The total cycle time was noted to be approximately 3 days. This fast-paced utilization of substrate was probably due to the increased available surface area of the bio-anode [14,15]. The amount of produced biohydrogen was about 800 ± 5 mm^3 by both MECs, but the maximum methane production rate and the total produced methane using this technique was only 26.4± 0.05 ml/l and 15.21 ± 0.15 ml respectively. This was not as high as the previously discussed technique (rate limited bio-anode – MEC). This reduced methane production rate was due to the difference in cycle duration time of both MECs because the rate limited bio-anode took roughly 4 folds more time than that of fully exposed bio-anode – MEC. The reduced experimental time might not allow methanogens to completely grow on the electrode surfaces because of their low growth rate, resulted in less production of methane [16].

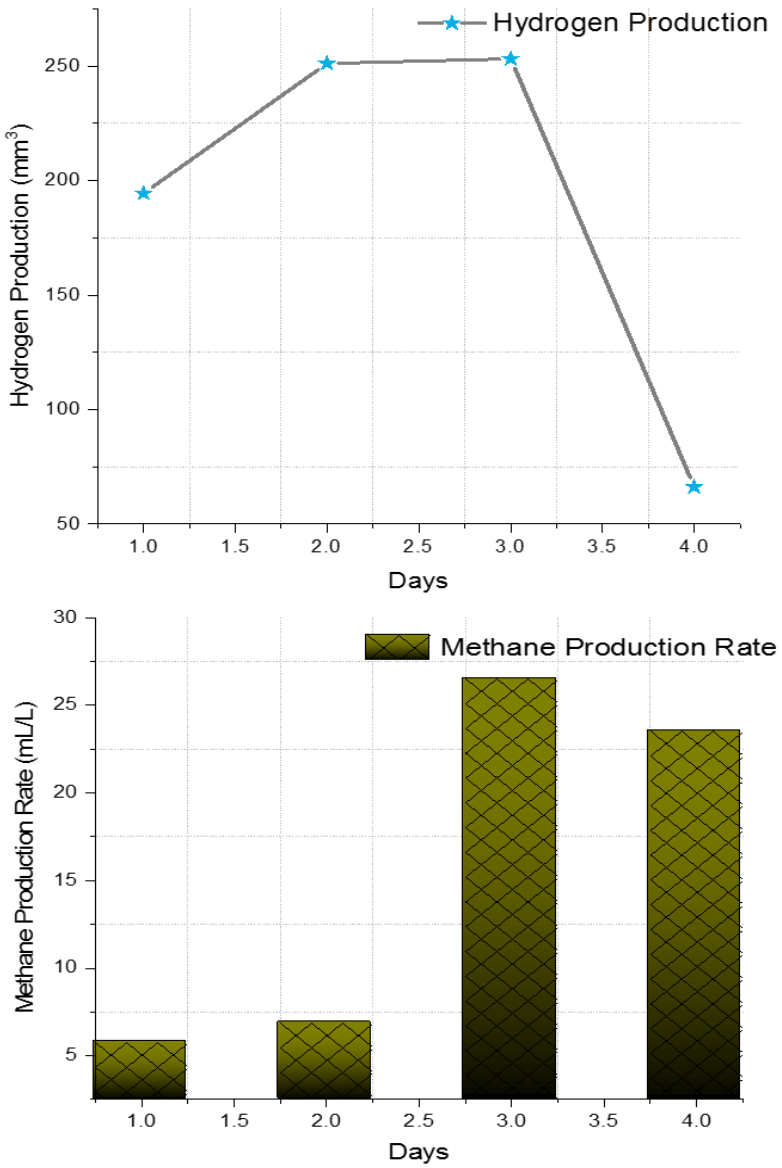

Figure 4-4: Bioenergy production Trends using fully exposed Microbial Electrolysis Cell (above) hydrogen production (below) Methane Production rate

An initially reported MEC, fed only with dairy manure wastewater, did not produce a measurable quantity of biogas with 0.43 mA current production [10], but this study successfully produced a measurable quantity of biogas (H_2 and CH_4; 800 mm^3 and 0.031 m^3/m^3/day respectively). This could be due to the use of highly porous anode material as well as due to the addition of additives in dairy manure wastewater.

The maximum methane production rate from this work (30.35 ml/l ≈ 0.031 m^3/m^3/day) using unassimilated bio-anode (rate limited separation technique) was equal to 11.54% of the CH_4 production rate obtained by the previously reported Anaerobic

Digestion (AD) coupled MEC system (0.26 m^3/m^3/day) [17]. This comparative analysis indicated that the obtainable CH_4 solely from unassimilated MEC fed with dairy manure wastewater, was of moderate quantity and more research work in this area could shift the focus towards the unassimilated MEC system.

4.3 Current generation from the unassimilated anode

The effects of the exposed surface area of the bio-anode on current (mA) generation were studied with the help of two electrode separation techniques (rate limited bio-anode MEC and fully exposed bio-anode MEC) and the results were plotted in Figure 4-5. The rate limited bio-anode - MEC completely utilized the substrate in about 13 days with a maximum current generation of 35.5 mA.

It was noted that the total biogas production as compared to such a high generated current was not satisfactory. This lack of biogas production could be due to the continuous oxidation of the organic substrate at the bio-anode, which results in the continuous generation of protons and electrons. Due to the less exposed surface area of the anode, the generated protons could not reach the cathode surface to combine with electrons coming from the external circuit for hydrogen evolution [18]. The unexploited electrons return to the anode surface, making the electron flow circuit complete. These electrons again travel from the outer circuit with newly generated electrons toward the cathode surface, resulting in an increased total amount of generated current [19].

All the suppositions about the rate limitation due to the bio-anode were confirmed by using the fully exposed bio-anode – MEC, as this technique completely digested the organic substrate within 3 days. The maximum generated current with this separation configuration was recorded to be 5.3 mA. This maximum generated current was achieved with in 24 hours after start of the MEC cycle. This may be due to the fact that microorganisms present in the inoculum readily started digesting the organic substrate as it feeds to the system and as there was no limitation, all the produced hydrogen ion and electrons move towards the cathode to produce bioenergy. The total biogas produced by the later configuration was half of that produced by the rate limited bio-anode – MEC.

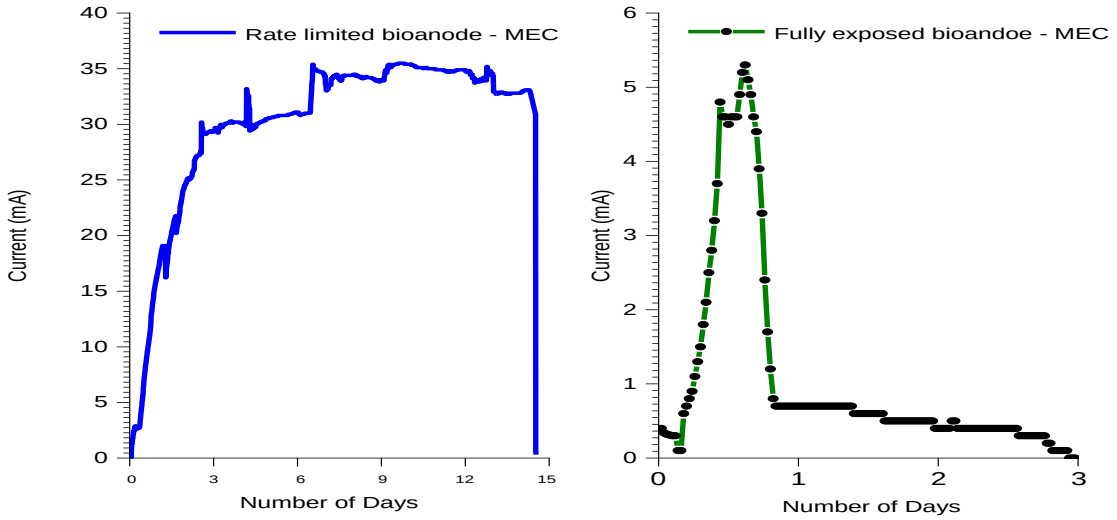

Figure 4-5: Current produced in Rate limited bio-anode - MEC (left) and fully exposed bio-anode - MEC (right)

4.4 Biofilm assimilation at electrodes

SEM was considered as the best and effective technique utilized for the examination of surface morphology of electrodes, characteristics of the assimilated biofilm or microbial community, and structural breakdown of the electrodes. Many previous studies have been reported till now that utilzied SEM to verify the formation of microbial community as well as the structural changes in the electrodes [20,21].

The anode and cathode of the rate limited bio-anode - MEC were of great interest because they produced more total biogas, had high current generation, and more chances of microbial community growth due to the long cycle time. Therefore, SEM-EDS analysis was performed on both electrodes before and after one complete cycle of the rate limited bio-anode – MEC. These characterization techniques were utilized to examine the growth of the microbial community, corrosion, structural damage on the electrode surfaces, and variations in the elemental composition of electrodes due to microbial community formation.

The Ni-foam anode has a highly porous structure (Figure 4-6). Therefore, it could promote the growth of an extensive microbial community by providing a large surface area for adherence and propagation of biofilm [20,21]. Figure 4-6 (A, B, C) and Figure 4-9 (A) presented the SEM images of the Ni-foam anode and the SSM 304L cathode, taken before

the startup of the MEC respectively. They demonstrated a flawless, smooth, and uniform surface of both electrodes.

Dairy manure debris got stuck on the major portion of the Ni-foam anode and caused clogging of the electrode pores, clearly visible at 200 µm magnification (Figure 4-6-D). This clogging of the pores may affect the assimilation of the microbial community as these clogged pores were not further exposed to the microorganisms. The presence of microbial community was also observed on the bio-anode surface as indicated by small bumps, encircled by white color and shown in Figure 4-6 (D, E, F) with the magnification of 200µm, 50 µm, and 20µm respectively.

The shape of the microbial community could only be recognized at higher magnification, like 10 µm, 5 µm, or 2 µm [22,23]. More magnification could also be utlized for the evaluation of the microbial community but it appeared to be less relevant as only the bacterial cell was visible at that scale and no electrode surface was sought. Therefore, the area marked with a red rectangular box in Figure 4-6 (G) was selected for in-depth analysis, and SEM images with a magnification of 10 µm and 2 µm were captured at that location. The grown microbial community on the bio-anode surface was shown in Figure 4-6 (H, I).

The rod-shaped (encircled green) along with round-shaped bacteria (encircled white) were formed in this community. The rod-shaped bacteria could be *Geobacter* or *Enterobacter* because they grow in an anaerobic environment, have exoelectrogenic nature, and a rod-shaped structure [24,25]. These microorganisms were reported for hydrogen production in previous studies [10,26,27]. The round-shaped microbial community observed on the anode surface could be *Methanococcus*, responsible for the conversion of produced CO_2 and H_2 into CH_4. [28–30].

The initiation of microbial community formation on the Ni-foam surface was almost identical to the previously reported studies [31,32], confirming the successful formation of the anodic biofilm community. It was also confirmed now that the production of the bioenergy was due to these bacteria growth as they started the breakdown of organic content present in the wastewater. The repeated exposure of electrodes in the bacterial environment and synergistic effect of microbial community could help in the proliferation and growth of formed community [33,34].

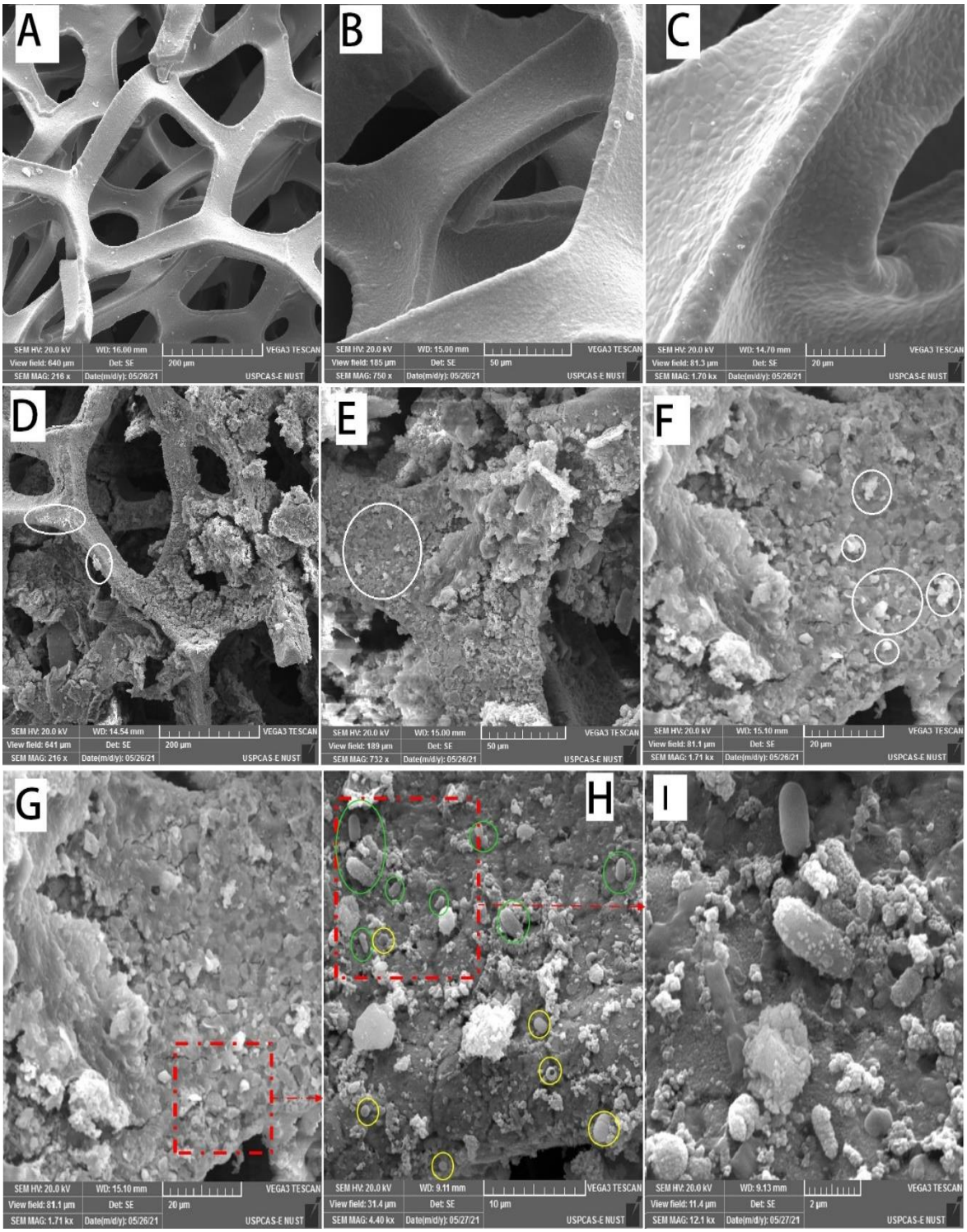

Figure 4-6 Scanning Electron Microscopy (SEM) images of Ni-foam bio-anode: (A, B, C) Ni-foam images before use with 200 um, 50 um and 20 um magnification respectively; (D, E, F) Ni-foam images after use in MEC with 200 um, 50 um and 20 um magnification respectively for comparison of morphological changes; (G, H, I) Images of biofilm formed on Ni-foam surface and shape of the microbial community formed with 20 um, 10 um and 2um. (Red box depicts the area from where magnified SEM image was taken in next frame) (Round or circular microbial community was marked with yellow circles and rod-like microbial community was marked with green circles)

Figure 4-7 presents the EDS results obtained from the Ni-foam anode. The SEM-EDS elemental analysis by weight percentage of the Ni-foam anode was presented in Figure 4-8. The comparison of both data revealed the reduction of nickel content by 15% weight percent, which was likely due to the removal of the oxide layer from the bio-anode surface. The change in the overall elemental composition of the electrode was also noticed and it was due to the accumulation of other elements on the Ni-foam surface, such as magnesium, silicon, calcium, and sodium that may come from SDMW. The variation in oxygen content from 0.8% to 13.7% was also a significant change in the elemental composition of the bio-anode and this was probably due to the assimilation of living microorganisms on the bio-anode surface.

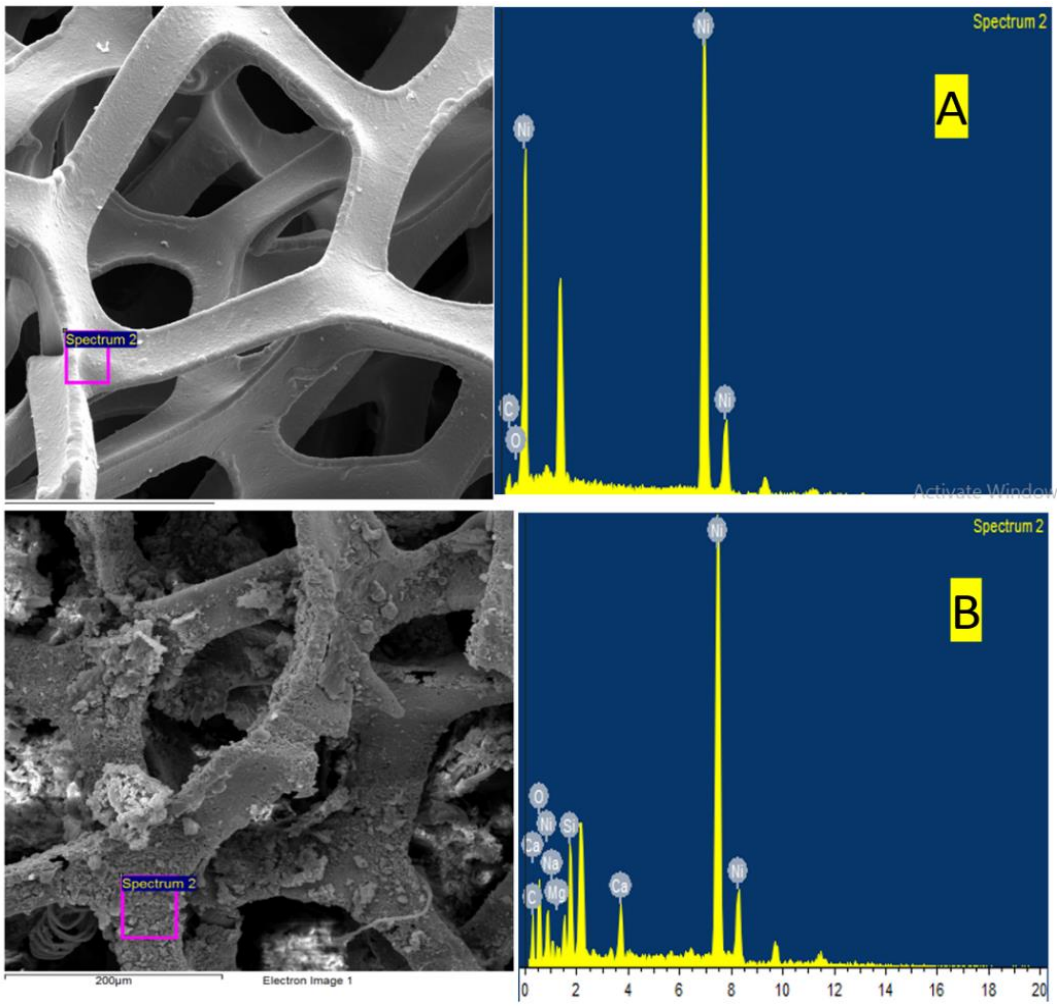

Figure 4-7 : EDS results of Ni-foam anode (a)before use and (b) after use

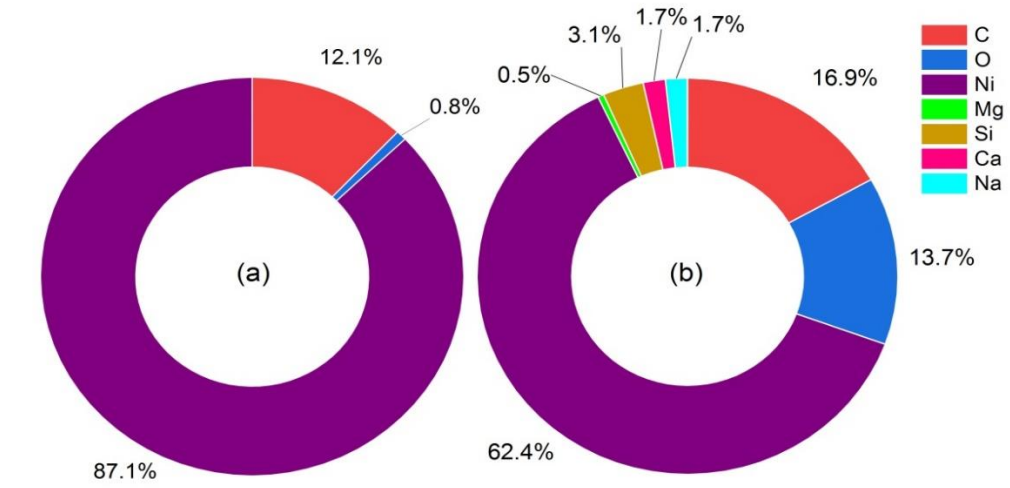

Figure 4-8 Elemental compositions (weight %) of Ni-foam anode (a) before and (b) after experimentation

The SSM grade 304L cathode was also examined by SEM-EDS, before and after utilization for one complete cycle in MEC and the captured images were presented in Figure 4-9. Figure 4-9 (b) revealed the attachment of debris to the electrode surface due to the presence of solid particles in the SDMW. It was noticeable in the red rectangular box that no pitting or wire distortion was observed on a debris-free section of the cathode, which was an indicator that no corrosion was noted after one complete cycle. This observation was also verified by comparing the elemental composition using EDS of the cathode material before and after the MEC cycle.

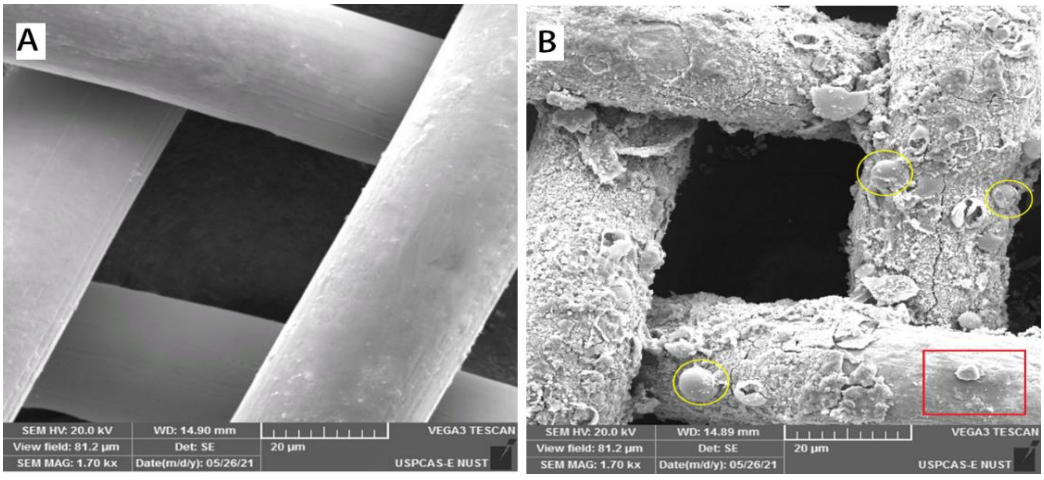

Figure 4-9: Scanning Electron Microscopy (SEM) images of Stainless Steel Mesh 304L cathode with 20um magnification (A) before utilization and (B) after use (Round or circular microbial community was marked with yellow circles and debris-free SSM wire was marked with red rectangular box)

Interestingly, the round-shaped microbial community (yellow circle), which was identical to the *Methanococcus* bacteria grown on the anode surface, was also detected on the cathode surface. Operating MEC under negative chamber pressure [35], shorter MEC cycle duration, high applied voltage, or adding bromoethanesulfonate as an additive to MEC [36], are some of the techniques that have been suggested in previously published works to limit the growth of methanogens and to reduce the conversion of hydrogen to methane. Figure 4-10 presents the EDS results obtained before and after the MEC cycle.

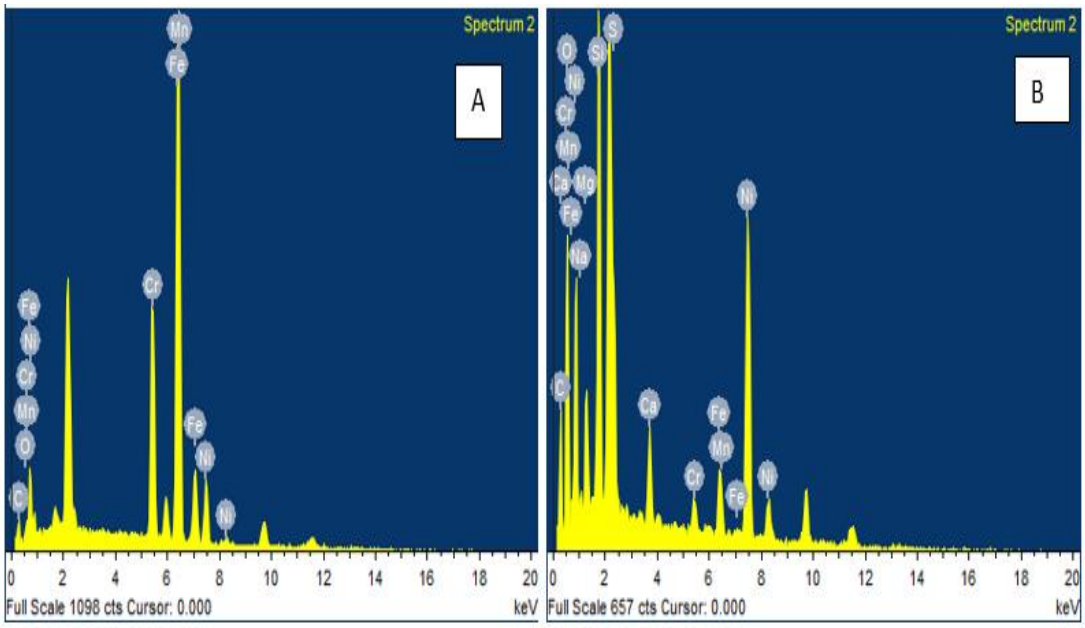

Figure 4-10 : EDS results of Stainless steel mesh 304L cathode (a) before use (b) after use.

Figure 4-11 presents the elemental composition analysis (weight percentage) of SSM grade 304L (a) before and (b) after experimentation. There was a significant reduction in chromium content by 15.8% weight after one complete MEC cycle, which was perhaps due to the effect of alkaline pH resulting in leaching and reduction of chromium content. The weight percentage content of carbon, oxygen, magnesium, silicon, calcium, and sulfur was also increased, possibly because of exposure to SDMW, alkaline buffer solution, and living bacteria.

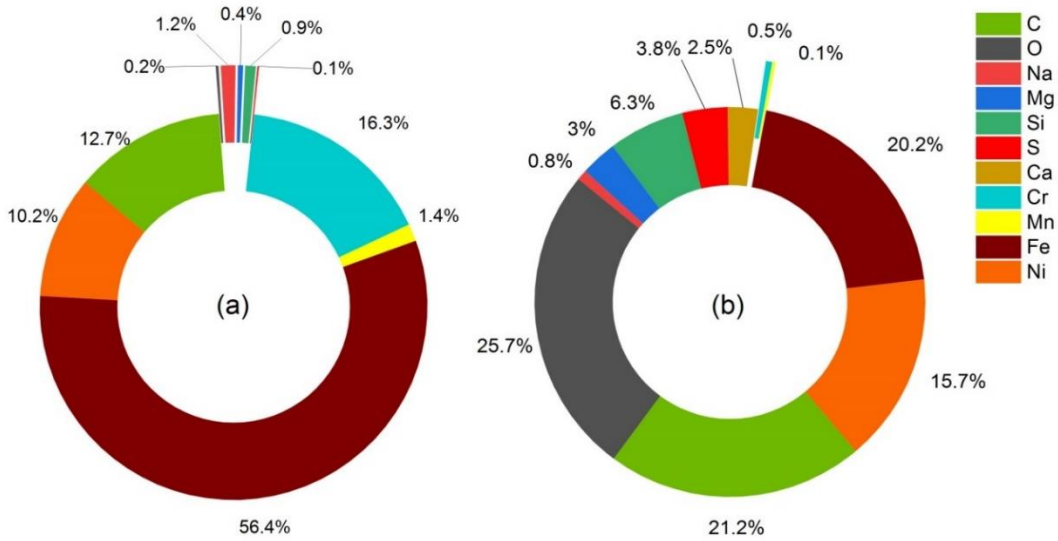

Figure 4-11: Elemental compositions (weight %) of Stainless-steel mesh 304L (a) before and (b) after experimentation

Summary

The most critical part of the research was described in this chapter. Results and discussions about the bioenergy production using an unassimilated bio-anode in the MEC was discussed in detail. The integration of solar cell with MEC and the electron transfer mechanism was discussed. It was suggested that the electron transfer took place as z-scheme of photosynthesis. The hydrogen produced using both separation techniques was about 800 mm^3. The methane production rate was 30.35 ± 0.03 ml/l by from rate limited bio-anode – MEC and 26.4 ± 0.03 ml/l from the fully exposed – MEC system.

The rate limited bio-anode – MEC produced 2 times more total methane as compared to the fully exposed bio-anode – MEC. Therefore, it was of more concentration. The highest generated current from the rate limited bio-anode – MEC was 35.5 mA. The SEM images of the anode confirmed the assimilation of the microbial community. The assimilated community was rod-shaped bacteria, could be *Geobacter* or *Enterobacter*, and round-shaped microbial community,could be *Methanococcus*. This growth of microbial community within 13 days of MEC operation along with bioenergy production was a breakthrough in this field.

References

[1] Chae K-J, Choi M-J, Kim K-Y, Ajayi F, Chang I-S, Kim I. A Solar-Powered Microbial Electrolysis Cell With a Platinum Catalyst-Free Cathode To Produce Hydrogen. Environ Sci Technol 2009;43:9525–30. https://doi.org/10.1021/es9022317.

[2] Lopez FB, Barclay GF. Chapter 4 - Plant Anatomy and Physiology. In: Badal S, Delgoda RBT-P, editors., Boston: Academic Press; 2017, p. 45–60. https://doi.org/https://doi.org/10.1016/B978-0-12-802104-0.00004-4.

[3] Kim JH, Jeon Y, Kim S. A Solar-driven Microbial Electrolysis Cell for Hydrogen Production from Acetate with Minimal CO2 and CH4 Emission Using p-Type Cu2O Nanoparticles. Bull Korean Chem Soc 2017;38:1385–6. https://doi.org/https://doi.org/10.1002/bkcs.11305.

[4] Zhuiykov SBT-NSO for the NG of E and FD, editor. 1 - Electrons and holes in a semiconductor, Woodhead Publishing; 2014, p. 1–49. https://doi.org/https://doi.org/10.1533/9781782422242.1.

[5] Blakers A, Zin N, McIntosh KR, Fong K. High Efficiency Silicon Solar Cells. Energy Procedia 2013;33:1–10. https://doi.org/https://doi.org/10.1016/j.egypro.2013.05.033.

[6] Cardeña R, Cercado B, Buitrón G. Chapter 7 - Microbial Electrolysis Cell for Biohydrogen Production. In: Pandey A, Mohan SV, Chang J-S, Hallenbeck PC, Larroche CBT-B (Second E, editors. Biomass, Biofuels, Biochem., Elsevier; 2019, p. 159–85. https://doi.org/https://doi.org/10.1016/B978-0-444-64203-5.00007-1.

[7] Varanasi JL, Veerubhotla R, Pandit S, Das D. Chapter 5.7 - Biohydrogen Production Using Microbial Electrolysis Cell: Recent Advances and Future Prospects. In: Mohan SV, Varjani S, Pandey ABT-MET, editors. Biomass, Biofuels Biochem., Elsevier; 2019, p. 843–69. https://doi.org/https://doi.org/10.1016/B978-0-444-64052-9.00035-2.

[8] Gupta KK, Aneja KR, Rana D. Current status of cow dung as a bioresource for sustainable development. Bioresour Bioprocess 2016;3:28. https://doi.org/10.1186/s40643-016-0105-9.

[9] Singh M. Isolation and characterization of bacteria from cow dung of desi cow breed

on different morpho-biochemical parameters in Dehradun, Uttarakhand, India. 2014.

[10] Kiely PD, Cusick R, Call DF, Selembo PA, Regan JM, Logan BE. Anode microbial communities produced by changing from microbial fuel cell to microbial electrolysis cell operation using two different wastewaters. Bioresour Technol 2011;102:388–94. https://doi.org/https://doi.org/10.1016/j.biortech.2010.05.019.

[11] Li F, Liu W, Sun Y, Ding W, Cheng S. Enhancing hydrogen production with Ni–P coated nickel foam as cathode catalyst in single chamber microbial electrolysis cells. Int J Hydrogen Energy 2017;42:3641–6. https://doi.org/https://doi.org/10.1016/j.ijhydene.2016.10.163.

[12] Wormald RM, Rout SP, Mayes W, Gomes H, Humphreys PN. Hydrogenotrophic Methanogenesis Under Alkaline Conditions . Front Microbiol 2020;11:3077.

[13] Wormald R. Hydrogenotrophic methanogenesis dominates at high pH. ACCESS Microbiol 2019;1.

[14] Guo K, Prévoteau A, Rabaey K. A novel tubular microbial electrolysis cell for high rate hydrogen production. J Power Sources 2017;356:484–90. https://doi.org/https://doi.org/10.1016/j.jpowsour.2017.03.029.

[15] Jwa E, Yun Y-M, Kim H, Jeong N, Park S-C, Nam J-Y. Domestic wastewater treatment in a tubular microbial electrolysis cell with a membrane electrode assembly. Int J Hydrogen Energy 2019;44:652–60. https://doi.org/https://doi.org/10.1016/j.ijhydene.2018.11.036.

[16] Liu X, Yan Z, Yue Z-B. 3.10 - Biogas. In: Moo-Young MBT-CB (Second E, editor., Burlington: Academic Press; 2011, p. 99–114. https://doi.org/https://doi.org/10.1016/B978-0-08-088504-9.00165-3.

[17] Hassanein A, Witarsa F, Lansing S, Qiu L, Liang Y. Bio-Electrochemical Enhancement of Hydrogen and Methane Production in a Combined Anaerobic Digester (AD) and Microbial Electrolysis Cell (MEC) from Dairy Manure. Sustain 2020;12. https://doi.org/10.3390/su12208491.

[18] Nemiwal M, Gosu V, Zhang TC, Kumar D. Metal organic frameworks as electrocatalysts: Hydrogen evolution reactions and overall water splitting. Int J Hydrogen Energy 2021;46:10216–38.

https://doi.org/https://doi.org/10.1016/j.ijhydene.2020.12.146.

[19] Lim SS, Yu EH, Daud WRW, Kim BH, Scott K. Bioanode as a limiting factor to biocathode performance in microbial electrolysis cells. Bioresour Technol 2017;238:313–24. https://doi.org/https://doi.org/10.1016/j.biortech.2017.03.127.

[20] Jeremiasse AW, Hamelers HVM, Saakes M, Buisman CJN. Ni foam cathode enables high volumetric H2 production in a microbial electrolysis cell. Int J Hydrogen Energy 2010;35:12716–23. https://doi.org/https://doi.org/10.1016/j.ijhydene.2010.08.131.

[21] Jayabalan T, Matheswaran M, Preethi V, Naina Mohamed S. Enhancing biohydrogen production from sugar industry wastewater using metal oxide/graphene nanocomposite catalysts in microbial electrolysis cell. Int J Hydrogen Energy 2020;45:7647–55. https://doi.org/https://doi.org/10.1016/j.ijhydene.2019.09.068.

[22] Gomes LC, Mergulhão FJ. SEM Analysis of Surface Impact on Biofilm Antibiotic Treatment. Scanning 2017;2017:2960194. https://doi.org/10.1155/2017/2960194.

[23] Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms. Curr Protoc Microbiol 2005;Chapter 1:Unit-1B.1. https://doi.org/10.1002/9780471729259.mc01b01s00.

[24] Tabares M, Dulay H, Reguera G. Geobacter sulfurreducens. Trends Microbiol 2020;28:327–8. https://doi.org/10.1016/j.tim.2019.11.004.

[25] Buckle J. Chapter 7 - Infection. In: Buckle JBT-CA (Third E, editor., St. Louis: Churchill Livingstone; 2015, p. 130–67. https://doi.org/https://doi.org/10.1016/B978-0-7020-5440-2.00007-3.

[26] Call D, Wagner R, Logan B. Hydrogen Production by Geobacter Species and a Mixed Consortium in a Microbial Electrolysis Cell. Appl Environ Microbiol 2009;75:7579–87. https://doi.org/10.1128/AEM.01760-09.

[27] Kumar N, Das D. Enhancement of hydrogen production by Enterobacter cloacae IIT-BT 08. Process Biochem 2000;35:589–93. https://doi.org/https://doi.org/10.1016/S0032-9592(99)00109-0.

[28] Chen S, Deng H, Zhao Y, Lu S, Zhao Y, Cheng X, et al. The effects of Methanococcus maripaludis on the corrosion behavior of EH40 steel in seawater. Bioelectrochemistry 2021;140:107824.

https://doi.org/https://doi.org/10.1016/j.bioelechem.2021.107824.

[29] Goyal N, Zhou Z, Karimi IA. Metabolic processes of Methanococcus maripaludis and potential applications. Microb Cell Fact 2016;15:107. https://doi.org/10.1186/s12934-016-0500-0.

[30] Gao T, Zhang H, Xu X, Teng J. Integrating microbial electrolysis cell based on electrochemical carbon dioxide reduction into anaerobic osmosis membrane reactor for biogas upgrading. Water Res 2021;190:116679. https://doi.org/https://doi.org/10.1016/j.watres.2020.116679.

[31] Zhen G, Kobayashi T, Lu X, Kumar G, Hu Y, Bakonyi P, et al. Recovery of biohydrogen in a single-chamber microbial electrohydrogenesis cell using liquid fraction of pressed municipal solid waste (LPW) as substrate. Int J Hydrogen Energy 2016;41:17896–906. https://doi.org/10.1016/j.ijhydene.2016.07.112.

[32] Lu L, Hou D, Fang Y, Huang Y, Ren Z. Nickel based catalysts for highly efficient H2 evolution from wastewater in microbial electrolysis cells. Electrochim Acta 2016;206. https://doi.org/10.1016/j.electacta.2016.04.167.

[33] Schink B. Synergistic interactions in the microbial world. Antonie Van Leeuwenhoek 2002;81:257–61. https://doi.org/10.1023/A:1020579004534.

[34] Shao Q, Li J, Yang S, Sun H. Effects of different substrates on microbial electrolysis cell (MEC) anodic membrane: biodiversity and hydrogen production performance. Water Sci Technol 2019;79:1123–33. https://doi.org/10.2166/wst.2019.107.

[35] Feng H, Huang L, Wang M, Xu Y, Shen D, Li N, et al. An effective method for hydrogen production in a single-chamber microbial electrolysis by negative pressure control. Int J Hydrogen Energy 2018;43:17556–61. https://doi.org/https://doi.org/10.1016/j.ijhydene.2018.07.197.

[36] Wang A, Liu W, Cheng S, Xing D, Zhou J, Logan BE. Source of methane and methods to control its formation in single chamber microbial electrolysis cells. Int J Hydrogen Energy 2009;34:3653–8. https://doi.org/https://doi.org/10.1016/j.ijhydene.2009.03.005.

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

This study utilized a single chamber, tubular MEC configuration for bioenergy production. The use of polycrystalline silicon solar cell was recommended because it could provide enough voltage for the MEC operation. The MEC and solar cell jointly work in a way similar to the Z-scheme in the natural photosynthesis process. The coaxial configuration of electrodes was a successful variation and could avoid rate limitation during the reaction. Unassimilated Ni-foam was proved to be very effective for instant bioenergy production from the wastewater. This experimental set-up successfully produced bioenergy (hydrogen and methane) without prior biofilm assimilation on the anode surface. Two different separation techniques were utilized to examine the effects of the exposed surface area of the bio-anode on bioenergy production. The rate limited bio-anode - MEC technique achieves a maximum methane production rate of 30.35 ± 0.03 ml/l, 14.2% more than that achieved by the fully exposed bio-anode - MEC technique (26.4 ± 0.05 ml/l). The total methane produced using formerly mentioned technique was 32.13 ± 0.15 ml, which was 2 times more than that produced by the later (15.21 ± 0.15 ml) mentioned technique. This was due to a prolonged retention time of 13 days. Hydrogen production was approximately 800 ± 5 mm^3 in both experimentations. The maximum generated current was 35.5 mA. SEM images helped to understand the characteristics of the grown microbial community after one complete cycle, and it was noted that both rod-shaped and round-shaped bacteria were formed on the anode surface, but only round-shaped (*Methanococcus*) bacteria on the cathode surface. The production of bioenergy during the first cycle using unassimilated bio-anode along with the growth of the microbial community, was a breakthrough in this field. These unique outcomes of using unassimilated bio-anode in the MEC make it a viable alternative strategy to produce bioenergy using wastewater instantly.

5.2 Recommendation

- The rate of bioenergy production directly depends upon the substrate utilized in the MEC, therefore future work using novel substrates (food processing wastewater, industrial wastewater) having high organic content can be utilized.
- The Ni-foam electrode produces significant amount of the bioenergy but the deposition of some highly efficient catalyst on bare Ni-foam (Molybdenum, disulfide, CNT's) could be an effective technique for future researchers.
- This research work focuses on the operation of MEC at 31 ^{0}C temperature, however it is possible to check the working of MEC at Psychrophilic, Thermophilic or hyper-thermophilic temperatures.
- There may be some leakage of the produced biogas while collecting in syringe and taking it for GC analysis, therefore, it is recommended to use a portable gas analyzer to avoid such error.

Appendix A

Research Article

Received: 12 June 2021 | Revised: 7 July 2021 | Accepted: 14 July 2021

DOI: 10.1002/er.7091

RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF ENERGY RESEARCH WILEY

Evaluating the use of unassimilated bio-anode with different exposed surface areas for bioenergy production using solar-powered microbial electrolysis cell

Muhammad Muddasar[1] | **Rabia Liaquat**[1] | **Ali Abdullah**[1] | **Asif Hussain Khoja**[1] | **Nadia Shahzad**[1] | **Naseem Iqbal**[1] | **Muhammad Ishtiaq Ali**[2] | **Azhar Uddin**[1] | **Sami Ullah**[1]

[1]U.S.-Pakistan Centre for Advanced Studies in Energy (USPCAS-E), National University of Sciences & Technology (NUST), Sector H-12, Islamabad, Pakistan

[2]Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

Correspondence
Rabia Liaquat, U.S.-Pakistan Centre for Advanced Studies in Energy (USPCAS-E), National University of Sciences & Technology (NUST), Sector H-12, Islamabad 44000, Pakistan.
Email: rabia@uspcase.nust.edu.pk

Summary

The microbial electrolysis cell (MEC) is an emerging technology for bioenergy production using organic wastewater. Normally, a preassimilated bio-anode is utilized by the MEC to break down the organic content, but the formation and assimilation of microbial community at the anode surface is a time-consuming process. This study utilized a novel unassimilated Ni-foam anode for the first time in solar-powered MEC for bioenergy production. Synthetic dairy manure wastewater (SDMW) was used both as substrate and an inoculum in the solar-powered tubular MEC. The impacts of the exposed surface area of the bio-anode on bioenergy production were evaluated by utilizing two different separation techniques (rate-limited bio-anode – MEC and fully exposed bio-anode - MEC). The former technique achieves a maximum methane production rate of 30.35 ± 0.03 mL/L, 14.2% more than that achieved by the later mentioned technique (26.4 ± 0.05 mL/L). Hydrogen production was approximately 800 ± 5 mm^3 in both experimentations. The maximum generated current in the rate limited bio-anode – MEC was 35.5 mA. Scanning electron microscope images confirmed the formation of rod-shaped along with round-shaped microbial communities on the anode surface, and, interestingly, round-shaped bacteria were also grown on the cathode surface. The bioenergy (H_2 and CH_4) produced using SDMW within first 13 days of operation, along with the formation of a microbial community, was a significant success in this area and has opened up many research opportunities for producing instant bioenergy from organic waste.

KEYWORDS

hydrogen production, methane production, microbial electrolysis cell, solar-powered MEC, unassimilated anode

1 | INTRODUCTION

The exponential growth of the world population along with rapid urbanization has resulted in huge demand for energy, which is expected to rise to 8.4×10^{14} MJ in 2060.[1] About 70% of the world's electricity generation is still from conventional fossil fuels, that is, coal, natural gas, and gasoline. The high concentration of greenhouse

