HETEROGENEOUS SULFONATED POLYSULFONE MEMBRANE IN MICROBIAL FUEL CELL FUELED BY DEWATERED SLUDGE AND FRUIT WASTE USING *BACILLUS SUBTILIS* AS BIOCATALYST

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by

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LIST OF SYMBOLS

μ_{max}	Maximum specific growth rate
K_s	Monod saturation constant
n	Number of electrons
R_Ω	Ohmic resistance
I_p	Peak of electric current
R_p	Polarization resistance
v	Potential scan rate
X_{red}	Reduced redox protein concentration
μ_o	Specific growth rate
R^2	Squared regression correlation coefficient
E^0	Standard potential of the redox couple
T	Temperature
t	Time
α	Transfer coefficient
V	Voltage
V	Volume of anode compartment
Y_M	Yield of oxidized redox protein

LIST OF ABBREVIATIONS

μ	Specific growth rate
μ	Micro
A	Ampere
A	Area
AAS	Atomic absorption spectroscopy
AEM	Anion exchange membrane
Ag_2NO_3	Silver nitrate
ANOVA	Analysis of Variance
BS	*Bacillus subtilis* sp.
Ca	Calcium
CAGR	Compound Annual Growth Rate
CCD	Central composite design
Cd	Cadmium
CEM	Cation exchange membrane
cm	Centimetre
CO	Carbon monoxide
CO2	Carbon dioxide
CO2	Carbon dioxide
COD	Chemical oxygen demand
CS	Chitosan
CSA	Chlorosulfonic acid
Cu	Copper
CV	Cyclic voltammetry

EB	Electrogenic bacteria
Fe	Iron
FTIR	Fourier-transform infrared spectroscopy
g	Gram
h	Hour
H	Hydrogen
h^{-1}	Per hour
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HNO_3	Nitric acid
I	Current
IEC	Ion exchange capacity
IEM	Ion exchange membrane
K	Potassium
$K_2Cr_2O_7$	Potassium dichromate
L	Litre
M	Mega
mA	Milliampere
MFCs	Microbial fuel cells
mg	Milligram
Mg	Magnesium
mg/L	Milligram per liter
min	Minute
mL	Millimetre

Mn	Manganese
mW	Milliwatts
W/m^2	Watt per meter square (Power density)
N	Nitrogen
O	Oxygen
^oC	Degree celcius
OFAT	One-Factor-At-A-Time
P	Power
P	Phosphorus
PEM	Proton exchange membrane
ppm	Parts per million
PSf	Polysulfone
R	Resistance
R^2	Correlation coefficient
RSM	Response Surface Methodology
Td	Doubling time
V	Voltage
vs	Versus
W	Watts
w/w	Weight per weight
Zn	Zinc
Ω	Ohms

LIST OF APPENDICES

MEMBRAN POLISULFON BERBENTUK HETEROGEN YANG DISULFONASI DALAM SEL BAHAN API MIKROB DIKUASAKAN OLEH ENAPCEMAR TERNYAHAIR DAN SISA BUAH MENGGUNAKAN *BACILLUS SUBTILIS* SEBAGAI BIOPEMANGKIN

ABSTRAK

Sel bahan api mikrob (MFC) digunakan sebagai sistem bioelektrokimia yang menukar sisa organik seperti enap cemar terdehidrasi dan sisa buah-buahan kepada tenaga elektrik dengan menggunakan membran polimer tersulfonasi yang direka khas untuk meningkatkan pemindahan proton dan prestasi keseluruhan. Pembuatan membran polimer tersulfonasi heterogen termaju untuk MFC ialah topik yang paling berpotensi pada masa ini untuk menangani isu substrat dan silang silang mikrob, kadar pemindahan proton yang rendah dan rintangan dalaman yang lebih tinggi dalam operasi MFC. Dalam kerja ini, polisulfon tersulfon heterogen yang disambung silang dengan kitosan tersulfonasi (SPSf/SCS) telah digunakan sebagai membran pertukaran proton dalam MFC. Pencirian fizikal, kimia dan elektrokimia membran SPSf dengan masa rendaman berbeza dalam agen sulfonasi telah disiasat sebagai prosedur saringan. SPSf yang paling berprestasi baik digunakan sebagai bes polimer bersulfonat untuk memaut silang dengan kitosan bersulfonat. Formulasi berbeza SPSf/SCS telah direka; iaitu SPSf/SCS-0.5 wt%, SPSf/SCS-0.75 wt% dan SPSf/SCS-1 wt% dan pencirian fizik, kimia dan elektrokimia yang komprehensif dibandingkan dengan membran dikomersialkan Nafion 212. Membran komposit mempamerkan kekuatan mekanikal dan kestabilan terma yang dipertingkatkan disebabkan oleh kekukuhan SPSf tulang belakang, manakala kelenturan tulang belakang SPSf yang lebih baik.. Secara kimia, kehadiran kumpulan asid sulfonik dalam kedua-dua komponen memudahkan kapasiti

pertukaran ion (IEC) yang tinggi dalam julat 1.01 – 2.01 meq/g dan kekonduksian proton, yang penting untuk pengangkutan proton yang cekap dalam MFC. Pencirian elektrokimia mendedahkan bahawa membran SPSf/SCS-0.75 wt% mencapai prestasi unggul kira-kira 0.0378 S/cm berbanding membran Nafion komersial, dengan rintangan dalaman yang lebih rendah dan kekonduksian ionik yang lebih tinggi. Prestasi sinergistik membran SPSf/SCS dan MFC yang menggunakan kepekatan berbeza enap cemar dewatered dan sisa buah sebagai substrat kemudiannya dinilai menggunakan pendekatan satu faktor pada satu masa (OFAT), diikuti dengan metodologi permukaan tindak balas (RSM) menggunakan model kuadratik melalui Reka Bentuk Komposit Pusat. SPSf/SCS – Membran 0.75 wt% menunjukkan ketumpatan kuasa tertinggi untuk 50% (b/v) enap cemar ternyah air dan 60% (b/v) sisa buah. Membran yang dioptimumkan menunjukkan 35 W/m², pertumbuhan biojisim 3.3 g/L dan penyingkiran COD sebanyak 88% untuk 50 substrat enapcemar ternyah air manakala ketumpatan kuasa 11.9 W/m², pertumbuhan biojisim 0.7 g/L dan penyingkiran COD terbuang sebanyak 85% (w/v) terbuang. kekonduksian proton. Kajian ini menyerlahkan potensi membran komposit SPSf/SCS sebagai bahan berprestasi tinggi untuk aplikasi MFC, menawarkan penyelesaian yang mampan untuk pemulihan tenaga daripada rawatan sisa organik.

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ABSTRACT

Microbial fuel cell (MFC) is used as a bioelectrochemical system that converts organic waste, such as dewatered sludge and fruit waste, into electricity using engineered sulfonated polymeric membranes to enhance proton transfer and overall performance.The fabrication of advanced heterogenous sulfonated polymeric membranes for MFCs is the most potential topic at present to address the issue of substrate and microbe's crossover, low proton transfer rates and higher internal resistance in the MFCs operation. In the present work, heterogenous sulfonated polysulfone crosslinked with sulfonated chitosan (SPSf/SCS) was utilized as proton exchange membrane in MFCs. The physical, chemical and electrochemical characterization of SPSf membrane with different immersion time in sulfonating agents were investigated as a screening procedure. The most well-performed SPSf was utilized as sulfonated polymer base to crosslink with sulfonated chitosan. Different formulations of SPSf/SCS were designed; namely SPSf/SCS-0.5 wt%, SPSf/SCS-0.75 wt% and SPSf/SCS-1 wt% and comprehensive physical, chemical, and electrochemical characterization was compared with the commercialized membrane Nafion 212. The composite membranes exhibited enhanced mechanical strength and thermal stability due to the robust SPSf backbone, while the incorporation of SCS improved flexibility and hydrophilicity. Chemically, the presence of sulfonic acid groups in both components facilitated high ion exchange capacity (IEC) within the range of 1.01 – 2.01

meq/g and proton conductivity, which are critical for efficient proton transport in MFCs. Electrochemical characterization revealed that the SPSf/SCS-0.75 wt% membranes achieved superior performance about 0.0378 S/cm compared to commercial Nafion membranes, with lower internal resistance and higher ionic conductivity. The synergistic performance of the SPSf/SCS membrane and MFCs utilizing different concentrations of dewatered dewatered sludge and fruit waste as substrate were then assessed using the one-factor-at-a-time (OFAT) approach, followed by response surface methodology (RSM) using a quadratic model through Central Composite Design. SPSf/SCS – 0.75 wt% membrane demonstrated the highest power density for 50% (w/v) of dewatered dewatered sludge and 60% (w/v) of fruit waste. The optimized membrane demonstrated a 35 W/m^2, biomass growth 3.3 g/L and COD removal of 88% for 50% (w/v) dewatered dewatered sludge substrate whereas power density of 11.9 W/m^2, biomass growth of 0.7 g/L and remediation efficiency of 85% for 60% (w/v) fruit waste, attributed to its balanced proton conductivity. This study highlights the potential of SPSf/SCS composite membranes as high-performance materials for MFC applications, offering a sustainable solution for energy recovery from organic waste treatment.

CHAPTER 1

INTRODUCTION

1.1 Research background

An accelerated supply and demand of energy from various sectors has resulted in an increased need for efficient energy storage options, where storing energy emerges as one of the most pivotal parts (Aghmadi & Mohammed, 2024). Today's most alarming environmental issues are pollution and energy crisis, which stand at the crux of organic waste treatment. Despite several types of biological, chemical and physical approaches to treat pollutants in organic waste resources especially organic waste such as dewatered sludge and lignocellulosic biomass, they all pose various drawbacks including high energy requirements as well as high operating cost, the huge consumption of chemicals and the generation of waste as by-products (Perea Moreno et al.,2019). Among those disadvantages, the high amount of energy needed to treat organic waste coincides with the worldwide energy crisis that we are facing now. As a result, much effort has gone into developing cost-effective and low-energy methods of removing pollutants from organic waste (Sevillano et al., 2021). Figure 1.1 demonstrated the energy consumption in 2024.

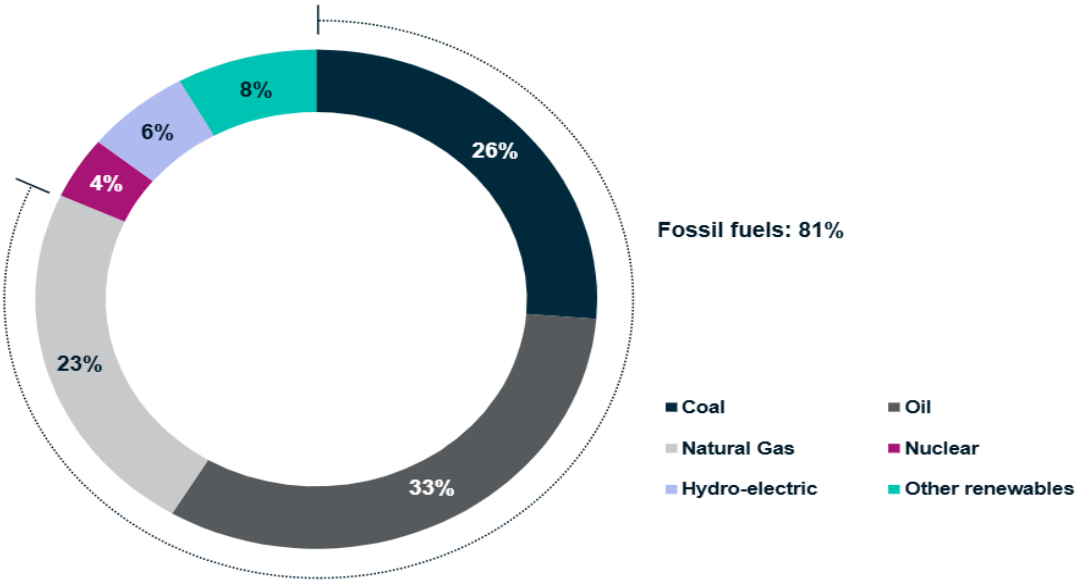

Figure 1.1: Global energy consumption in 2024 (IEA, 2025)

Owing to their sustainable nature, MFCs have been recognized as a viable technology for producing energy and simultaneously remediating pollutants from waste. Its emergence as a promising approach to convert chemical energy into electricity in the presence of biocatalysts and at the same time remediating pollutants from organic waste (Omenesa Idris et al., 2023). The electroactive bacteria species used to degrade the organic waste and then emitted electrons from its body subsequently it passes from anode to cathode compartment for electrical energy generation. Several parameters directly affect the performance of MFCs such as their scale and design, most importantly the proton exchange membranes (PEMs) efficiency and types of organic substrate.

In MFCs, PEMs serve as a crucial separator between the anolyte and catholyte chambers. The use of synthetic PEMs especially commercial options like the perfluorinated polymer Nafion raises concerns regarding the long-term sustainability of

MFC technology. Among these commercial membranes, the choice of material plays a significant role in determining MFC performance, as PEMs are responsible for proton conduction and thus enhance hydrogen ion transfer (Luo et al., 2018a). Researchers have explored a variety of polymeric materials for PEMs, such as polyimide (PI), polybenzimidazole (PBI), polyetheretherketone (PEEK), and polysulfone, which have shown promising results in MFC applications (Cha et al., 2021). As the PEM lies at the heart of MFC functionality, it must act as a durable electrolyte facilitating the transport of positively charged ions while effectively blocking unwanted electrical and chemical interference.

Eco friendly biopolymers and nanoparticles as organic filler in polymeric membrane can improve performance by increasing thermal and mechanical properties as well as generating preferential permeation routes (Hanna Rosli et al., 2020). Certain changes are made to polymeric membranes to improve their properties; these modified membranes are known as the incorporation of organic filler into the organic membranes. These composite membranes are doped or incorporated with a particular filler material at the best concentration using the solvent casting method. Additional methods of modification, including layer-by-layer assembly, surface modification, and cross-linking, have been tried and shown to enhance the membrane's characteristics (Kheirieh et al., 2018).

Heterogenous membrane-based polymers and nanoparticles are sought to be desirable candidates for membranes because of their outstanding physical and thermal durability, high electrochemical stability, and affordable price (Kamal et al., 2020). Furthermore, polymer membrane with sulfonation (SO_3H) is proven to improve the wettability of the membrane, the conductivity of positively charged ions and membrane

stability (Roshanravan et al., 2022a). An effective membrane should also possess certain characteristics such as chemical stability, low gas permeability, good ionic conductivity and no toxicity to microbes with resistance to biofouling. The incorporation of nanoparticles in polymeric membranes for instance chitosan, titanium dioxide, silicon dioxide, zinc oxide, multi walled carbon nanotubes and graphene will upgrade the intensive properties of the overall membrane.

Membrane technologies offer a one-of-a-kind opportunity in bioprocessing especially the proton exchange membrane act as a separator between anolyte and catholyte in MFCs (Kiss et al., 2016). They are particularly appealing for a variety of reasons such as prevent the crossover of substrate (organic waste), improved the efficiency of electrical energy, enhanced bioremediation process as well as improved ionic conductivity of membrane (He et al., 2012). The utilisation of PEM via membrane technology meets the 12th goal of Sustainability Development Goals (SDG's) in which it ensures sustainable consumption and production patterns towards 2030 timeline (Johnston, 2016). This is due to the membrane can reduce the resistivity by enhancing the selectivity of hydrogens ion from anode to cathode compartment in MFCs. According to Malaysian Science, Technology, Innovation and Economic (MySTIE) Framework of Malaysia, the fifth key driver is bioscience and technology which is the most appropriate criteria fulfilled by the membrane separation technology. The major goal of bioscience and technology is to develop technology that uses biological processes, systems, or live beings to create products or to develop technology based on molecular biology, bionics, bioengineering, genetic engineering, and nanotechnology (Nair et al., 2020). This clearly shows that the innovative bio-based membrane technology capable to recover and transports ions and this could be one of the ways to transform Malaysia as eco-friendly nation by 2030.

On the other hand, choosing the right substrate is crucial for optimizing the performance of MFCs. Organic wastes such as domestic waste (DW), dewatered sludge, and lignocellulosic biomass (LCB) are among the most plentiful renewable energy sources available (Gheibi et al., 2021). Globally, approximately 200 billion tons of organic waste are generated annually (Yaser et al., 2022). Much of this biomass is either discarded or incinerated, leading to resource depletion and environmental hazards. Consequently, harnessing nutrient-rich biomass for energy production has become a major focus for researchers. Projections suggest that by 2050, such biomass could contribute up to 38% of global biofuel supply and 17% of electricity generation (Neupane, 2023). Innovative technologies like MFCs are now being developed to not only generate electricity from biomass but also treat pollutants present in organic waste.

MFCs aligned with several Sustainable Development Goals (SDGs) and Environmental, Social, Governance (ESG) criteria by addressing energy sustainability, environmental remediation and social equity. The United Nations SDGs are the blueprint to achieve a better and more sustainable future for all. Renewable energy and organic waste treatment integration MFCs lower greenhouse gas emissions from conventional organic waste treatment plants by offsetting energy demands and reducing chemical usage. Policies incentivizing low-carbon technologies often include MFCs for their dual environmental benefits. Malaysia's National Green Technology Policy (NGTP) which supports MFCs as part of circular economy strategies, emphasizing waste-to-energy conversion and environmental education. MFCs are recognized for their role in treating organic waste while generating electricity, aligning with policies aimed at reducing greenhouse gas emissions and enhancing energy efficiency in organic waste treatment plants.

Using MFC, biomass energy could be directly harvested with electricity, the most convenient, widespread, and clean energy (Hoang et al., 2022). Therefore, MFCs was considered as another promising way to harness the sustainable energies in biomass and add a new dimension to the biomass energy industry. MFCs are directly align with several United Nations Sustainable Development Goals (SDGs) such as clean water and sanitation (SDG 6), affordable and clean energy (SDG 7), industry, innovation, and infrastructure (SDG 9) and responsible consumption and production (SDG 12). In that case, MFC technology exemplifies a multidisciplinary approach to addressing global sustainability challenges by integrating renewable energy generation, waste management, and environmental protection.

Based on the aforesaid introduction, the main aim of this study is to reveal the efficiency of PEM in dual MFCs, composite sulfonated polysulfone and sulfonated chitosan (SPSf/SCS) is utilised as an alternative compared to Nafion ionomer for the development of proton exchange electrolytic membranes with low cost, environmentally friendly and high-performance PEM fuel cells. To date, there is the limited number of studies regarding utilisation of chitin-based biopolymer in a SPSf based MFCs. In addition, MFCs are largely untested, which limits the applicability of this technology for major waste-producing sectors, including agriculture (fruit waste) and domestic waste (dewatered sludge) management. This research also focuses on evaluating the potential for electricity production from solid substrate, cellulosic wastes; dewatered sludge and lignocellulosic biomass in dual MFCs. Last but not least, the biocompatibility of PEM and substrate in MFCs was studied through the electrochemical behaviours of energy generation.

1.2 Problem statement

Microbial Fuel Cells (MFCs) are emerging as a sustainable solution for energy production and organic waste treatment. However, despite their potential, they remain impractical for large-scale commercial or industrial use due to low energy output, poor waste degradation efficiency, and high internal resistance. Malaysia, in particular, faces significant challenges in managing large quantities of organic waste such as dewatered sludge and lignocellulosic fruit waste. Current waste disposal methods, including landfilling and open burning, are unsustainable and environmentally damaging. As highlighted by Razak et al. (2015), there is an urgent need for green technologies like MFCs to tackle these waste management issues while generating renewable energy.

Key obstacles in MFC efficiency include the poor ionic conductivity of proton exchange membranes (PEMs), substrate crossover due to ineffective separators, and suboptimal substrate selection. The PEM is a critical component influencing power generation by selectively allowing proton transfer while restricting other particles. Commercial Nafion membranes are widely used due to their superior electrochemical and mechanical properties. However, their high cost, susceptibility to substrate loss, and biofouling limit long-term performance. Alternatively, sulfonated polymeric membranes offer a cost-effective and thermally stable substitute with good mechanical and film-forming capabilities. Recent innovations involve integrating biopolymers and nanoparticles into these membranes, enhancing thermal and mechanical properties while improving selectivity and ion transport. Techniques such as surface grafting of sulfonated groups have shown potential in improving PEM performance (Tiwari et al., 2016).

In Malaysia, approximately 3 million metric tons of dewatered sludge are generated annually, with forecasts predicting a rise to 7 million tons by 2020 due to urbanization and population growth (Hanum et al., 2019). This sludge is often underutilized, despite its energy-rich content. Likewise, the country generates about 1.2 million tons of agricultural waste annually, including fruit and palm oil residues, much of which is poorly managed. The integration of dewatered sludge and lignocellulosic fruit waste as a mixed feedstock in MFCs could enhance substrate synergy and energy recovery, offering a novel waste-to-energy strategy.

Nevertheless, limited research exists on the combined use of these two substrates in MFCs, particularly under optimized operational conditions. Most prior studies focus on single substrates like glucose or sludge alone. Furthermore, the application of statistical tools such as Response Surface Methodology (RSM) using Central Composite Design (CCD) to optimize MFC performance with mixed substrates remains scarce. Such optimization techniques are crucial for fine-tuning variables like membrane formulation and substrate concentration to maximize power output and remediation efficiency.

Another crucial aspect often overlooked is the influence of internal resistivity and redox potential on MFC performance. High internal resistance impairs energy conversion, while fluctuations in redox potential disrupt microbial stability and substrate degradation. Despite their importance, these electrochemical parameters are rarely studied under optimized MFC conditions. Techniques such as Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) offer valuable insights into charge transfer resistance, ionic behavior, and redox activity. Yet, few studies have systematically correlated these factors with MFC performance, particularly in systems using heterogeneous PEMs and dual-substrate feedstocks.

This study addresses these gaps by exploring the performance of a Dual-Chamber MFC powered by dewatered sludge and lignocellulosic fruit waste, with optimization via CCD-RSM and in-depth electrochemical analysis using EIS and CV. The research aims to uncover the interplay between membrane characteristics, substrate synergy, and electrochemical behavior, ultimately advancing MFC design for improved bioelectricity generation and environmental sustainability.

1.3 Research objectives

1. To synthesize and characterize the heterogenous composite proton exchange membrane with different organic loading.
2. To evaluate the electrochemical properties and ionic flux of heterogeneous PEMs using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry.
3. To optimize via Response Surface Methodology regarding the effect of membrane and substrate on microbial fuel cell performance in terms of, electrogenic bacteria growth, power generation and COD removal.
4. To evaluate the proximate composition, resistivity and redox potential of optimum condition microbial fuel cell system using electrochemical impedance spectroscopy and cyclic voltammetry.

1.4 Scope of study

The first step in this study was to examine the possible immersion time of PSf sulfonation methods and then to characterize the performance of SPSf. Then, the heterogeneous composite PEM using sulfonated polysulfone and sulfonated chitosan

(SPSf/SCS) with different formulations were fabricated. The synthesized membrane was characterized using various physicochemical techniques such as back-end titration testing for ion exchange capacity, nuclear magnetic resonance (NMR), moisture content analysis, contact angle analysis, Fourier transform infrared (FTIR), X- ray diffraction analysis (XRD), scanning electron microscopy analysis (SEM), thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), mechanical testing, antimicrobial testing, electrochemical impedance spectroscopy analysis and cyclic voltammetry.

Secondly, experimental setup of single and dual microbial fuel cells, with and without PEM respectively were built up. This study presents an investigation into the performance of MFCs, focusing on the effectiveness of different substrate utilisation as well as with various concentration at anode compartment together with the performance of proton exchange membrane. A comprehensive approach has been taken by conducting the experimental and electrochemical studies together with the kinetic studies of anode reaction, including microbial and electrode reactions in a batch system. Various experiments were conducted in a batch mode at varying parameters, including the effect of reactor design, different substrates, the effect of initial substrate concentrations as well as PEM formulations.

The microbial and the electrochemical methods such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were applied to get the important profile such as substrates utilization, electric current, power production, internal resistance and voltage losses to present the performance studies of the microbial fuel cell. A statistical tool, response surface methodology (RSM) via CCD was utilised in this project to further study the relationship of three (2) independent

variables (substrate concentration; A) and PEM; B) to the responses; power density (Y1), biomass growth (Y2), and remediation efficiency (Y3).

1.5 Thesis outline

This thesis has five chapters, each describing the sequence of the entire study.

- Chapter 1 (Introduction): This chapter briefly describes the global issues related to organic waste; dewatered sludge and lignocellulosic fruit waste accumulation as well as the potential and capability of organic substrate in MFCs, the constraints in MFCs technology without proper proton exchange membrane, electrochemical and electrical processes within the system. Problem statements that provide the basis and rationale that justified the direction of this research are highlighted. The specific objectives of this research are also listed in this chapter.

- Chapter 2 (Literature Review): A literature review of the microbial fuel cells processes is presented in this chapter. This review focuses on the basic of microbial fuel cell reaction, the operational factors of PEM that may affect the microbial fuel cells performance and the bioelectrochemical theories, electrochemical methods and analyses. The theory for the development of a Response Surface Methodology approach is also described in detail.

- Chapter 3 (Research Methodology): This chapter describes in detail all the experimental works done in this study. This chapter is divided into five sections.

The first section provides information on the types of equipment and chemicals used in this study. In the second section, the fabrication and characterization of the composite proton exchange membrane is followed by organic waste preparation and physiochemical analysis. The subsequent section is the experimental set-up, material, and equipment used for the MFCs process in the batch system. The following section described the optimization of the membrane and concentration of dewatered sludge and lignocellulosic fruit waste in MFCs. The last section describes the procedures for in situ electrochemical studies for the optimized condition of MFCs.

- Chapter 4 (Results and Discussion): This chapter describes the results from the experimental and modelling works that have been conducted. Results of the experiments are further subdivided into five sections. The first section and second section discuss the physical and chemical characterization of fabricated PEM. The second section discusses the physiochemical and proximate analysis of organic waste together with power output, voltage losses and internal resistance in the batch microbial fuel cell by using polarization curve and EIS approaches. These performances are discussed based on experiments conducted in the single and dual chamber of microbial fuel cells. The next section is regarding the effect of different of substrate on the performance MFCs. The third section explains the effect of various initial substrate concentrations and formulations of PEM through optimization of Response Surface Methodology. Additionally, the growth profile, substrate utilization and inhibition studies are also discussed in detail. The fourth section discusses the EIS and CV studies for the optimized condition of PEM and MFC.

- Chapter 5 (Conclusions and Recommendations): This chapter concludes all the major findings obtained in the present study. At the end of this thesis, suggestions and recommendations to improve the present research work, as well as the future direction of the research, are presented

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of microbial fuel cells (MFCs)

Microbial fuel cells (MFCs) is a bioelectrochemical system that utilises electroactive microorganisms as biocatalysts in oxidising organic and inorganic matter to generate electrical energy (Logan et al., 2006). The direct conversion of substrate chemical energy from the chemical bond of organic waste into electrical energy within the system is achieved through the microorganism's catalytic and enzymatic activities of microbes (Logan et al., 2006). An MFCs would generally be comprised of an anode and a cathode which are separated by a membrane or more specifically, a cation exchange membrane (CEM) (Pandey et al., 2011). Microbes or the biocatalysts at the anode compartment would oxidise the substrate (electron donor) present in the MFCs which results in the transfer of the produced-electrons to the anode (negative terminal) by the bacteria as shown in Figure 2.1. Once transferred, the electrons as well as protons would then mobile towards an electron acceptor at the cathode (positive terminal) through the circuit and membrane. Through the transfer of electrons from an electron donor to an electron acceptor, the bacteria gain energy for its metabolism (Pandey et al., 2011).

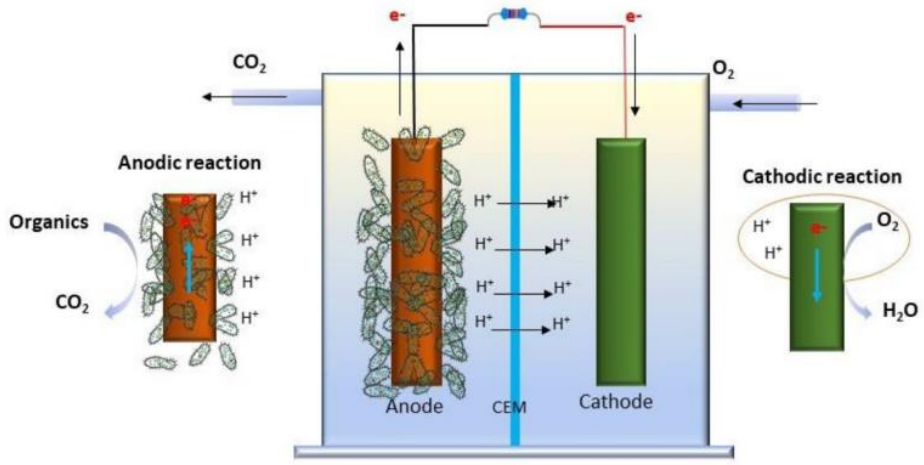

Figure 2.1: Schematic diagram of microbial fuel cells (Pandey et al., 2011)

The power generation segment held the largest share of 49.63% in the MFCs market, in terms of revenue, in 2023, which can be attributed to the growing global demand for renewable and sustainable energy solutions, driven by the urgent need to reduce greenhouse gas emissions and combat climate change (Al Lafi et al., 2025). MFCs, which generate electricity by converting chemical energy from organic matter using microbial processes, offer a clean and renewable source of power. This technology is particularly advantageous in settings where organic waste is abundant, such as in organic waste treatment plants, agricultural operations, and food processing industries. The organic waste treatment segment is expected to grow at a significant compound annual growth rate (CAGR) from 2024 to 2030, fuelled by the growing focus on environmental regulations and sustainability goals is pushing industries and municipalities to adopt cleaner technologies. MFCs, with their ability to convert organic pollutants into energy, align perfectly with these goals, offering a green alternative to conventional treatment processes. Additionally, technological advancements in MFC design and microbial engineering are enhancing their efficiency and scalability, making them more viable for large-scale organic waste treatment applications. Asia Pacific dominated the global MFCs market and accounted for the largest revenue share of

38.68% in 2023, driven by rapid industrialization and urbanization. Countries such as China and India are facing significant environmental challenges due to the increased generation of organic waste and the rising demand for clean energy. MFCs provide a sustainable solution to these issues by offering an integrated approach to energy production and waste management.

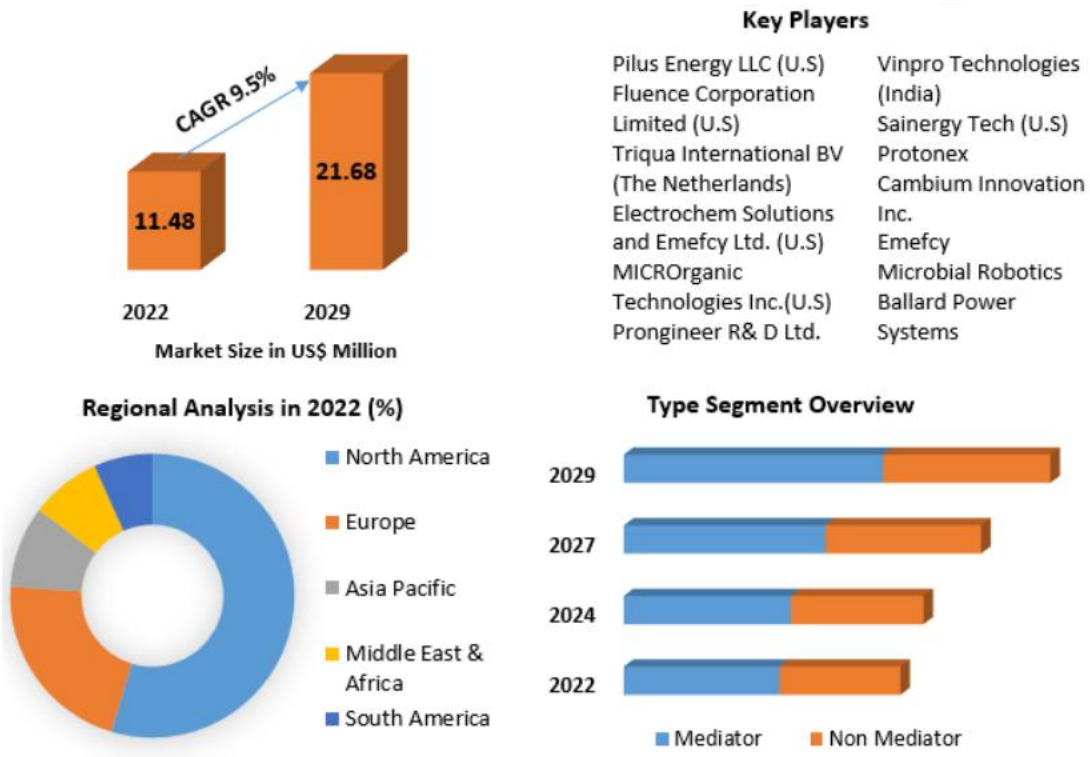

Figure 2.2: Global trend of microbial fuel cells from prominent countries and the key players (Rojas Flores et al.,2023)

In MFCs the electrons can be transferred from the anode to cathode by various approaches namely by direct membrane electron transfer, by electron mediators, or through the addition of chemical mediators (Logan et al., 2006). On the other hand, a system that employs catalysts or more specifically enzymes which were not produced sustainably or in the system's device, is better known as enzymatic biofuel cells as opposed to an MFCs. In the context of MFC system, the utilization of power and voltage

involves the analysis of power curves, which serve as indicators of MFC performance. These curves are constructed by varying the electrical resistance of the external circuit. When the resistance is very high or in an open circuit, the voltage (V) reaches its maximum value, but the current and power (P = VI) are zero. As the resistance is reduced, the voltage decreases while the current increases, resulting in a bell-shaped curve. The MFC achieves its maximum current output when the resistance is very low or when the cell is short-circuited (Zhang et al., 2020).

The maximum power represents the optimal operating condition for energy generation in an MFC. However, when the objective is organic waste treatment, the optimal operating point has yet to be determined. A short-circuited MFC generates the highest currents, indicating a faster rate of organic matter oxidation. If the bell-shaped power/current curve were perfectly symmetrical, shifting from the maximum power to the maximum-current point would double the rate of organic waste treatment (a 100% increase) (Maktabifard, 2018). The potential difference between the anode and cathode, together with the flow of electrons will result in an electrical current. The general half-cell reactions using glucose as a carbon source of substrate are shown below:

An MFCs has the capability to operate on a diverse range of organic compounds as its substrate ranging from proteins, fatty acids as well as carbohydrates. During the MFCs operation, organic substrate such as glucose, acetate sucrose are oxidized by bacteria to generate the electrons as shown in Figure 2.3. The oxidation reaction at anode and reduction reaction at cathode can be written as follow with different organic substrate. Table 2.1 depicted the general standard electrode potential of half reactions for different types of substrates in MFCs.

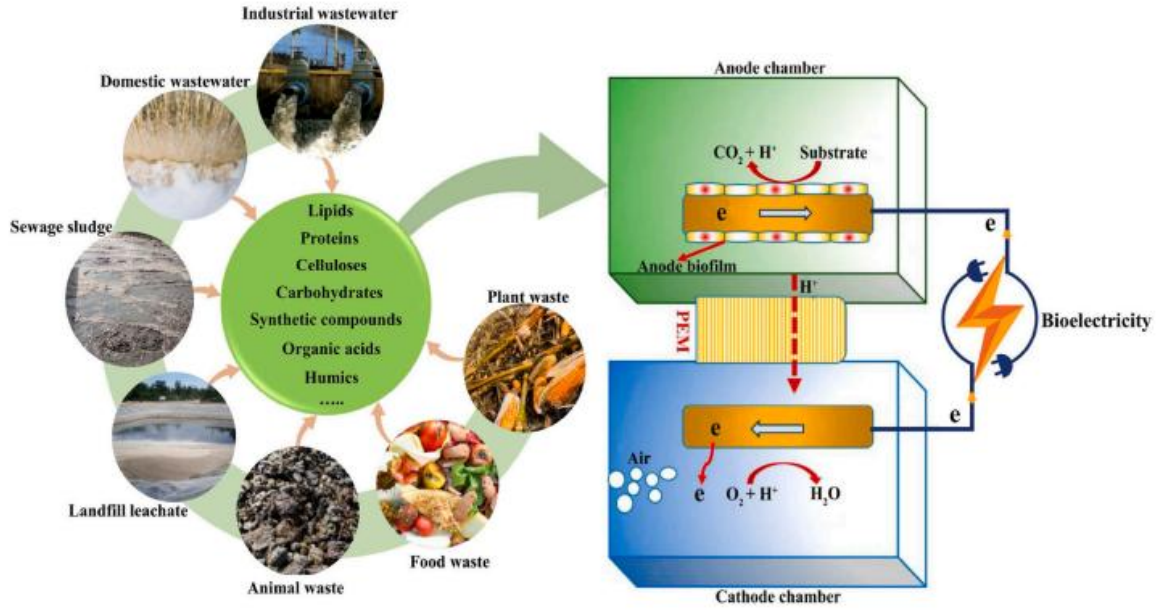

Figure 2.3: Utilisation of potential organic waste for bioelectricity generation (Hoang et al., 2022)

(a) If glucose is used as organic substrate

Oxidation reaction at anode: $C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24H^+ + 24e$

Reduction reaction at cathode: $24H^+ + 24e^- + 6O_2 \longrightarrow 12H_2O$

Overall reaction: $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$ + Electricity + Biomass

(b) If acetate is used as organic substrate

Oxidation reaction at anode: $CH_3COOH + 2H_2O \longrightarrow 2CO_2 + 8H^+ + 8e$

Reduction reaction at cathode: $8H^+ + 8e^- + 2O_2 \longrightarrow 4H_2O$

Overall reaction: $CH_3COOH + 2O_2 \longrightarrow 2CO_2 + 2H_2O$ + Electricity + Biomass

(c) If sucrose is used as organic substrate

Oxidation reaction at anode: $C_{12}H_{22}O_{11} + 13H_2O \longrightarrow 12CO_2 + 48H^+ + 48e$

Reduction reaction at cathode: $4H^+ + 4e^- + O_2 \longrightarrow 2H_2O$

Overall reaction: $C_{12}H_{22}O_{11} + 12O_2 \longrightarrow 12CO_2 + 11H_2O$ + Electricity + Biomass.

Further, the produced electrons move from anode to cathode electrode while different electrochemical measurements are carried out to measure the performance of the electrode. The general standard electrode potential for half reactions in microbial fuel cells depicted in Table 2.1.

Table 2.1: General standard electrode potential of half reactions for different types of substrates in microbial fuel cells

Half reactions	Standard electrode potential (E^o)
$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$	-0.43 V
$24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$	+0.82 V
$C_6H_{12}O_6 + 6O_2 \rightarrow 6O_2 + 6H_2O$ + electricity + biomass	1.25

2.2 Applicability of membrane technology in MFCs

Membrane is a pivotal part in a MFCs since it allows as ion exchange and this can lead to a more effective ions management layer. Among these are Nafion, cation exchange membranes (CEM) that offer a modest degree of proton conductivity. However, they are costly and prone to biofouling, which has prompted research into substitutes such as sulfonated polymers (Jenani et al., 2024). CEM functionality being enhanced with sulfonation allows for enhanced proton transport and stability. Trade-offs in cost, durability, and performance are evident when contrasting perfluorinated with non-fluorinated membranes (Genova-Dimitrova et al., 2001). Membrane can be further optimized using membrane modification techniques as well as material selection to improve the cost-benefit analysis of MFCs for sustainable energy and organic waste treatment.

In the beginning of MFCs technology development, a salt bridge was utilized instead of a membrane separator. The salt bridge is a form of separator used specifically in MFCs (Luo et al., 2018a). It leads the ions through a tube filled with electrolytes. Typically, inert electrolytes are used, such as saturated solutions of potassium chloride and phosphate buffer solution, while agar, an organic polymer, is added to counteract the exchange of fluids. The elaboration of a salt bridge as a separator is cheap, even more so than porous membranes. However, the MFCs performance results obtained with the salt bridge have not been very encouraging. The power output is usually one order of magnitude lower when using devices with a salt bridge compared to using Nafion as a separator. This difference in power is attributed to the high value of internal

resistance (R_{int}) observed for the MFC using a salt bridge as a separator, 20,000 Ω, while the R_{int} observed for the MFC operated with Nafion was significantly lower, 1300 Ω.

Furthermore, in both systems, using Nafion and the salt bridge, the oxygen diffusion phenomenon was observed from the cathode to the anode. Besides, Kargi and Eker et al., (2019) evaluated a double chambered MFCs to generate bioelectricity and organic waste treatment using a low-cost separator: a salt–agar slab. However, copper and gold-covered copper wires were used as anodes and cathodes, respectively. The results showed a low power density, 3 mW/m^2. Salt bridge use is limited to double chambered MFCs only. Also, their use represents high values of Rint and low values of power density. For that reason, and despite their low cost; they have not continued to be utilized as separators. On the other hand, agar–agar membranes are inexpensive and have shown low Rint values and encouraging volumetric power values in single chambered MFCs. The membrane or separator used in these devices represents a key part of proper operation and good performance.

2.2.1 Ion exchange membranes in MFCs

Ion exchange membranes (IEMs) have been established as a key component in industrial water desalination and electrolysis processes. Thus, nowadays, they are being studied and developed for application in new energy conversion and storage systems as well as efficient desalination and organic waste treatment processes. These processes include redox flow batteries, reverse electrodialysis, membrane capacitive deionization, microbial fuel cells, and ion exchange membrane bioreactors. Based on the charge and distribution of fixed ionic groups, IEMs are generally classified into five groups: CEMs,

AEMs, amphoteric IEMs, bipolar membranes, and mosaic IEMs. CEMs and AEMs are also called monopolar IEMs, to be distinguished from bipolar membranes as displayed in Figure 2.4. Since an IEM contains hydrophilic/hydrated ionic groups and relatively more hydrophobic polymer chains, the membrane is essentially heterogeneous at a length scale equivalent to the polymer chain segments. Thus, when the membrane is brought into contact with water, micro-phase separation between the ionic groups and hydrophobic domain is assumed.

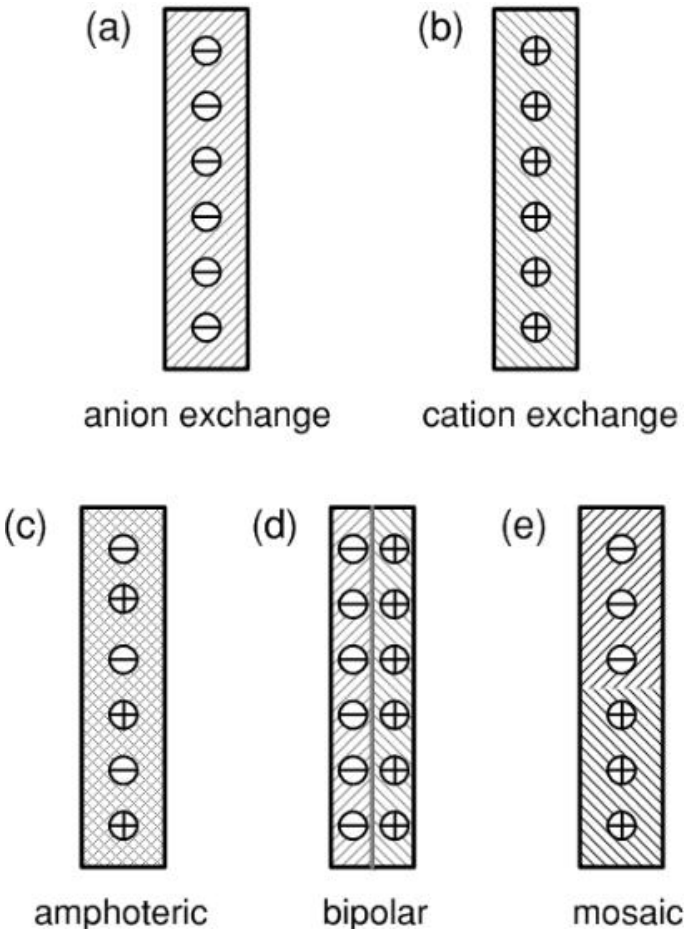

Figure 2.4: Schematic illustration of different ion exchange membrane (IEM) types: (a) anion exchange membranes (AEMs), (b) cation exchange membranes (CEMs), (c) amphoteric IEMs, (d) bipolar IEMs and (e) mosaic IEMs. The circles depict the counter-ions in the membrane matrix, while the background patterns differentiate between the fixed ionic groups ((Luo et al., 2018)

In most MFC configurations, the CEM and AEM is considered an essential component as it performs several important functions: 1) providing physical separation between the anolyte and catholyte so that the two do not mix and cause unwanted cross-reactions; 2) maintaining the overall electrochemical neutrality in the cell by completing the inner circuit; 3) blocking the back-diffusion of oxygen from the cathodic chamber to the anodic chamber; and 4) preventing the loss of microorganisms and substrates (Leong et al., 2013). In the early designs of MFCs, this function was performed by an Agar–KCl salt bridge. However, owing to their larger resistance and consequently reduced power densities, salt bridge designs have been largely abandoned in favor of low-resistance membrane-based MFCs.

Cation exchange membranes (CEM) and anion exchange membranes (AEMs) contain different functional groups that determine their selectivity, charge transport properties, and overall performance in ion exchange applications as shown in Figure 2.5. The functional groups in these membranes play a crucial role in facilitating the selective transport of cations or anions. CEM selectively allow cations (e.g., H^+, Na^+, K^+) to pass through while rejecting anions (M. Ghosh et al., 2018). Their backbone is usually made of polymeric materials like polystyrene-divinylbenzene, sulfonated poly(ether ether ketone) (SPEEK), or perfluorosulfonic acid. Common functional groups, such as sulfonic acid (-SO_3H): Strong acid group with high ion exchange capacity and excellent cation selectivity, carboxylic acid (-COOH): Weaker acid group, offering moderate cation selectivity with tunable properties and phosphonic acid (-PO_3H_2): Less common, but provides good thermal and chemical stability.

AEM selectively allow anions (e.g., Cl^-, OH^-, SO_4^{2-}) to pass through while rejecting cations (Cha et al., 2021). They are typically composed of polymeric

backbones such as polystyrene, poly(arylene ether), or quaternized polybenzimidazole. The common functional groups such as quaternary ammonium ($-NR_4^+$, e.g., $-N^+(CH_3)_3$): The most widely used functional group, offering high anion exchange capacity. Phosphonium ($-PR_4^+$): Provides higher stability in alkaline environments, Imidazolium ($-C_3H_4N_2^+$): Used for enhanced alkaline stability and pyridinium ($-C_5H_5N^+$): Offers specific anion transport properties. Quaternary ammonium and other positively charged groups provide excellent anion exchange capabilities. Table 2.2 demonstrated the properties of functional groups in CEM and AEM.

Table 2.2 Differences between CEM and AEM (Aghmadi & Mohammed, 2024)

Properties	CEM (Cation Exchange Membrane)	AEM (Anion Exchange Membrane)
Functional Groups	Sulfonic acid ($-SO_3H$), Carboxyl ($-COOH$), Phosphonic acid ($-PO_3H_2$)	Quaternary ammonium ($-NR_4^+$), Phosphonium ($-PR_4^+$), Imidazolium ($-C_3H_4N_2^+$)
Charge Type	Negatively charged (-)	Positively charged (+)
Ion Selectivity	Allows cation transport (H^+, Na^+, K^+)	Allows anion transport (Cl^-, OH^-, SO_4^{2-})
pH Stability	Stable in acidic conditions	Stability depends on functional group; quaternary ammonium can degrade in high pH