OPTIMIZATION OF ENERGY GENERATION AND REMOVAL OF POLLUTANTS USING MICROBIAL FUEL CELLS (MFCs) WITH MODIFIED GRAPHENE-STEEL ANODES

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by

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DECLARATION BY AUTHOR

This dissertation is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my master research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. In addition, the present work (around 98 %) was already published in various journals by us (Najwa Najihah Binti Mohamad Daud), main supervisor (Prof. Dr. Mohamad Nasir Bin Mohamad Ibrahim), and co-supervisor (Dr. Amira Suriaty Binti Yaakop and Dr. Asim Ali Yaqoob). The list of publications was given at the end of the thesis.

Best regards,

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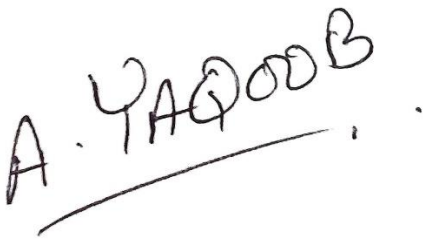

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LIST OF SYMBOLS

%	Percentage
∞	Infinity
Ω	Ohms
°C	Degree of Celcius
˚C/min	Degree of Celcius per minute
Å	Angstrom
A/cm^2	Ampere per square centimetre
A/m^2	Ampere per square metre
A/m^3	Ampere per cubic metre
cm^{-1}	Reciprocal centimetre
cm^2	Centimetre square
cm^3/g	Centimetre cubic per gram
eV	Electronvolt
F/g	Farad per gram
g	Gram
g/L	Gram per litre
h	Hour
kHz	Kilohertz
kV	Kilovolt
L	Litre
mA	Milliampere
mA/cm^2	Milliampere per square centimetre
mA/m^2	Milliampere per square metre
m^2/g	Metre square per gram

mg	Milligrams
mg/g	Milligrams per gram
mg/L	Milligrams per liter
mg/mL	Milligrams per milliliter
min	Minute
mL	Milliliter
mm	Millimetre
mM	Millimolar
MPa	Megapascal
mV	Millivolt
mV/s	Millivolt per second
mW	Milliwatts
mW/cm^2	Milliwatts per square centimetre
mW/m^2	Milliwatt per square metre
mW/m^3	Milliwatt per cubic metre
nm	Nanometre
ppm	Parts per million
psi	Pound per square inch
USD	United States Dollar
USD/m^2	United States Dollar per square metre
USD/ton	United States Dollar per ton
V	Volt
W/m^2	Watts per square metre
W/m^3	Watts per cubic metre
w/w	Weight by weight
$\mu A/cm^2$	Microampere per square centimetre
µL	Microlitre

µm	Micrometre
µS/cm	Microsiemens per centimetre
$\mu W/cm^2$	Microwatt per square centimetre
$\mu W/m^3$	Microwatt per cubic metre

LIST OF ABBREVIATIONS

3D	Three dimensional
A	Cross-sectional area
AAS	Atomic Absorption Spectrometry
AFM	Atomic Force Microscopy
APA	Acetaminophen
ATP	Adenosine trisphosphate
β_a	Anodic beta coefficient
β_c	Cathodic beta coefficient
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
BLAST	Basic Local Alignment Search Tool
BMFCs	Benthic Microbial Fuel Cells
BOD	Biochemical oxygen demand
BPA	Bisphenol A
CB	Carbon brush
CC	Carbon cloth
CCB	Centre of Chemical Biology
CCV	Closed circuit voltage
CD	Current density
CE	Coulombic efficiency
CF	Carbon felt
CFB	Carbon fibre brush
CM	Carbon mesh
COD	Chemical oxygen demand
C_p	Specific capacitance
CP	Carbon paper
CPE	Constant phase element
CPs	Capital costs
CR	Carbon rod
CS	Carbon steel
CV	Cyclic voltammetry

CWACs	Coffee waste-derived activated carbon
DCM	Dichloromethane
DET	Direct electron transfer
DI	Distilled water
DMFCs	Double-chamber Microbial Fuel Cells
DMRB	Dissimilatory metal-reducing bacteria
DNB	Denitrifying bacteria
E	Electromotive force
ECM	Equivalent circuit modeling
EDX	Energy-Dispersive X-ray
EIS	Electrochemical Impedance Spectroscopy
EPS	Extracellular Polymeric Substances
FFV	Free fraction volume
FS	Forward scan
FTIR	Fourier Transform Infrared
GAC	Granular activated carbon
GB	Graphite brush
GE	Graphite electrode
GO	Graphene oxide
GF	Graphite felt
GO-GE	Graphene-graphite
GO-ME	Graphene-mild steel
GO-SE	Graphene-stainless steel
GP	Graphite plate
GR	Graphite rod
GS	Graphite sheet
HFR	High-frequency resistance
i_{corr}	Corrosion current
IET	Interspecies Electron Transfer
IOB	Iron-oxidizing bacteria
IUPAC	International Union of Pure and Applied Chemistry
LED	Light emitting diode
ME	Mild steel electrode
MET	Mediator electron transfer

MEGA	Molecular Evolutionary Genetics Analysis
MFCs	Microbial fuel cells
MIC	Microbially-induced corrosion
MO	Methyl orange
MS	Mild steel
NPF	Nipah palm frond
NPF-GO	Nipah palm frond – graphene oxide
NPV	Net present value
OCP	Open circuit potential
OCV	Open circuit voltage
OMCs	Operating and maintenance costs
OPEFB	Oil palm empty fruit bunch
OTC	Oxytetracycline
PCP	Pentachlorophenol
PCR	Polymerase chain reaction
PD	Power density
PDP	Potentiodynamic polarization
PEM	Proton exchange membrane
PKS-GO	Palm kernel shell-derived graphene oxide
PKS-RGO	Palm kernel shell-derived reduced graphene oxide
PLA	Polylactic acid
PP	Polypropylene
PSF	Polysulfones
Pt	Platinum
PVA	Polyvinyl alcohol
r	Internal resistance
RE	Removal efficiency
$R_{f\text{-}anode}$	Anode resistance
$R_{f\text{-}cathode}$	Cathode resistance
rGO	Reduced graphene oxide
RS	Reverse scan
R_p	Polarization resistance
SA	Syringic acid
SCE	Saturated calomel electrode

SE	Stainless steel electrode
SEM	Scanning Electron Microscope
SMFCs	Single-chamber Microbial Fuel Cells
SRB	Sulfate-reducing bacteria
SS	Stainless steel
SSM	Stainless steel mesh
TAE	Tris-acetate-EDTA
TEM	Transmission Electron Microscope
TGA	Thermal Gravimetric Analysis
TRL	Technology Readiness Level
USM	Universiti Sains Malaysia
UV-DRS	UV-Vis Diffuse Reflectance Spectroscopy
WCA	Water contact angle
XRD	X-ray diffraction

PENGOPTIMUMAN PENJANAAN TENAGA DAN PENYINGKIRAN BAHAN CEMAR MENGGUNAKAN SEL BAHAN BAKAR MIKROB (MFCs) DENGAN ANOD KELULI-GRAFIN TERUBAHSUAI

ABSTRAK

Sel bahan api mikrob (MFCs) menawarkan penjanaan tenaga dan penyelesaian penyingkiran bahan pencemar yang menjanjikan. Namun, ketidakstabilan elektrod dan bahan substrat organik menghalang pengangkutan dan penjanaan elektron yang cekap. Penyelidikan ini menyiasat dan membandingkan prestasi anod biasa komersial—grafit (GE), keluli lembut (ME), dan keluli tahan karat (SE)—dengan salutan terbitan grafin yang disintesis daripada pelepah sawit Nipah (NPF)—grafin-grafit (GO-GE), grafin-keluli lembut (GO-ME), dan grafin-keluli tahan karat (GO-SE). Prestasi penjanaan elektrik dan penyingkiran bahan pencemar MFCs dipantau dalam tempoh operasi yang berbeza: 65 hari untuk anod biasa dan 105 hari untuk anod bersalut. Keputusan menunjukkan bahawa GO-ME secara konsisten mengatasi prestasi anod lain, menjana 29.10 mW/m^2 berbanding GO-SE (26.50 mW/m^2), GO-GE (7.60 mW/m^2), SE (5.85 mW/m^2), ME (2.75 mW $/m^2$), dan GE (1.07 mW/m^2). MFCs menunjukkan kecekapan penyingkiran bahan pencemar bisfenol A, (BPA) yang tinggi untuk GO-ME pada 98.03%, diikuti oleh GO-GE pada 96.95%, GO-SE pada 83.73%, SE pada 96.25%, ME pada 94.55%, dan GE pada 81.08 %, dengan semua tindak balas melebihi 96% dalam kecekapan penyingkiran plumbum (II) nitrat ($Pb(NO_3)_2$). Nira sap berfungsi sebagai substrat organik dalam pembiakan *Bacillus* sp. untuk semua anod. Penyelidikan diakhiri dengan membandingkan keputusan terdahulu dan mencadangkan cadangan lanjut.

OPTIMIZATION OF ENERGY GENERATION AND REMOVAL OF POLLUTANTS USING MICROBIAL FUEL CELLS (MFCs) WITH MODIFIED GRAPHENE-STEEL ANODES

ABSTRACT

Microbial fuel cells (MFCs) offer promising energy generation and pollutant removal solutions. However, instabilities in electrode and organic substrate materials hinder efficient electron transport and generation. This research investigates and compares the performance of commercial plain anodes—graphite (GE), mild steel (ME), and stainless steel (SE)—with graphene derivative coatings synthesized from Nipah palm frond (NPF)—graphene-graphite (GO-GE), graphene-mild steel (GO-ME), and graphene-stainless steel (GO-SE). The electricity generation and pollutant removal performance of the MFCs were monitored over distinct operational periods: 65 days for plain anodes and 105 days for coated anodes. Results show that GO-ME consistently outperforms other anodes, generating 29.10 mW/m^2 compared to GO-SE (26.50 mW/m^2), GO-GE (7.60 mW/m^2), SE (5.85 mW/m^2), ME (2.75 mW/m^2), and GE (1.07 mW/m^2). MFCs demonstrated high bisphenol A (BPA) pollutant removal efficiencies for GO-ME at 98.03%, followed by GO-GE at 96.95%, GO-SE at 83.73%, SE at 96.25%, ME at 94.55%, and GE at 81.08%, with all reactions surpassing over 96% in lead (II) nitrate ($Pb(NO_3)_2$) removal efficiency. Nira sap serves as a beneficial organic substrate, fostering *Bacillus* sp. dominance across all anodes. The research concludes by comparing prior results and suggesting further recommendations.

CHAPTER 1

INTRODUCTION

1.1 Background study

The imminent energy crisis and the critical water pollution concern are salient contemporary environmental challenges. Addressing these challenges has led to exploring various biological, chemical, and physical methods. However, these approaches exhibit inherent limitations, such as elevated energy requirements, substantial operational costs, extensive reliance on chemicals, and the generation of waste by-products (Aleid et al., 2023). Consequently, the substantial energy needs for wastewater treatment converge with the prevailing global energy crisis, compelling the scientific community and industry to pursue a unified and integrated strategy. In response to these challenges, microbial fuel cells (MFCs) emerge as a promising and sustainable solution for wastewater treatment. Generally, MFCs comprise two compartments: an anode and a cathode, separated by a proton exchange membrane (PEM). Within the anode compartment, the oxidation of organic matter that serves as an electron donor, as exemplified by glucose, occurs in the absence of oxygen. This process leads to the liberation of both electrons and protons. The electrons are subsequently transferred to the surface of the anode, whereby these electrons traverse an external circuit route to the cathode compartment. Simultaneously, the protons directly migrate through the PEM to the cathode compartment. MFCs harness the metabolic activities of microorganisms to generate electrical energy while concurrently addressing water pollution. The distinctive capability of MFCs to convert organic matter into electrical power offers an innovative approach that mitigates the drawbacks associated with conventional methods. In the energy domain, MFCs are a remarkable technology, powering small-scale devices like pollutant monitoring

biosensors, life-saving equipment such as pacemakers, and remote applications like wireless temperature sensors, LED lighting, digital wristwatches, and mobile phone chargers (Roy et al., 2022; Srivastava et al., 2020). Furthermore, MFCs offer the supplementary benefit of wastewater treatment by effectively removing toxic pollutants from water. Significant advantages of MFCs include the generation of stable sludge compared to aerobic treatment processes, minimal CO_2 emissions compared to biological treatment methods, and higher conversion efficiency than enzymatic fuel cells (Yaqoob et al., 2021d). Hence, MFCs emerge as a promising and sustainable solution, providing dual benefits for sustainable energy production and environmental remediation.

Despite the well-documented advantages of MFCs, two significant challenges impede their power performance. Firstly, the utilization of low-quality anode materials hinders efficient electron transport from microbes to the anode surface. Secondly, the limited availability of carbon sources constrains electron production. These issues pose persistent challenges to scaling up MFCs for commercial applications. Various materials were used in the literature, including carbon or graphite, one of the most commonly used anode materials for MFCs, owing to its high biocompatibility and conductivity, good microbial adhesion capability, and chemical stability (Agrahari et al., 2022). Sonawane et al. (2017) investigated using different carbon materials as anodes in MFCs. These carbon materials encompass a range of forms, including particulate porous carbon or bulk, powdery carbon, and fibrous carbon materials. In contrast to metal materials, carbon materials exhibit suboptimal mechanical properties and relatively low specific conductivity, presenting challenges for large-scale and commercial applications. Metals like stainless steel (SS) and mild steel (MS) exhibit superior current collector capabilities and better electrical conductivity, presenting a

substantial difference of three orders of magnitude (Shahid et al., 2021). Research indicates that forming an oxide layer on SS possesses electrochemical and semiconductor characteristics, contributing to the material's complex behavior (Pu et al., 2018). Saadi et al. (2020) confirmed these findings by demonstrating the competitiveness of SS as an anode under controlled electrochemical conditions with a power density (PD) of 3.7 mW/m^2. However, Banerjee et al. (2022) argue that metals have yet to undergo extensive study in MFCs due to their corrosive nature, poor biocompatibility, and high overpotential. As per the existing literature, our investigation reveals a notable gap in examining and exploring MS for MFCs compared to SS. This disparity may be attributed to MS's relatively inferior corrosion resistance. Hence, coating metallic surfaces is strongly recommended to enhance metal-anode performance, as it facilitates the development of desirable characteristics for anode electrodes.

A prior literature review suggested the potential of utilizing graphene derivatives for anode development (Starowicz et al., 2023). Graphene oxide (GO) materials derived from waste exhibit significant oxygenation, leading to a pronounced alteration of Van der Waals interactions and diverse water solubilities. The electroactive properties are predominantly concentrated in the edge plane nano bands located on the heterogeneous surfaces of GO, whereas the basal plane islands maintain electrochemical inertness. The high surface area of GO facilitates the attachment of microbes to the electrode surface, enabling inner electrode reduction at the anode. The exceptional conductivity, surface area, biocompatibility, and mechanical stability of graphene derivatives have been unveiled (Aiswaria et al., 2022; Starowicz et al., 2023). Consequently, they can be considered outstanding performers in contrast to alternative materials. However, due to the complex and energy-intensive production processes,

the issue arises with the high cost of commercially available graphene derivatives. Therefore, using waste materials as a starting point for GO synthesis is one possible approach to reducing the cost of graphene.

Earlier studies have suggested that GO produced from forest waste has the potential to induce microorganisms to release signalling molecules, which, in turn, may expedite microbial growth and function as mediator molecules, enhancing electron transfer efficiency (Cai et al., 2020). For example, Yaqoob et al. (2021c) used lignin obtained from the waste of oil palm empty fruit bunches as a primary material for the manufacture of GO. The results indicate that modifying the anode using waste-derived GO has proven effective in promoting the healthy growth of bacteria on the anode surface, thereby facilitating the flow of electrons. The present study employed Nipah palm frond (NPF) from Nipah palm (*Nypa fruticans Wurmb.)* as a primary material for GO production. Nipah palm is underutilized since there is less scientific literature on it than coconut and palm oil. Due to its fast development, the Nipah palm is seen as a threat to the mangrove forest; hence, there is an increasing need to remove it (Aini et al., 2020). Based on the findings of Akpakpan et al. (2011), it has been determined that NPF had a cellulose content of 42.22% and a lignin content of 19.85%. In another investigation, they provided insight into the Nipah palm's chemical composition and corrosion-inhibiting capabilities (Orubite-Okorosaye et al., 2007; Orubite-Okorosaye et al., 2004). Furthermore, the Nipah palm is known for its sugary liquid sap, commonly referred to as 'nira.' The composition of nira sap consisted of glucose (84%), fructose (36%), and sucrose, as well as several minor constituents, including carbohydrates (12%), protein (56%), fat (6%), calcium (56%), phosphorus (6%), and moisture (74%) (Aini et al., 2020). The choice of substrate used as an anolyte is a crucial determinant that impacts both the comprehensive composition of the

bacterial community and the energy production derived from MFCs. According to Lempang (2013), nira sap has the highest percentage of sugar (13.28%) compared to fresh sap from aren (10.52%), siwalan (10.96%), and coconut (10.37%). Therefore, opting for locally accessible nira sap as an organic substrate is an ideal solution to address the electron generation problem effectively.

This comprehensive study also considered the wastewater treatment application, which is a secondary application of MFCs. The present study aims to remove bisphenol A (BPA) and lead (II) nitrate (Pb^{2+}), focusing on examining two specific types of toxic pollutants commonly found in wastewater: BPA, introduced in the anode chamber, and Pb^{2+}, introduced in the cathode chamber. BPA and Pb^{2+} are prevalent in industrial, medical, and household wastewater and are known for their hazardous nature and non-biodegradability, which are linked to various health concerns (Razak et al., 2021; Shehab et al., 2020). However, removing Pb^{2+} from a cathode chamber using MFCs presents challenges due to its low redox potential. Hence, we explored the feasibility of utilizing biocathodes, where microbial communities in the cathode chamber assist in metal removal and enhance electricity generation within MFCs. Moreover, incorporating BPA into the anode chamber in MFCs offers additional benefits, as it acts as an organic substrate that promotes the respiration of electrogenic bacteria, thereby increasing their metabolic activity (T.-J. Zhu et al., 2023).

This study proposes using NPF waste to produce GO and nira sap as organic substrates for MFCs microbial species. Initially, MFCs were constructed utilizing commercially available plain graphite rod (GR), MS, and SS, identified as GE, ME, and SE. The outcomes obtained from these setups were then compared with anodes

coated with graphene derived from NPF waste (NPF-GO), identified as GO-GE, GO-ME, and GO-SE. Lastly, this work uniquely combines inorganic and organic dual cathodic-anodic pollutant treatment and generates energy without any external support. The results obtained have undergone comprehensive validation through a series of electrochemical tests and biological characterizations. The report finishes by examining MFCs' operating mechanisms, comparing prior results, and suggesting further recommendations. Figure 1.1 illustrates the overview of the present study, highlighting the benefits of MFCs technology.

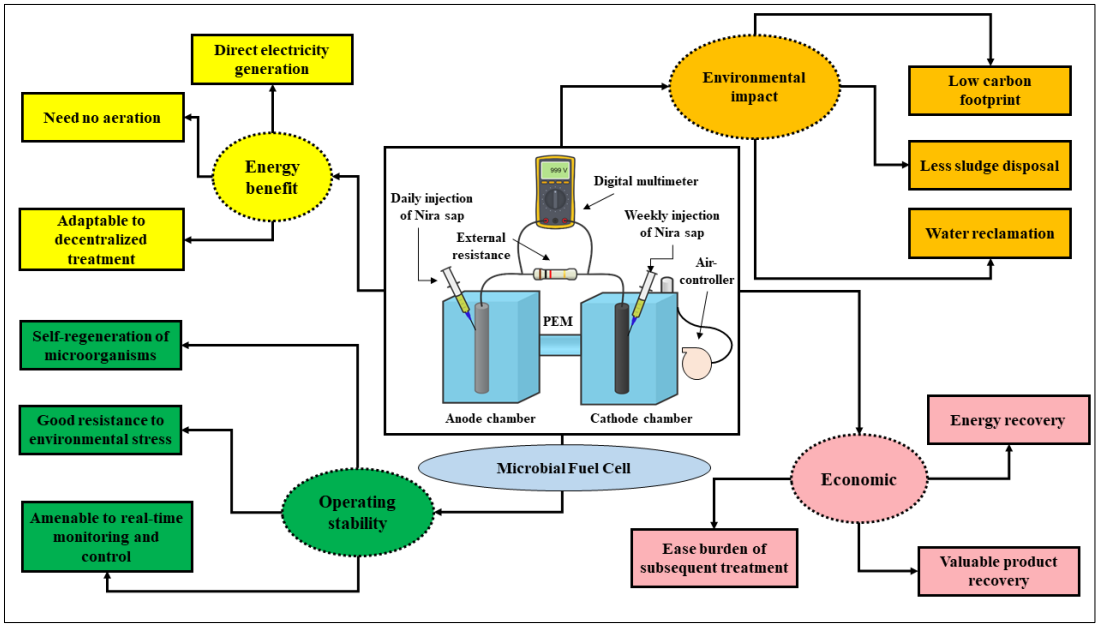

Figure 1.1: Overview of the present study, along with its benefits.

1.2 Problem statement

Industrial wastewater from various industries is a major contributor to environmental pollution, containing both organic pollutants (e.g., phenols, azo dyes, and pesticides) and inorganic pollutants (e.g., heavy metals like cadmium and lead). These pollutants, particularly recalcitrant organic and non-biodegradable inorganic types, pose serious threats to environmental safety and human health, necessitating

effective treatment before final disposal into the environment. In the quest for sustainable solutions, MFCs have emerged as a promising approach for treating such wastewater. MFCs utilize microorganisms to degrade pollutants while generating electricity, offering benefits such as environmental friendliness, cost-effectiveness, and minimal sludge production (Khan et al., 2024; Pan & Bhattacharyya, 2023). However, MFCs have yet to receive the recognition they merit in wastewater treatment or the renewable energy sector due to constraints like low power generation and limited efficiencies, which have led to relatively lower investments in MFCs technology (Roy et al., 2022).

The primary challenge in MFCs is poor electron transport from bacteria to the electrode due to insufficient bacterial attachment to the anode, which hampers electricity production. Over the past decade, efforts have focused on improving electron transport to enhance energy generation and durability in MFCs performance (Suresh et al., 2022; Yaqoob et al., 2021a). In pursuit of this goal, the choice of anode materials with desirable qualities such as high porosity, conductivity, stability, and cost-effectiveness is crucial. While previous research predominantly focused on non-metal or carbon materials, these materials displayed limitations such as high resistivity, low mechanical strength, and challenges in large-scale applications. In comparison, metal electrodes have been considered for use as anodes due to their robust mechanical strength and conductivity, making them suitable for large-scale implementation (Prathiba et al., 2022). Some studies have found that surface-modified SS outperforms other metallic anodes. For instance, a comparison conducted by Han et al. (2018) between oxidized SS and nickel revealed that SS can achieve a maximum current density (CD) of 6.74 A/m^2 compared to nickel's 3.84 A/m^2. Despite its advantages, the high cost of producing SS, attributed to the addition of chromium for corrosion

resistance, has prompted interest in MS as a cheaper alternative. However, corrosion and low biocompatibility in metal anodes limit their effectiveness. In order to address these challenges, graphene derivatives have been explored as coatings to enhance biocompatibility, prevent corrosion, and improve electron transport on metal anodes. Waste-derived GO has shown promise in increasing bacteria-anode interactions and biofilm formation, leading to better performance in MFCs (Yaqoob et al., 2021d). The potential to reduce graphene costs by utilizing waste materials for GO preparation adds to its attractiveness for commercial-scale applications.

Additionally, the research explores the utilization of locally available organic materials as substrates to address the challenge of electron generation. According to Prathiba et al. (2022), substrates have emerged as a pivotal biological determinant within MFCs, exerting a profound influence on the composition of bacterial populations within the anode biofilm. The stability and composition of the substrate directly impact pollutant removal and electricity generation. Sarma et al. (2022) asserted that a broad range of substrates, from simple carbon structures to complex organic matter found in wastewater, could be effectively utilized in MFCs. A complex substrate helps determine an electrochemically active, diverse microbial community, while a simple substrate is easier to degrade and enhances electrical output. The use of nira sap as an organic medium in the present study exemplifies the diverse range of substrates that support bacterial growth, demonstrating its versatility in MFC applications.

Thus, MFCs offer a sustainable approach to wastewater treatment by integrating innovative strategies, such as the application of waste-derived graphene

derivatives on anode electrodes and the utilization of locally available organic substrates, addressing key limitations in electron transport and generation.

1.3 Research objectives

The study aimed to achieve the following objectives:

1. To assess the performance of the commercially available plain anodes (GE, ME, and SE), and the prepared graphene-modified anodes (GO-GE, GO-ME, and GO-SE), through electrochemical and biological tests.
2. To investigate the efficacy of nira sap as an organic substrate for enhancing the operational efficiency of MFCs.
3. To evaluate the removal efficiency of BPA and Pb^{2+} by utilizing the prepared graphene-modified anodes and comparing them with commercial plain anodes in MFCs.

1.4 Scope of study

The scope of this research entails a comprehensive examination of MFCs, focusing on evaluating the performance of commercially available plain anodes and graphene-modified anodes. The biomass material targeted for this study is Nipah palm frond (NPF), which was chosen as the primary source for producing GO. The NPF underwent a simple carbonization process, resulting in NPF carbonized carbon. The NPF carbonized carbon was not characterized since it served as the primary source before conversion into GO. Subsequently, the prepared GO was utilized to coat SS, MS, and GR electrodes for MFCs applications. Commercial plain graphites (GRs) were employed as cathodes in all MFCs reactions throughout the project. The MFCs setup consisted of double chambers separated by PEM.

The prepared GO underwent comprehensive characterization using techniques such as Fourier Transform Infrared (FTIR), UV-Vis Diffuse Reflectance Spectroscopy (UV-DRS), X-ray diffraction (XRD), Scanning Electron Microscope (SEM), Energy-Dispersive X-ray (EDX), Transmission Electron Microscope (TEM), Thermal Gravimetric Analysis (TGA), Raman spectroscopy, Atomic Force Microscopy (AFM) and Brunauer-Emmett-Teller (BET). The prepared anode performances concerning electron transportation were analyzed. The study aimed to investigate the impact of using nira sap as an organic substrate in terms of electron generation, enhancing the rate of oxidation and reduction reactions.

The performance was evaluated through various electrochemical measurements such as open circuit voltage (OCV), closed circuit voltage (CCV), polarization behavior, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and potentiodynamic polarization (PDP) tests. Biological characterizations such as SEM, EDX, and bacteria identification tests were conducted. However, an in-depth study of bacterial isolation and identification was not the primary objective. Hence, a simplified process was employed to identify the bacterial species present in the reactions. Furthermore, the research aimed to evaluate the removal efficiency of both organic and inorganic pollutants, specifically BPA and Pb^{2+}. The target pollutants were incorporated with collected wastewater to use as an inoculation source in the double-chamber MFCs (DMFCs). The removal efficiency was assessed using UV-Vis spectrophotometry for BPA and atomic absorption spectrometry (AAS) for Pb^{2+}. This study did not include a detailed investigation into multiple parameter optimization, such as temperature, pH, and conductivity.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

MFCs represent a bio-electrochemical device that converts the chemical energy found in organic substrates into electrical energy through microbial activity. Structurally, MFCs consist of two chambers, the anode and cathode, which are separated by PEM. In the anode chamber, electrochemically active microorganisms form biofilms and utilize organic substrates as a carbon source for their metabolic processes, producing electrons and protons. These electrons are then transferred to the anode and subsequently transported to the cathode through an external circuit. Meanwhile, protons migrate through the PEM to the cathode chamber, where they combine with oxygen to form water. Through the utilization of organic materials like wastewater, MFCs exemplify eco-friendly practices, providing a two-fold benefit of bioelectricity generation and waste management. Recent advancements have further broadened the scope of MFCs technology, extending its application to include the degradation of organic pollutants in the anode chamber, thereby enhancing its environmental remediation capabilities (Idris et al., 2022). With advantages such as reduced activated sludge production, minimal aeration energy requirements, straightforward operation, and environmental compatibility, MFCs emerge as a compelling solution for addressing power generation and waste treatment challenges. Despite these advantages, the low efficiency of MFCs remains a significant challenge, as highlighted in several studies (Cai et al., 2020; Pareek et al., 2019b; Siddiqui et al., 2023).

2.2 Electrode study

Given these challenges, a comprehensive understanding of electrode properties and their impact on MFCs performance is essential. Electrodes serve as the foundation of MFCs, which are crucial for facilitating essential processes like electron transfer and substrate oxidation. In electron transfer, electrodes act as conduits for exchanging electrons between microbial biofilms and the MFC's external circuit, enabling the conversion of chemical energy into electrical energy. Additionally, electrodes play a pivotal role in substrate oxidation, particularly in the anode compartment, where electrochemically active microorganisms catalyze the oxidation of organic compounds, generating electrons and protons. While optimizing the anode is vital for enhancing MFCs performance, challenges persist in the cathode, notably the oxygen reduction reaction. Efforts to address this challenge have led to the exploration of biocathodes, offering advantages such as cost reduction and by-product utilization (Anjum et al., 2021). Nevertheless, it is noteworthy that optimizing the anode holds greater significance for enhancing MFCs performance compared to the cathode. Studies demonstrate that anode electrodes incorporating modified graphene exhibit higher energy generation than cathode electrodes, as depicted in Figure 2.1 (Aiswaria et al., 2022). Hence, a primary objective in MFCs research revolves around designing and synthesizing low-cost advanced anode electrode materials with increased efficiency and improved durability.

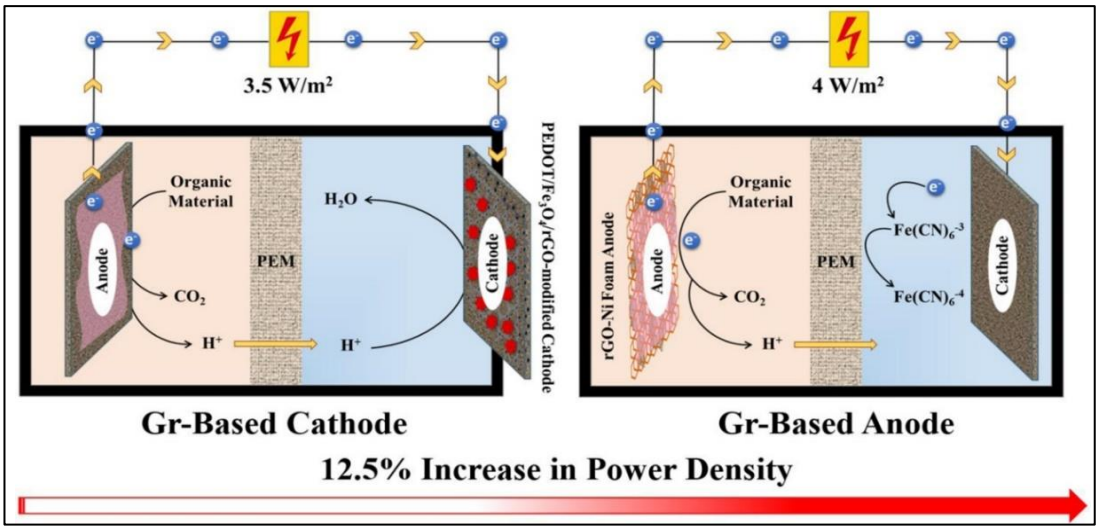

Figure 2.1: Anode electrodes featuring modified graphene outperform cathode electrodes (Adapted from Yaqoob et al., 2020b with Elsevier permission).

2.3 Essential attributes of anode electrode materials

The material preference for anode electrodes in MFCs continues to be a complicated subject for researchers to achieve the desired electrochemical performance, electron transfer, and bacterial adherence. Figure 2.2 lists and illustrates several of the most crucial features that an optimal anode electrode must have.

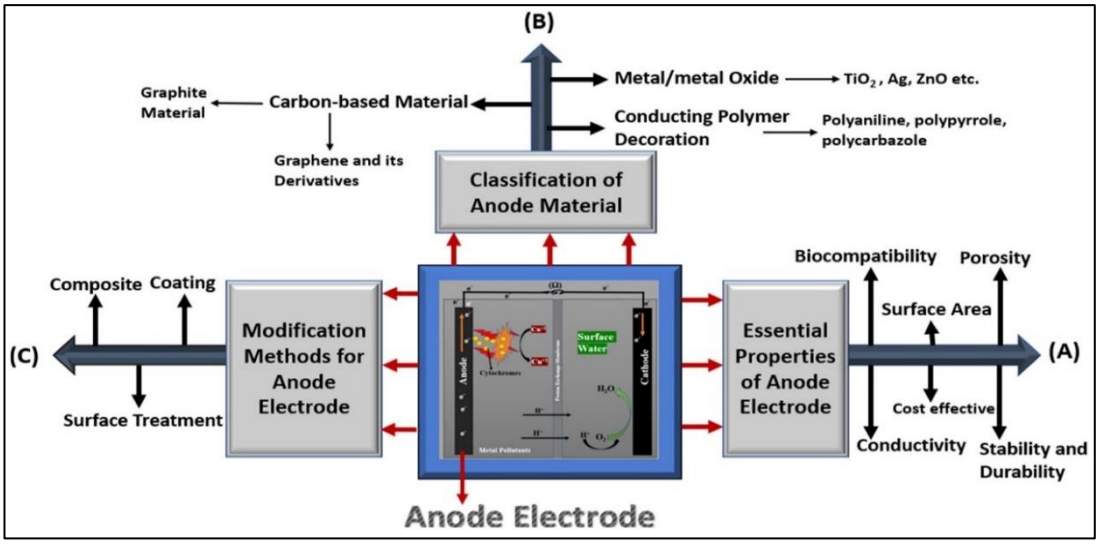

Figure 2.2: Diagram of an anode; (A) Required characteristics of an anode; (B) Classification of anode material; (C) Modification procedures of an anode (Adapted from Yaqoob et al., 2020a with Elsevier permission).

2.3.1 Conductivity

The fundamental attribute of electrode materials is their conductivity. Conductivity plays a pivotal role as it facilitates the transmission of electrons generated by bacteria, allowing them to pass from the anode to the cathode through an external circuit. Thus, the anode material is responsible for enabling the flow of electrons and increasing their speed. Highly conductive materials help to reduce the bulk solution resistance and increase the transfer of electrons (Din et al., 2020). In addition, the contact resistance between the substrates and electrodes needs to be minimal to maximize the electron transfer (Din et al., 2020). Before fabricating the anode electrode, the conductivity of materials is often investigated.

2.3.2 Area and porosity of electrode surface

The area of the electrode surface has a significant effect on the energy output of MFCs. Anode resistance, directly linked to fuel cell ohmic losses, can be effectively mitigated by increasing the surface area. Furthermore, a high surface area provides more bacterial growth sites, thereby optimizing the kinetics of fuel cell electrode efficiency. Various bacterial species, including *Geobacter* sp., *Pseudomonas* sp., and *Escherichia coli,* were immobilized successfully on the electrode surfaces, resulting in successful electron transmission. Anode electrode surfaces are subjected to biological processes; hence, the surface area significantly impacts the MFCs' performance (Banerjee et al., 2022). Notably, graphene and its derivatives exhibit a more extensive surface area than conventional carbon materials (Yaqoob et al., 2021a; S. Zhang et al., 2020).

2.3.3 Biocompatibility

The biocompatibility of the anode electrode is critical in MFCs operations when it comes to direct interference with bacteria and cellular respiration. Certain elements, including gold, silver, and copper, are unsuitable as electrodes in MFCs because of their corrosive nature (Banerjee et al., 2022; Yaqoob et al., 2021b). The toxic effect of such compounds will hinder the development of bacteria in MFCs, resulting in reduced power production.

2.3.4 Stability and long-term durability

In general, prolonged contact of standard electrodes between the substrate and inoculation microorganisms results in swelling due to mechanical and chemical instability, resulting in a profound effect on the electrode's physical stability. Corrosion, thermal fluctuation, and low mechanical strength are the factors that contribute to the swelling (Sauerteig et al., 2018). Additionally, a rough electrode surface provides more active sites for bacterial adhesion (Banerjee et al., 2022). The cost and availability of electrode materials are critical elements in the selection process since they significantly affect the overall price of MFCs. For instance, gold, platinum, and silver are costly and difficult to obtain. In MFCs, electrode materials made of metal composites and carbon-based materials might be intriguing alternatives to expensive metals (Yaqoob et al., 2021a).

2.4 Comparative analysis of anode materials

Conventional anodes in MFCs are classified into two main categories: carbon-based and metal-based. These categories encompass a variety of materials, including carbon rod (CR), carbon cloth (CC), carbon paper (CP), carbon mesh (CM), carbon

felt (CF), carbon brush (CB), GR, graphite brush (GB), graphite sheet (GS), graphite felt (GF), graphite plate (GP), SS, stainless steel mesh (SSM), carbon steel (CS), and MS. Among these options, carbonaceous materials are widely preferred in MFCs due to their numerous advantages, such as high biocompatibility, resistance to environmental conditions, chemical stability, and a high specific surface area conducive to biofilm development (Banerjee et al., 2022). However, power generation efficiency in MFCs can vary significantly depending on the specific material used. For instance, utilizing CF as the anode yielded a higher cell voltage than CC or CP, with CF achieving a maximum PD of 680.0 mW/m^2, whereas CC and CP exhibited significantly lower PD at 40.0 mW/m^2 and 38.1 mW/m^2, respectively (Sayed et al., 2020; Xian et al., 2021; K. Zhang et al., 2020). Notably, GF electrodes demonstrated the highest voltage and power output at 346 ± 5 mV and 24.0 mW/m^2, respectively, whereas GP electrodes showed substantially lower values at 130 ± 5 mV and 4.5 mW/m^2, respectively (Nosek et al., 2020).

Despite their advantages, many carbon-based anode materials are deemed unsuitable for practical engineering applications for various reasons identified in previous studies. For example, CC, commonly used for anode electrodes in MFCs, poses challenges such as high cost and chemical instability, leading to fouling and reduced long-term stability. CPs that offer high porosity are often considered cost-prohibitive for use as an anode electrode. Although CM is commercially available at a reasonable cost, it suffers from low electrical conductivity and poor mechanical stability, leading to decreased durability. CF boasts high porosity and good electrical conductivity but can hinder substrate diffusion and microbial colonization due to its thickness. With its large surface area and optimal area-to-volume ratio, CB shows promise for power generation in MFCs. Nevertheless, their association with titanium

for electrical conductivity increases economic costs. Graphite is available in various shapes such as plates, rods, granules, cloth, and brushes offer enhanced efficiency compared to simple carbon materials. However, smooth surfaces of graphite electrodes can limit biofilm accumulation, affecting MFCs power output (Banerjee et al., 2022; Yaqoob et al., 2021a).

While carbonaceous materials have long been favoured in MFCs for their biocompatibility and stability, metal-based anodes offer superior conductivity, prompting a shift towards exploring alternative materials. Traditionally, various noble metal electrodes, such as gold (Au), platinum (Pt), palladium (Pd), titanium (Ti), and silver (Ag), have been used in electrochemical applications. Yet, deploying these precious metals in large-scale MFCs setups proves economically impractical. Consequently, there is a growing inclination towards substituting these costly electrodes with more economical options such as iron (Fe), rhodium (Rh), copper (Cu), nickel (Ni), aluminium (Al), SS, SSM, and MS. Unfortunately, these low-cost electrode materials suffer from high corrosion rates in aqueous solutions, hindering the long-term performance of MFCs. Furthermore, the smooth surface of metals typically does not facilitate bacterial adhesion, potentially hindering their ability to attain higher PD compared to carbon materials. Research indicates that due to their smooth surface, metals such as SS anodes have not yielded optimal outputs, with the maximum reported output PD reaching around 3.7 mW/m^2 (Saadi et al., 2020). In addition, SSM may experience biofilm detachment over time due to gravitational effects. This phenomenon can hinder bioelectricity production despite the initial suitability of SSM electrodes (Masoudi et al., 2020). Therefore, finding a balance between conductivity, cost-effectiveness, and corrosion resistance is essential for developing effective metal-based anode materials for MFCs.

In recent years, there has been a notable transition towards adopting newly developed anode materials in MFCs, characterized by superior performance compared to conventional options. These alternatives, categorized into natural material-based and graphene derivative-based anodes, present promising avenues for enhancing MFCs efficiency. Natural material-based anodes derived from biomass and natural waste capitalize on readily available resources and serve as a valuable carbon source in MFCs. The carbonization process, typically conducted through thermochemical decomposition such as pyrolysis, yields biochar, among other products, with treatment conditions dictating the resulting attributes. Pyrolysis parameters, including heating rate and residence time, are crucial in determining product composition and quality. Biomass carbonization initiates within the temperature range of 200 °C–300 °C, progressing through the breakdown of hemicellulose (15–30% of biomass dry weight), followed by the decomposition of cellulose (40–50% of biomass dry weight) between 240 °C and 400 °C, and ends with the decomposition of lignin (15–30% of biomass dry weight) at temperatures exceeding 900 °C (Chakraborty et al., 2020). The electron-donating capacity and specific surface area of biochar contribute to the enhanced performance of biofilms (Qin et al., 2020).

Furthermore, the production process for low-temperature biochar yields lower carbon emissions and incurs reduced production costs compared to graphite, rendering it a promising alternative for anode materials in MFCs (Gorrazzi et al., 2023). In recent research by Vempaty et al. (2023), datura peels were subjected to carbonization at 800 °C, after which the resulting biochar was examined for its suitability as an anode material. It was found that the biochar exhibited a maximum PD of 584.2 mW/m². In another study by Li et al. (2020a), mango wood underwent carbonization at various temperatures, and the resulting carbon was coated onto a CF electrode using a binder.

Their findings revealed a significant enhancement in PD, with the coated electrode achieving 277.0 mW/m^2, nearly double that of the uncoated counterpart at 589.8 mW/m^2. Similarly, Chaijak et al. (2020) employed anodes derived from sawdust biochar in MFCs to treat rubber industry wastewater, achieving a maximum PD of 3.3 $\mu W/m^3$, along with notable removal efficiencies for chemical oxygen demand (COD) at 89.8 % and sulfate at 88.3 %. Hung et al. (2019) investigated the feasibility of utilizing activated carbon derived from spent coffee waste in MFCs anodes, achieving a high PD of 3927 mW/m^2, surpassing commercial activated carbon. Additionally, Q. Chen et al. (2018) investigated the performance enhancement of chestnut shell-based anodes through chemical activation, resulting in significant improvements with the PD reaching 850 mW/m^2. Given the abundance of waste as a readily available carbon source and the utilization of straightforward synthesis techniques, researchers have investigated its potential as an input material for anodes in MFCs. Nonetheless, biochar encounters challenges stemming from inherent limitations in durability and electrical conductivity. Thus, further examination of biochar's application as an anode material is imperative to optimize the performance of MFCs.

Anode modifications using allotropes of carbon, such as graphene, have shown promise in improving MFCs performance. Studies have highlighted graphene's capability to stimulate microorganisms to release signalling molecules, accelerating bacterial cell growth and acting as a mediator molecule to enhance electron transfer efficiency (Roy et al., 2023). Its unique nanostructure establishes physical contact with bacterial membrane cytochromes and/or conductive pili, facilitating direct electron shuttling and enabling rapid electron transfer through a pseudo-direct mechanism involving bacterial endogenous electron mediators. According to Mohamed et al. (2023), over 70% of the total resistance in MFCs stems from diffusion resistance and

activation losses. Further investigations by Aiswaria et al. (2022) reveal that optimal performance in MFCs anodes is achievable with macro-porous structures (>50 nm), surpassing microporous (<2 nm) and mesoporous (2–50 nm) counterparts, aligning with the size of bacterial cells (approximately 1–2 μm). The absence of macro-porous anodes restricts biofilm growth to the surface, impeding access to the electrode's interior and significantly reducing anode productivity. Consequently, graphene-based anode materials present advanced solutions capable of tackling multiple challenges, such as enhancing conductivity, increasing surface area, ensuring biocompatibility, and promoting bacterial adhesion, thereby demonstrating promising potential for applications in MFCs.

However, considering the cost constraints of commercial graphene, exploring alternatives like GO emerges as a viable option for large-scale production due to its inherent advantages. GO synthesis methods, such as the widely adopted Hummer's method, offer cost-efficiency and eco-friendliness, aligning with the pursuit of affordability in MFCs (Yaqoob et al., 2021a). Moreover, the integration of waste-derived materials into GO production, coupled with its straightforward synthesis from biomass, positions it as an attractive option for anode fabrication in MFCs. Additionally, GO demonstrates considerable potential as an electronic insulator, owing to the presence of oxide groups such as epoxide, hydroxide, carbonyls, and carboxyls. These functional groups contribute to GO's insulating and hydrophilic behavior while retaining its mechanical strength, surface area, and gas impermeability, making it a versatile and promising material for various applications in MFCs (Aiswaria et al., 2022). Recent studies have highlighted the potential of employing waste-derived GO materials, exemplified by palm kernel shell-derived GO (PKS-GO), to enhance the efficiency of MFCs. The incorporation of PKS-GO resulted in MFCs achieving a

maximum PD of 13.8 mW/m² and a maximum CD of 63.7 mA/m² (Idris et al., 2023b). In a study by Sayed et al. (2021), the performance of MFCs utilizing GO-coated CB electrodes was compared with that of MFCs using plain CB anodes. The results revealed a substantial enhancement in MFCs performance with the GO-CB, as evidenced by a more than 10-fold increase in PD, from 33 mW/m^2 to 381 mW/m^2. Sustained efforts in research and innovation concerning material development are imperative to deliver enhanced energy generation capabilities and contribute to environmental sustainability. Table 2.1 offers an overview of conventional and advanced anode materials utilized in MFCs.

Table 2.1: Overview of utilized conventional and advanced anode materials in MFCs.

Conventional anode							
Anode material	Size of electrode (cm × cm)	Surface area (cm^2)	Organic substrate/ Inoculum source	Microbes	Power density (mW/m^2)	Time (days)	Reference
CC	-	-	Diesel soil	-	15.0	25	(Zafar et al., 2024)
CC	-	6.0	Wastewater from the water treatment plant	-	40.0	3	(Sayed et al., 2020)
CM	26.0 × 6.0	-	Acetate	*Geobacter* sp.	1.4 W/m^3	6	(Luo et al., 2020)
CP	-	-	Acetate	*Shewanella loihica*	38.1	3	(Xian et al., 2021)
CF	2.5 × 2.5	-	Glucose	Mixed inoculum	680.0	15	(K. Zhang et al., 2020)
CB	2.5 × 2.5	-	Glucose	Mixed inoculum	1350.0	15	(K. Zhang et al., 2020)
GR	15.0 × 1.0	201.0	Palm sugar	Mixed inoculum	130.2	80	(Yaqoob et al., 2023)
GS	-	-	Acetate	*Shewanella putrefaciens*	285.0	3	(Tripathi et al., 2022)
SS	-	19.6	Acetate and sludge	Mixed inoculum	100	30	(Shahid et al., 2021)
SS	1.0 × 1.0	1.0	Acetate and sludge	Mixed inoculum	3.7	16	(Saadi et al., 2020)
SSM	6.0 × 6.0	174.0	Dairy wastewater	-	237.1 mW/m^3	35	(Masoudi et al., 2020)
MS	4.5 × 4.5	3.4	Sludge	Mixed inoculum	1184.0	30	(Sreelekshmy et al., 2020)
CS	-	-	Acetate and mud	Mud	260.1	8	(Raba'atun Adawiyah Shamsuddin et al., 2020)

Advanced anode							
Anode material	Size of electrode (cm × cm)	Surface area (cm^2)	Organic substrate/ Inoculum source	Microbes	Power density (mW/m^2)	Time (days)	Reference
Bamboo	-	16	-	*Bacillus licheniformis, Shewanella putrefaciens*	12.9 W/m^3	5	(Kumar et al., 2023)
Datura peels	-	-	Acetate	*Pseudomonas aeruginosa*	584.2	38	(Vempaty et al., 2023)
Cedar wood	-	-	Acetate	Mixed inoculum	9.9	12	(Bataillou et al., 2022)
Sludge-CF	-	-	Sodium acetate	Mixed inoculum	615.2	15	(Li et al., 2020b)
Mango Wood	-	75.3	Sodium acetate	Mixed inoculum	277.0	15	(Li et al., 2020a)
Corncob waste	-	-	Acetate	Mixed inoculum	4990.0	47	(Wang et al., 2020)
Cellulose waste	7.5 × 1.2	71.0	Sweet potato waste	Mixed inoculum	0.1	40	(Yaqoob et al., 2020c)
Rubber tree sawdust	-	-	Rubber wastewater sludge	*Galactomyces reessii*	3.3 $\mu W/m^3$	7	(Chaijak et al., 2020)
Coffee waste-CC	1.0 × 1.0	1.0	-	*Escherichia coli*	3927.0	5	(Hung et al., 2019)
Chestnut-CC	-	-	Sludge	Mixed inoculum	850.0	7	(Q. Chen et al., 2018)
Pomegranate peels-GO	-	-	Waste stream	Mixed inoculum	12.5 W/m^2	15	(Mohamed et al., 2023)
PKSGO	8.0 × 1.3	76.0	Rotten sweet potatoes	Mixed inoculum	35.1	70	(Idris et al., 2023b)

Advanced anode							
Anode material	Size of electrode (cm × cm)	Surface area (cm^2)	Organic substrate/ Inoculum source	Microbes	Power density (mW/m^2)	Time (days)	Reference
Lignin-GO	8.0 × 1.3	76.0	Oil palm trunk sap	Mixed inoculum	0.3	90	(Yaqoob et al., 2021c)
Lignin-lemon peel-GO/ZnO	8.0 × 1.3	76.0	Oil palm trunk sap	Mixed inoculum	0.9	90	(Yaqoob et al., 2021c)
Lignin-lemon peel-GO/TiO_2	8.0 × 1.3	76.0	Oil palm trunk sap	Mixed inoculum	0.6	90	(Yaqoob et al., 2021c)
Carbon nanotube-GO	1.0 × 1.0	1.0	-	*Escherichia coli*	3291.0	-	(Liu et al., 2020)
CC/GO	-	4.0	Glucose	Mixed inoculum	1.6	3	(Pareek et al., 2019a)
GO-Ni	-	2.0	*Luria-Bertani*	*Shewanella putrefaciens*	26.2	3	(Zhu et al., 2019)
MS/Ni-P composite	1.0 × 1.0	1.0	Sugarcane bagasse effluent	Mixed inoculum	2100.0	90	(Chandraserkharan Meenu et al., 2018)
CF/GO	3.0 × 3.0	9.0	Sludge septic tank	Mixed inoculum	184.9	3	(Paul et al., 2018)

Note: "–" specifies the relevant information was not stated in the reference.