JNRID || ISSN 2984-8687 || © April 2024, Volume 2, Issue 4

AN INVESTIGATION INTO GRAPHENE AS AN ELECTRODE MATERIAL IN MICROBIAL FUEL CELLS FOR BIOELECTRICITY GENERATION

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Abstract - The study investigates the potential of Graphene as an electrode coating material in microbial fuel cells for bioelectricity generation as well as a comparison with other electrode coating materials as copper , driven by the demand for materials with superior mechanical and thermal properties in the realm of advancing technology. Rigorous testing, including mechanical, physical, chemical, electrical resistance, and defect inspections, was conducted on a Graphene sheet (0.70 x 1200 mm) and compared with Copper, carbon isotope values, and NIS standards (NIS-119:1984 and NIS-487:2010). State-of-the-art laboratory equipment, such as an atomic absorption spectrometer, coating thickness gauge, universal tensile machine, and defect inspection system, was employed. The findings indicate that most elements in the graphene material align well with standard values, affirming its suitability for intended applications. However, manganese exceeds the standard limit, necessitating further scrutiny and potential refinement in the production process. In the ultimate tensile strength test, graphene surpasses the NIS requirement of 175, registering an impressive 182. The electrical resistivity values also showcase favourable results, with graphene exhibiting 0.02610-6 Ohm/cm compared to the NIS standard of 0.03410-5 Ohm/cm. Impact resistance, a critical parameter, demonstrates graphene's robustness with a measured value of 5 J, exceeding the NIS range of 2 J - 5 J. The gloss result for graphene falls within the specified NIS requirement of 40-60%, recording 58%. This research significantly contributes to our understanding of graphene’s applicability. It provides valuable insights for optimized production processes and identifies potential applications that demand enhanced mechanical properties. The observed discrepancies, particularly the elevated manganese level, highlight areas for further investigation and process refinement. Overall, the study underscores graphene’s promising role in microbial fuel cells and sets the stage for continued advancements in material science and bioelectricity generation technologies.

Index Terms - *Graphene Electrode, Microbial Fuel Cells, Bioelectricity Generation, Mechanical Properties, Electrical Resistivity*

I. INTRODUCTION

II. 1.1 BACKGROUND OF STUDY

microbial fuel cells (mfcs) represent an innovative and sustainable technology for bioelectricity generation by harnessing the metabolic activities of microorganism.. in recent years, the exploration of advanced materials for mfc electrodes has been identified as a key component in the generation of bioelectricity. one such material that has emerged as a promising candidate is graphene, graphene is a single layer of carbon atoms arranged in a hexagonal lattice, forming a two-dimensional structure. it is the basic building block of other carbon allotropes, such as graphite, carbon nanotubes, and fullerenes. graphene has remarkable properties that make it an exciting material in various fields of science and technology. these attributes make graphene an attractive material for electrode applications in mfcs, where efficient electron transfer between microorganisms and electrodes is crucial for enhanced bioelectricity generation (mirala et al., 2022).

this investigation into the utilization of graphene as an electrode material in mfcs holds great potential for addressing several issues facing the conventional microbial fuel cell (mfc), such as limited conductivity and susceptibility to biofouling. this study focuses on establishing graphene as an electrode material that would significantly contribute to not only the ecosystem but also national economic development.

in order to establish the above, i explored several methods of confirming the positive properties of graphene as a material some of which include electrical efficiency, chemical composition analysis, weathering and durablility testing .

notably, substantial energy reservoirs lie within organic matter, such as carbohydrates abundantly present in agricultural and municipal waste. operating as a bio-electrochemical system (bes), mfcs directly convert the chemical energy stored in organic compounds into electrical energy through microbial metabolism, offering advantages such as low maintenance, environmental friendliness, high conversion efficiency, and operation at ambient and low temperatures (wen et al., 2013; alatraktchi et al., 2012).

III. LITERATURE SURVEY

OVERVIEW ON MICROBIAL FUEL CELLS (MFCS)

The origins of mfc technology can be traced back to the early 20th century, with initial experiments exploring microbial involvement in electricity production. however, significant advancements were made in the late 20th and early 21st centuries, marked by the identification of electroactive microorganisms and the optimization of mfc designs. over time, mfc technology has evolved from basic laboratory experiments to diversified applications, including wastewater treatment, power generation, and biosensor development.

at its core, mfcs operate on the principle of utilizing living microorganisms as biocatalysts for the conversion of chemical energy stored in organic matter into electrical energy. this unique technology holds immense potential for sustainable energy generation due to its ability to directly convert organic substrates, such as wastewater and organic waste, into electricity (cecconet et al., 2018).

the fundamental principle of mfcs involves the interaction between microorganisms and electrodes within a controlled environment. microorganisms, commonly bacteria, oxidize organic matter, releasing electrons and protons (jain et al., 2021). these electrons are then harnessed as electrical energy when they flow through an external circuit towards a cathode, where they combine with protons and oxygen to form water. the anode and cathode constitute the key components, with the anode facilitating microbial metabolism and electron release, and the cathode enabling electron acceptance for the overall electrochemical reaction {shabani et al., (2020); kim and patel (2020)}.

the significance of mfcs in sustainable energy generation lies in their potential to address pressing environmental and energy challenges. by providing a means to harness energy from organic waste, mfcs contribute to the reduction of pollutants and offer a sustainable alternative to traditional energy sources (ni et al., 2020). furthermore, mfcs align with the principles of the circular economy by transforming waste into a valuable resource, exemplifying their role in creating a more sustainable and environmentally friendly energy landscape (wei et al., 2011; logan, 2010).

mfcs can be grouped into two general categories, those that use a mediator and those that are mediator-less (kalathil et al., 2017). a mediator is a chemical that transfers electrons from the bacteria in the cell to the anode. in the mediator-less mfc the bacteria typically have electrochemically active redox proteins such as cytochromes on their outer membrane that can transfer electrons directly to the anode (singh et al., 2019). a microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms (yazdi et al., 2016). a typical microbial fuel cell consists of anode and cathode compartments separated by a cation specific membrane. in the anode compartment, fuel is oxidized by microorganisms, generating co2, electrons and protons. power production was very low and required the addition of exogenous mediators to shuttle electrons from inside to outside the cell. in new systems, exogenous mediators are not needed, and power production from mfcs has increased dramatically in just the past few years, in part because of designs that lower the reactor’s internal resistance {ivars-barcdo et al., (2018); samsundeen et al., (2015); coedova-bautista et al., (2017)}. extensive optimization is required to exploit the maximum microbial potential for novel wastewater treatment process and biosensor for oxygen and pollutants.

2.2 APPLICATIONS OF MICROBIAL FUEL CELLS

A) WASTE WATER TREATMENT

MFC-based wastewater treatment and energy harvesting research, and analyze various biocatalysts used in MFCs and their underlying mechanisms in pollutant removal as well as energy recovery from wastewater. Lastly, we highlight key future research areas that will further our understanding in improving MFC performance for simultaneous wastewater treatment and sustainable energy harvesting.

B) DESALINATION

Microbial desalination cells (MDCs) with common liquid anodic substrate exhibit a slow startup and destructive pH drop, and abiotic cathodes have high cost and low sustainability. A biocathode MDC with dewatered sludge as fuel was developed for synergistic desalination, electricity generation and sludge stabilization. Experimental results indicated that the startup period was reduced to 3d, anodic pH was maintained between 6.6 and 7.6, and high stability was shown under long-term operation (300d). When initial NaCl concentrations were 5 and 10g/L, the desalinization rates during stable operation were 46.37±1.14% and 40.74±0.89%, respectively. The maximum power output of 3.178W/m(3) with open circuit voltage (OCV) of 1.118V was produced on 130d. After 300d, 25.71±0.15% of organic matter was removed. These results demonstrated that dewatered sludge was an appropriate anodic substrate to enhance MDC stability for desalination and electricity generation.

C) HYDROGEN PRODUCTION

The primary product of the electrochemical reaction in Fuel cells is Hydrogen. Hydrogen from the beginning is known for its ability to produce energy/release of electrons Hydrogen gas has immense potential as an ecologically adequate energy carrier for vehicles. MFC is bioelectrochemical process that utilize microorganism as the impetuses to oxidize organic and inorganic matter and create electricity, whereas MEC are a reactor for biohydrogen production by combining MFC and electrolysis. MEC and MFC have been the cleanest, eco-friendly, and efficient method for biohydrogen production.

D) Remote Power Source Generation

The advancement of microbial fuel cell technologies in the 1980s was largely spurred by the dream of providing cheap, accessible power to remote regions of Africa, where 74% of the population lives without electricity . Although implementation of homemade MFC use in Africa is just beginning, microbial fuel cells that run on manure and dirt have been developed and tested. The electrical current produced by a simple homemade MFC is enough to recharge a cell phone battery, an important communication and lighting tool to rural African communities. . The materials required to construct a simple MFC are soil, manure, copper wire, buckets, and graphite cloth which are relatively accessible.

DIAGRAMATICAL REPRESENTATION OF MICROBIAL FUEL CELLS (MFC)

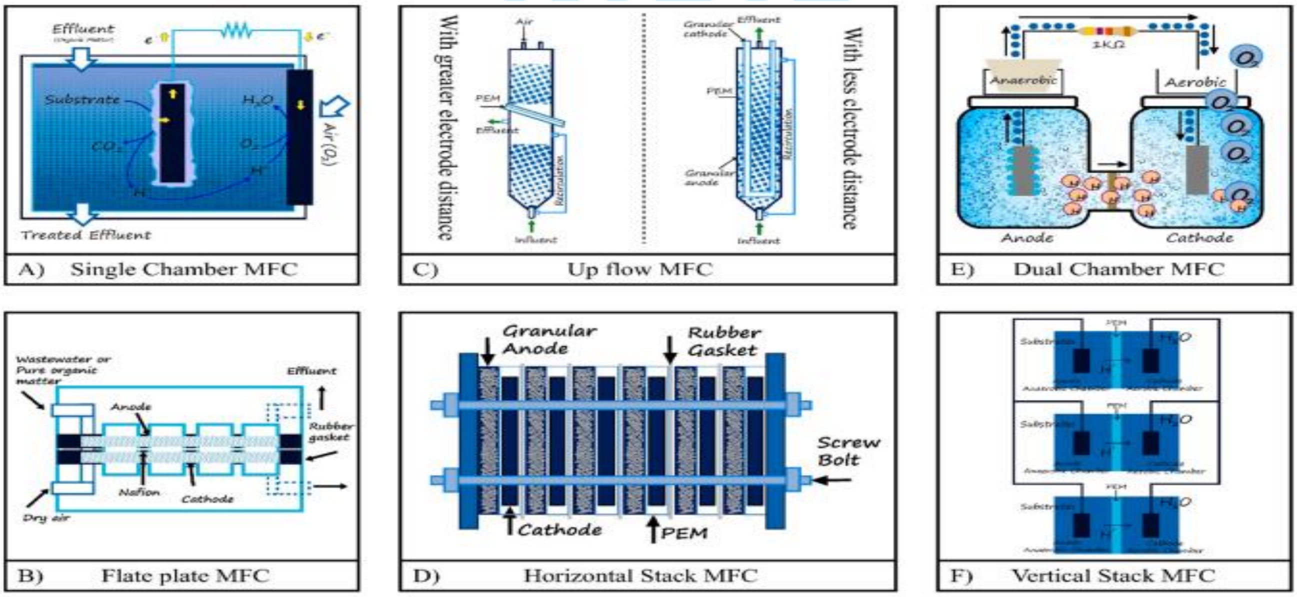


Figure 2.1: Diagrammatic representation of different types of MFCs.
Source: Nawaz *et al.*, (2022)

2.3 Electrode Materials in Microbial Fuel Cells

Microbial Fuel Cells (MFCs) have gained significant attention as sustainable bioenergy devices that harness the metabolic activity of microorganisms to generate electricity. Central to the efficiency and performance of MFCs are the electrode materials, which play a pivotal role in electron transfer processes.

The electrodes in MFCs serve as interfaces for electron transfer between microbial biofilms and external circuits. Anode and cathode materials play distinct roles in facilitating the oxidation and reduction reactions, respectively. The efficiency of electron transfer is crucial for maximizing power output in MFCs. Understanding the properties and characteristics of electrode materials is essential for optimizing MFC performance.

2.3.1 Traditional Electrode Materials and Their Limitations

(a) Graphite-Based Anodes: Traditional anodes are often composed of graphite due to their conductivity and stability. Limitations include biofilm fouling, low surface area, and vulnerability to corrosion (Logan *et al.*, 2006; Cheng and Logan, 2007).

(b) Platinum-Based Cathodes: Platinum and other noble metals are commonly used as cathode materials. High cost and scarcity of noble metals pose economic and sustainability challenges. (Liu *et al.*, 2008; Kim *et al.*, 2018)

2.3.2 Emerging Trends in Advanced Electrode Materials

Nanostructured Anodes: Recent advancements involve the use of nanostructured materials (e.g., carbon nanotubes, graphene) to enhance surface area and conductivity. Nanostructured anodes mitigate biofilm fouling and improve electron transfer rates (Wang *et al.*, 2011; Santoro *et al.*, 2017).

2.4 Introduction to Graphene: Properties and Characteristics

Graphene, a single layer of carbon atoms arranged in a hexagonal lattice, has garnered immense interest in recent years due to its extraordinary properties. Its high electrical conductivity, exceptional mechanical strength, large surface area, and chemical stability make graphene an attractive material for various applications, including its use as electrodes in Microbial Fuel Cells (MFCs). This literature review provides an in-depth examination of graphene's properties, its historical applications across industries, and the theoretical framework supporting its suitability as an electrode material in MFCs. Table 2.1 presents the properties and characteristics of graphene.

Table 2.1 Properties and Characteristics of Graphene.

Properties	Description
High Electrical Conductivity	Graphene exhibits an outstanding electrical conductivity, surpassing that of traditional electrode materials. Enhanced electron transfer capabilities make graphene a promising candidate for electrode materials in MFCs (Novoselov *et al.,* 2004; Geim and Novoselov, 2007).
High Power Density and Columbic Efficiency	The columbic efficiency of a microbial fuel cell where its electrodes are coated with graphene are higher than conventional Electrode Materials (Wu S, Liang P, Zhang C, Li H, Zuo K, Huang X, 2015)
Large Surface Area	The two-dimensional structure of graphene provides an extensive surface area for microbial attachment and biofilm formation. Increased surface area contributes to improved microbial activity and electron transfer in MFCs (Choi *et al.,* 2015; Shao *et al.,* 2016).
Mechanical Strength and Flexibility	Graphene's exceptional mechanical properties ensure durability and flexibility in various applications. Robustness is crucial for long-term stability and performance of MFC electrodes (Lee *et al.,* 2008; Wang *et al.,* 2019).
Chemical Stability	Graphene exhibits high chemical stability, resisting corrosion and degradation over time. Chemical stability is essential for maintaining electrode integrity in the complex environment of MFCs (Stoller *et al.,* 2008; Pei and Cheng, 2012).

Graphene oxide (GO) has garnered immense interest in recent research, spanning back to studies as early as 1859. Despite its widespread application, the characterization of GO has lacked standardized protocols, leading to a multitude of methods that hinder comparison and reproducibility. This review delves into GO characterization approaches, particularly in two subcategories: GO as a dispersant and GO for emulsion stabilization.

GO is characterized by a unique structure, featuring islands of oxygen-functionalized sp3 carbon amidst sp2 carbon, giving rise to both hydrophobic and hydrophilic regions. Investigations highlight GO as a surfactant, lowering water surface tension at water–air interfaces, though it doesn't exhibit micellization typical of surfactants. The review emphasizes GO's role as a 2D surfactant by reducing interfacial tension.

The synthesis of GO typically involves oxidizing graphite, followed by exfoliation into mono- or few-layer sheets. Various methods, including Brodie, Staudenmaier, and Hummers, have evolved since the 19th century, with the latter gaining prevalence due to safety considerations. Structural models depict GO as a 2D hexagonal carbon sheet with sp3 islands featuring oxygen functionalities. GO's hydrophilicity and intercalation of water molecules between layers are crucial characteristics.

The review categorizes recent literature into studies employing GO as a dispersant or emulsification agent. GO's application as a dispersant involves unique challenges, with diverse approaches utilizing methods like Kovtyukhova and Hirata (2018) modifications. Emphasizing the hydrophilic nature of GO, the material can form stable colloidal suspensions in various solvents.

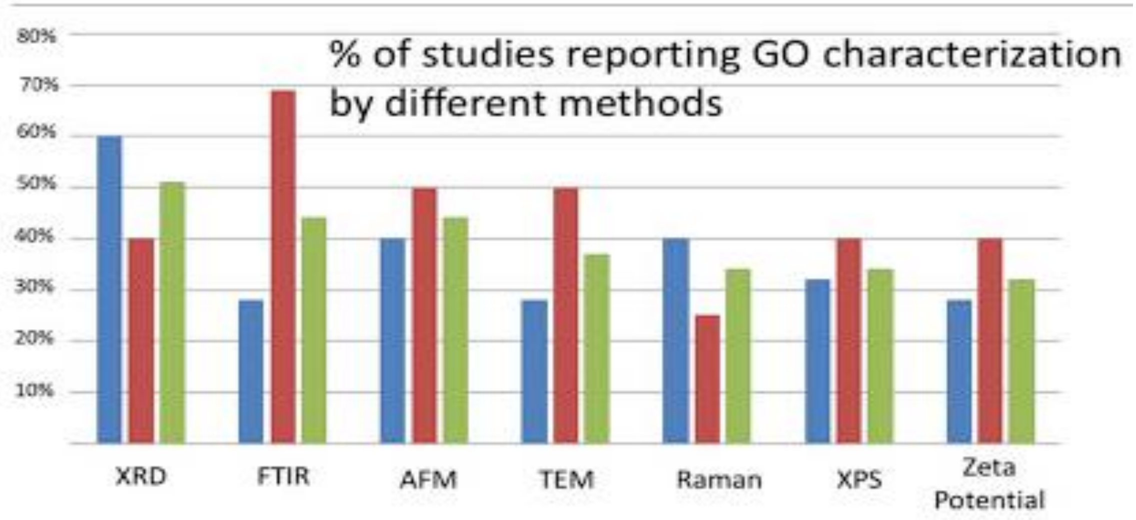


Figure 2.3: Different Experimental Characteristics of Graphene Oxide
Source: Slate *et al.*, (2019)

In summary, the review provides an insightful exploration of GO characterization methodologies, steering away from critique and focusing on showcasing the diversity and frequency of techniques used. Understanding the nuances in GO characterization is pivotal for advancing research in fields utilizing this versatile material.

Some notable applications of graphene in various industries as graphene's being an exceptional electrical property have been exploited in electronic devices, such as transistors and sensors.

Previous successes in electronics highlight graphene's potential as a high-performance electrode material (Avouris *et al.*, 2007; Schwierz, 2010).

Graphene-based materials have been extensively studied for energy storage applications, including supercapacitors and batteries. Insights from energy storage applications inform the use of graphene in MFCs for efficient energy conversion. (Choi *et al.*, 2012; Wang *et al.*, 2017).

Albuquerque *et al.*, (2023), investigated on degradation modes estimation tool for second life li-ion batteries with inhomogeneous lithium distribution. The authors introduced a Diagnostic tool utilizing Incremental Capacity Analysis (ICA) to estimate Degradation Modes (DMs) in Li-ion batteries as shown in figure 2.4. The tool considers an estimation of inhomogeneous loss of cyclable lithium within the negative electrode to enhance precision. Tested on second-life Li-ion batteries with Graphite/NMC622 chemistry, the tool effectively characterizes and predicts battery aging, allowing valuable insights into the behavior and performance of these critical energy storage systems, as shown in figure 2.5 (a) & (b), illustrating experimental differential voltage for the three analyzed cells and degradation modes in function of capacity loss.

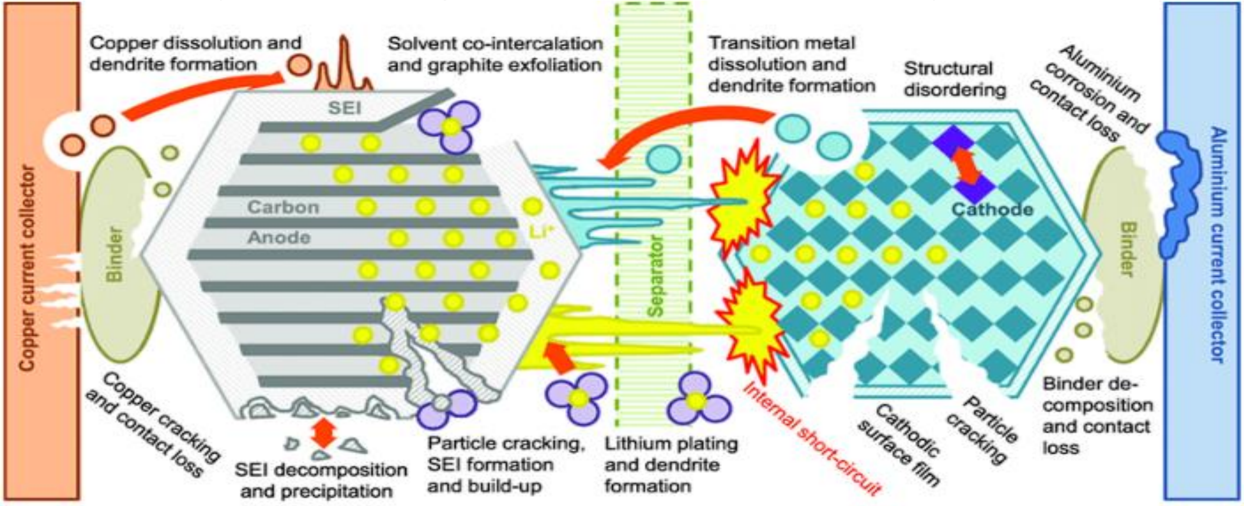
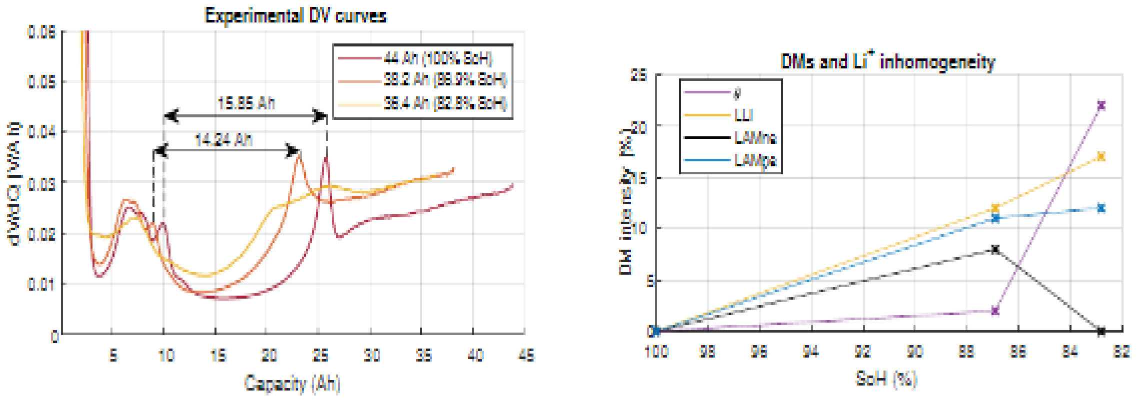


Figure 2.4: The Degradation mechanisms in a Li-ion battery
Source: Original Diagram by Birkl *et al.*, (2017), modified by Albuquerque *et al.*, (2023).



(a) **(b)**

Figure 2.6: **(a)** Experimental Differential Voltage for the three analyzed cells and **(b)** Degradation Modes in function of capacity loss.
Source: Albuquerque *et al.*, (2023)

On the area of materials science graphene has found applications in materials science, contributing to the development of composites and coatings. Graphene nanoplatelets have garnered considerable attention in the field of composites due to their remarkable properties. GNPs exhibit strong interfacial interactions with fibre matrices, leading to enhanced mechanical characteristics, including stiffness and tensile strength (Anderson *et al.*, 2022). Moreover, GNPs offer superior barrier properties when incorporated into composites, making them an attractive candidate for ballistic shielding materials (***Mirela et al., 2022*****).** Previous successes in diverse industries underscore graphene's versatility and potential for MFC electrodes. (Dreyer *et al.*, 2010; Kuilla *et al.*, 2010)**,**

2.5 Theoretical Framework for Graphene's Suitability in MFCs

According to Logan *et al.*, 2019; Theoretical studies highlight graphene's ability to facilitate rapid electron transfer between microbial biofilms and electrodes. The unique electronic structure of graphene promotes efficient electron transport in MFCs (Santoro *et al.*, 2014). The large surface area and biocompatible nature of graphene support strong biofilm adhesion and microbial activity. Theoretical frameworks explore the interactions between graphene electrodes and microbial communities in MFCs (Xie *et al.*, 2016; Feng *et al.*, 2018). One of the notable theoretical frameworks for graphene's suitability in MFCs is long-term stability Theoretical models assess the mechanical strength and chemical stability of graphene electrodes over extended MFC operation. Predictions of long-term stability contribute to the practical viability of graphene as an electrode material (Wei *et al.*, 2021; Zhang *et al.*, 2022).

2.6 Factors Influencing MFC Performance

The microbial fuel cell (MFC) stands out as a promising technological innovation for simultaneous wastewater treatment and efficient electricity generation (Bose *et al.*, 2019). Various factors play a crucial role in shaping MFC performance and electricity generation, including electrode and membrane materials, temperature, pH, substrate loading rate, biofilm formation, and external resistance. To enhance energy output, optimization of these factors is essential. Figure 5 illustrates a web diagram of the factors influencing MFC performance and electricity generation, with each factor's impact discussed in detail in the subsequent sections.

2.6.1. Electrode and Membrane Material (Anode)

The anode compartment serves as a vital component of the microbial fuel cell, being the site where electron production occurs. Referred to as the heart of the MFC, the anode facilitates the oxidation of organic pollutants, generating protons and electrons, biofilm formation, and hydrogen production. The selection of a suitable material for the anode is imperative to enhance MFC performance. The chosen material should exhibit high electrical conductivity, compatibility with microorganisms, chemical stability, a substantial surface area, and cost-effectiveness (Jaiswal *et al.*, 2020). Compatibility with microbial communities is a critical aspect of anode material. While materials like gold, copper, stainless steel, and platinum showcase high electrical conductivity, their use as anodes is limited due to high costs and inhibitory effects on cellular growth. Alternatively, cost-effective carbonaceous anode materials such as graphite rod, graphite fiber brush, graphite plates, carbon fiber, carbon cloth, carbon paper, carbon brush, carbon mesh, carbon felt, carbon rods, and carbon nanotube have been employed. Carbon brush, with its fibrous nature, provides increased surface area, while carbon rods offer affordability and significant electrical conductivity, though with a smaller surface area. Porous and cost-effective materials like carbon veil, carbon paper, and carbon mesh exhibit high porosity but are fragile for mechanical applications. Carbon felt, porous with a large surface area and commendable conductivity, has also been employed (Mustakeem, 2015; Singh *et al*., 2019; Jatoi *et al*., 2020).

Several modifications have been made to anode materials to enhance the working potential of MFCs. The introduction of carbon nanotubes as anode material has proven effective, offering substantial conductance, mechanical stability, and biocompatibility (Yazdi *et al.,* 2016). In a study evaluating the impact of multi-walled carbon nanotubes (MWCNTs) on MFC performance and microbial growth, anodes modified with MWCNTs, MWCNT-COOH, and MWCNT-NH2 were compared with a bare carbon cloth anode. The study revealed that the MWCNT-modified anode exhibited denser microbial colonization, with the highest biomass of 39.27 nmol p/cm2 observed for MWCNT-NH2. For power density, the MFC with MWCNT-COOH-modified anode demonstrated the highest value (560.4 mW/m2), signifying a 49% increase in power output compared to the unmodified anode. This indicated the improved performance of MFCs using MWCNTs due to their large surface area supporting microbial growth and enhanced electron transfer potential (Fan *et al.*, 2017).

The application of nitrogen for anode material modification has enhanced the bioelectricity generation potential of MFCs. Anode modification with nitrogen-doped porous carbon material has shown increased MFC performance by promoting microbial growth and facilitating electron transfer from microbes to the electrode (Bi *et al.,* 2018). The use of nanocomposites consisting of various metals and their oxides for anode modification improves MFC efficiency by enhancing microbial cell adherence and reducing ohmic loss. The integration of titanium (Ti), iron (Fe), tin (Sn), manganese (Mn) oxides onto carbon-based materials forming nanocomposites for anode modification has also been employed to enhance MFC efficiency. Additionally, the application of conductive polymers such as Polyaniline for anode modification has garnered attention due to their high conductivity potential, enhanced bacterial adherence, and resilience to environmental conditions (Hindatu *et al.*, 2017; Mehdinia *et al.*, 2014). Yellappa *et al.* (2020) investigated the potential of a stainless-steel mesh integrated with a polyaniline-functionalized activated carbon (PANi-FAC) composite as an anode. Compared to the power output of a stainless-steel mesh anode (169 mW/m2), SSMPANi/FAC resulted in higher power output (322 mW/m2). Similarly, the charge-transfer resistance (Rct) displayed by SSM-PANi/FAC was considerably lower than SSM, suggesting it as an effective anode composite improving biocatalytic potential with lesser ohmic losses in MFC.

Other modifications in the anode for the improvement of MFC performance involve anode treatment with heat and acid. Heat treatment aids in improving power density by eliminating impurities at the anode that hinder electrical conductance (Kalathil *et al.*, 2017). Ni *et al*. (2020) reported the effect of acid–heat-treated anode carbon cloth on MFC performance, comparing it with untreated carbon cloth (CC), acid–heat-modified carbon cloth (N_H_CC), nitric acid-modified carbon cloth (N_CC), and ferric chloride-modified carbon. Acid–heat treatment resulted in the highest power density of 883.62 mW/m2, displaying a 350% increment compared to the unmodified anode. Similarly, acid–heat treatment led to the highest COD removal (80.1%). This treatment also resulted in the selective growth of microbial communities, contributing to improved MFC performance, revealing the positive effect of acid–heat treatment on the properties of the anode carbon cloth.

2.6.2. Cathode

The cathode electrode in the microbial fuel cell (MFC) plays a crucial role in receiving electrons and protons generated at the anode. In double-chamber MFCs, the cathode is separated by a membrane, while in single-chamber MFCs, which lack a membrane, an air cathode is employed to receive the generated electrons. Upon reaching the cathode, electrons undergo reduction facilitated by electron acceptors present in the cathode (Flimban *et al.*, 2019).

Various materials have been employed for cathodes, including carbon cloth, graphite fiber brush, graphite rod, carbon paper, graphite felt, and stainless steel (Zhang *et al.*, 2012; Janicek *et al.*, 2015; Chen *et al.*, 2018; Cecconet *et al.*, 2018). Oxygen is a preferred electron acceptor for its high redox potential, but its use is limited due to insufficient contact with the electrode and increased power consumption for its supply. Alternatively, different electron acceptors have been explored as substitutes for oxygen, including tetrachloroethane, hexavalent chromium, ferric ions, perchlorate, nitrite, sodium bromate, dichloroethane, potassium ferricyanide, and fumarate (Jaiswal *et al.*, 2020).

Additionally, the cathode requires catalysts, such as platinum, to enhance its performance. However, these catalysts are expensive, less sustainable, and involve increased preparation time, making the overall process costly (Chaturvedi and Verma, 2016; Zhang *et al.*, 2012). Alternatively, biocathodes have been introduced, utilizing microorganisms as catalysts to carry out the reduction process by accepting electrons and protons generated at the anode. Bio-cathodes offer certain advantages, as their application can generate beneficial substances during the operational process and contribute to eliminating unnecessary chemical components. Furthermore, bio-cathodes utilizing algae as catalysts do not require the external provision of oxygen (Jung and

Pandit, 2019). The use of microbes in bio-cathodes as biocatalysts is highly advantageous due to their cost-effectiveness, sustainability, satisfactory working efficiency at neutral pH, and self-regeneration potential, thereby enhancing the reduction action at the cathode.

2.6.3. Separating Membranes

Protons, generated alongside electrons in the oxidation reaction at the anode, play a pivotal role in influencing the power output of the Microbial Fuel Cell (MFC). These protons traverse to the cathode through a separating membrane (Rahimnejad *et al.*, 2014). Positioned between the anode and cathode electrodes, the separating membrane serves the purpose of compartmentalization, proton transport, and prevention of oxygen transfer from the cathode to the anode. An ideal proton exchange membrane for MFC operation should exhibit high proton conductivity, chemical and thermal tolerance, mechanical stability, suitable surfafce area, and cost-effectiveness.

Various types of separating membranes include cation exchange membranes, anion exchange membranes, salt bridges, bipolar membranes, ultrafiltration membranes (UFM), microfiltration membranes (MFM), and ceramic separators. Nafion, a cation exchange membrane, is widely used due to its high proton and electrical conductivity. However, Nafion's negative charges can lead to unwanted cation transfer, affecting the pH in both the anode and cathode compartments, thus impacting MFC efficiency. Furthermore, Nafion is relatively expensive. Anion exchange membranes enhance MFC efficiency by facilitating proton transport and preventing the transfer of other cations, maintaining the desired pH gradient (Jung and Pandit, 2019).

Materials like glass fiber and glass wool, chosen for their low cost and positive impact on MFC performance, have also been used as separating membranes. Glass fiber, for instance, exhibits efficient proton transport, reduced oxygen transfer rate, and decreased resistance (Aghababaie *et al.*, 2015).

Despite Nafion's widespread use, challenges like reduced methanol resistance, decreased proton conductivity at high temperatures and low humidity, have led to the exploration of alternative materials. Sulfonated hydrocarbon polymers such as sulfonated poly (ether-ether ketone)s (SPEEK), sulfonated polyimides (SPI), and sulfonated poly(ether sulfone) (SPES) show promise due to their greater proton conductivity and stability at elevated temperature and humidity (Jiao *et al.*, 2021). Nano composite membranes, incorporating nanoparticles, have demonstrated superior power generation potential, Columbic efficiency, proton conductivity, cost-effectiveness, and antifouling characteristics compared to Nafion, making them suitable as Proton Exchange Membranes (PEM) in MFCs (Das *et al.*, 2018).

Other potential separating membranes include sulfonated polybenzimidazole (S-OPBI), graphene oxide/SPEEK (GO/SPEEK), and Quaternized poly(ether-ester ketone) (QPEEK), suggesting that non-fluorinated membranes, especially those modified with nanoparticles, serve as promising alternatives capable of enhancing energy output from microbial fuel cells (Shabani *et al.*, 2020; Kim and Patel, 2020).

2.6.4 Effect of pH in Microbial Fuel Cell (MFC)

In the course of Microbial Fuel Cell (MFC) operation, protons generated at the anode are transported to the cathode, where they, alongside electrons, react with oxygen to form water. The speed of proton transport from the anode to the cathode significantly impacts the pH and operational efficiency of the MFC. A slow proton transport rate leads to an accumulation of protons at the anode, causing anode acidification. Simultaneously, at the cathode, the utilization of protons in the reduction of oxygen, coupled with a slower rate of proton transfer, elevates the pH. This rise in cathode pH is associated with a decrease in oxygen reduction, leading to a reduction in power generation (Ivars-Barceló *et al.*, 2018; Samsudeen *et al.*, 2015). Maintaining pH within an optimal range is crucial for regulating microbial metabolism and directly affects MFC power generation potential (Singh *et al.*, 2019). Bacterial cells exhibit peak metabolic activity at neutral pH conditions. pH alterations can impact physiological parameters such as ionic concentration, biofilm formation, proton motive force, and membrane potential. Anode pH is particularly critical for bacterial cell activity, and deviations can affect electrogenic potential. For instance, a study focusing on B. Subtilis strain in a double-chamber MFC demonstrated optimal growth, B. Subtilis activity, and the highest power density of 405 mW/m² at an anodic pH of 8.6 (Córdova-Bautista *et al.*, 2017). Another study, using wastewater from the pulp industry and Pseudomonas fluorescens for electricity generation, found that a pH of 7 yielded the highest voltage and current, while deviations from pH 7 resulted in reduced bioelectricity generation potential (Kaushik and Jadhav, 2017).

2.6.5 Temperature Impact in Microbial Fuel Cell (MFC)

Temperature exerts a substantial influence on MFC performance, impacting various kinetic and thermodynamic properties. It directly affects the production of bioelectricity, with an increase in temperature leading to higher power density output and decreased MFC resistance (Jatoi et al., 2020). Given the involvement of microorganisms in the MFC operational mechanism, temperature assumes greater significance than in conventional fuel cells. Most electricigens are mesophilic microorganisms, although some psychrophiles, thermophiles, and hyperthermophiles also exhibit electroactive properties (Butti *et al.*, 2016). Studies have reported that microorganisms exhibit optimum activity in the temperature range of 30–45 °C, resulting in elevated electric power generation in MFCs (Liu *et al.*, 2011). Temperature variations from the recommended optimum can hinder biofilm formation and reduce MFC potential for bioelectricity generation. Moreover, such temperature deviations can cause physical damage to microbes and alterations in bacterial processes. Different bacteria types have varying temperature requirements for biofilm development, and once the desired temperature is reached, bacterial populations carry out metabolic functions accordingly (Jatoi *et al.*, 2020; Song *et al.*, 2017). Operating within elevated temperature ranges enhances voltage output, likely due to increased microbial growth and biofilm formation supporting heightened electrochemical activity (Tee *et al.*, 2017). In a study investigating the denitrification of wastewater and electricity generation in a dual-chamber MFC, the highest power output was observed at 35 °C, providing the highest coulombic efficiency and current density (Wang *et al.*, 2018). Another study exploring various parameters, including temperature, on power productivity in a dual-chamber MFC, noted an increase in power density with temperature elevation, with the highest efficiency achieved at 35 °C (Tremouli *et al.*, 2017).

2.6.6 Substrate Loading Rate Impact

The substrate loading rate remains a factor of utmost significance, dictating the specific influx of organic substrate into the cell and the requisite microbial population for its degradation. Achieving an enhanced working potential in Microbial Fuel Cells (MFCs) involves the optimization of the substrate loading rate or organic loading rate, given that energy output and coulombic efficiency are predominantly contingent on the rate of substrate utilization (Abdallah *et al.*, 2019). An augmentation in the organic loading rate brings about a substantial increase in power yield and improved substrate degradation. However, surpassing a certain threshold in organic loading rate may result in a decline in energy output, accompanied by heightened substrate degradation (Goswami and Mishra, 2018).

A study meticulously examined the influence of diverse organic loading rates on electricity production using wastewater from the surgical cotton industry. Notably, a surge in power density, escalating from 42 mW/m² to 116.03 mW/m², was observed as the organic loading rate increased from 0.7 gCOD/L d to 1.9 gCOD/L d. This upswing in power output could be attributed to the efficient utilization of substrate by microbes. Nevertheless, further elevating the organic loading rate led to a subsequent reduction in power generation (Tamilarasan *et al.*, 2017).

2.6.7 Microbial Biofilm in MFC

Microorganisms stand as the fundamental component in Microbial Fuel Cells (MFCs) for both wastewater treatment and subsequent electricity generation. Within the MFC framework, the anodic biofilm holds paramount importance, as the overall performance is intricately tied to the development and characteristics of this biofilm. The production of bioelectricity in MFC is directly proportional to the growth of the biofilm at the anode. A thicker and denser biofilm facilitates enhanced substrate utilization, given the elevated proportion of bacterial cells acting as catalysts in a substantial biofilm structure (Arbianti *et al.*, 2018; Choudhury *et al.*, 2017). As the biofilm thickness grows at the anode, it diminishes the polarization resistance, consequently amplifying power generation in the MFC (Baranitharan *et al.*, 2015).

Several advancements have been implemented to augment biofilm formation, such as employing hydrophilic, positively charged anodes, aligning with the negative surface charge and hydrophilic nature of most bacteria (Kalathil *et al.*, 2017). Reports have highlighted the enhancement of MFC power generation through the application of an improved hybrid biofilm incorporating bacterial multiwalled carbon nanotubes. This hybrid biofilm demonstrated a noteworthy reduction in startup time by 53.8%, showcasing superior adsorption properties and increased tolerance to abrupt concentration changes in the substrate compared to naturally developed biofilms. Consequently, this led to an augmented current density (P. Zhang *et al.*, 2017; L. Zhang *et al.*, 2017).

The primary objective of microbial modification on the electrode is to produce a biofilm with high conductivity, activity, and penetration capabilities. Genetic modifications of microorganisms have been employed to boost their electrogenic potential, resulting in an increased electric output of MFCs utilizing wastewater. Conventional microbial strains are replaced by genetically engineered microbes, and the careful selection of an appropriate genetically manipulated strain is pivotal for ensuring extended MFC performance. Various molecular biology methods, including synthetic biology, metagenomics, and microbiomes, have contributed to efforts aimed at augmenting the microbial biofilm population in MFCs (Palanisamy *et al.*, 2019; Angelaalincy *et al.*, 2018).

In the context of a single-chamber microbial fuel cell with an air cathode, the absence of a separating membrane and exposure to diffused organic matter can have detrimental effects on air cathode activity. Biofilm development on the air cathode obstructs active sites of catalyst coatings and can negatively impact catalyst performance through extracellular substances, thereby affecting the oxygen reduction potential. Additionally, biofilm accumulation at the cathode poses competition to anodic microbial biofilm for substrate utilization. Aerobic biofilm growth obstructs proton and charged moieties' passage to the catalyst, hinders pH at the electrode, and consumes oxygen, affecting the reduction reaction at the cathode. Various strategies, including physical cleaning methods, chemical treatment, application of electric fields for biofilm removal, and modification of electrode surfaces with antimicrobial components integrated into catalyst coatings, have been proposed to counteract biofouling issues (Al Lawati *et al.*, 2019).

2.6.8 Impact of External Resistance

The external resistance significantly influences the performance of Microbial Fuel Cells (MFCs) in terms of electricity generation, Chemical Oxygen Demand (COD) elimination, and microbial diversity. An elevation in external resistance results in reduced current output, while decreased external resistance leads to heightened current generation. Operating the MFC under decreased external resistance accelerates reactions at the cathode and electrical activity, owing to the enhanced transport of electrons to the cathode. This, in turn, improves the current output in the MFC. Moreover, low resistance enhances the positive potential of the anode, resulting in greater energy gain by microorganisms and increased electron flux. This selective promotion of the electrogenic microbial community further contributes to overall efficiency (del Campo *et al.*, 2014).

A study investigating the impact of external resistance on power generation, employing a combination of the wood industry and municipal wastewater, revealed that the maximum current density of 440 mA/m² was achieved at 100 Ω, representing the lowest resistance among those examined (Kloch and Toczyłowska-Mamińska, 2020).

2.7 Key Metrics for Assessing MFC Performance

Traditional electrode materials in MFCs, such as carbonaceous materials, face challenges such as limited surface area, susceptibility to fouling, and high cost (Liu et al., 2005). Microbial Fuel Cells, with their unique ability to harness energy from organic matter, are subject to various performance metrics. Addressing challenges associated with traditional electrode materials and leveraging graphene's properties hold promise for enhancing MFC performance, some notable key performance assessment matrix includes:

a. Power Density

Power density is a crucial metric indicating the amount of electrical power generated per unit of electrode surface area. It reflects the efficiency of energy conversion in MFCs (Logan *et al.*, 2006).

b. Columbic Efficiency

Columbic efficiency measures the percentage of electrons transferred from the substrate to the anode that contribute to current generation. It assesses how effectively microorganisms convert substrate into electricity (Rabaey *et al.*, 2004).

c. Open Circuit Voltage (OCV)

OCV is the voltage generated by an MFC when no current is drawn. It provides insights into the electrochemical potential of the microbial processes occurring within the cell (Logan *et al.*, 2006).

d. Internal Resistance:

Internal resistance impacts the efficiency of electron transfer within the MFC. Lower internal resistance results in higher power output (Logan *et al.*, 2006). Table 2.2 presents some potential of graphene to enhance MFC performance metrics.

Table 2.2: Highlights and Description of some Potential Graphene/MFC Performance Metrics

Highlight	Description
High Surface Area	Graphene, with its exceptional surface area, offers enhanced microbial adhesion and colonization, promoting efficient electron transfer (Santoro *et al.,* 2012).
Power Density and Columbic Efficiency	The columbic efficiency of the microbial fuel cell where its electrodes are coated with graphene are higher than conventional Electrode Materials (Wu S, Liang P, Zhang C, Li H, Zuo K, Huang X, 2015)
Conductivity and Biocompatibility	Graphene's high electrical conductivity and biocompatibility contribute to improved charge transfer kinetics and overall MFC performance (Zhang *et al.,* 2011).
Reduced Internal Resistance	The integration of graphene into MFC electrodes has shown promise in reducing internal resistance, leading to higher power densities (Liu *et al.,* 2010).

2.8 Review of Previous Studies on Graphene Utilization in MFCs

Arvaniti and Fountoulakis (2021) researched on the efficacy of an up-flow constructed wetland-microbial fuel cell (CW-MFC) in treating greywater, utilizing a novel graphite-cement composite as the electrode material for the first time. Over a seven-month period, the study monitored the removal of organic matter, nitrogen, and solids, as well as bioelectricity production, comparing the results with a conventional up-flow constructed wetland (CW) lacking electrodes. Both systems exhibited a mean chemical oxygen demand (COD) removal of approximately 93% under an applied organic loading rate (OLR) of 20.6 g COD m^{-2} d^{-1}. NH_4^+-N removal efficiency ranged from 75% to 80%, while total suspended solids (TSS) removal consistently exceeded 95%. Increasing the OLR and prolonging the feeding period resulted in a significant increase in bioelectricity production, escalating from 6.9 mV to 60.8 mV. At higher OLR, the CW-MFC demonstrated a mean power density of 11.6 ± 3.0 mW m^{-3} and a coulombic efficiency of 0.48 ± 0.22%. The study concludes that the examined graphite-cement electrodes proved successful in facilitating bioelectricity production in CW-MFCs. Additionally, the proposed system exhibited highly efficient removal of organic pollutants from greywater. However, the presence of complex and potentially toxic organic compounds in greywater led to a comparatively lower bioelectricity generation when compared to low molecular weight carbon sources {Tran *et al.*, (2010); Bi *et al.*, (2018)}.

According to Han *et al.*, (2023), the constructed wetland-microbial fuel cell (CW-MFC) has garnered considerable attention due to its dual role in wastewater treatment and energy recovery. However, its efficacy in handling high-concentration wastewater is compromised by reduced dissolved oxygen at the cathode and inadequate electron acceptors. This study employed two connected CW-MFC systems with cathodic aeration in series to explore the impacts of aeration rate and hydraulic retention time (HRT) on pollutant removal and electricity production performance in high-concentration wastewater. Results indicated that aeration significantly improved NH_4^+-N and TP removal by 45.0–49.8% and 11.5–18.0%, respectively, compared to unaerated conditions, with no substantial change observed in COD removal. Aeration contributed to increased output voltage and power density, particularly in the first-stage CW-MFC, enhancing power production by 1 to 2 orders of magnitude. The extension of HRT improved pollutant treatment efficiency and power generation performance for high-concentration wastewater, but a 2-day extension did not significantly enhance removal efficiency. Under optimized conditions, the two-stage tandem CW-MFC system achieved maximum total removal rates of 99.3 ± 0.2% for COD, 92.4 ± 1.6% for NH_4^+-N, and 79.5 ± 3.4% for TP. Simultaneously, the first-stage CW-MFC reached a maximum output voltage of 405 mV and a maximum power density of 138.0 mW/m^3, while the second stage exhibited a maximum output voltage of 105 mV and a maximum power density of 14.7 mW/m^3.

Traditionally, oxygen has been considered the optimal terminal electron acceptor in microbial fuel cell (MFC) cathodes (Bose *et al.*, 2019). However, the common use of environmentally unfriendly and expensive catalysts to mitigate the overpotential of oxygen reduction at the cathode surface raises concerns (Butti *et al.*, 2016). Han *et al.,* (2023), explored the potential of employing a high surface area electrode to reduce cathodic reaction overpotential instead of relying on catalyzed materials. A dual-chambered MFC reactor was designed with graphite-granule electrodes and a permeable membrane, and its performance in electricity generation and organic removal rate was evaluated under continuous-feed conditions. Results presented in figure 2.7 & 2.8 demonstrated a maximum volumetric power of 4.4+/-0.2 W/m^3 in the net anodic compartment (NAC) at a current density of 11+/-0.5 A/m^3 NAC. Increasing the electrolyte ionic strength improved power output, and

acceptable effluent quality was achieved at an organic loading rate (OLR) of 2 kgCOD/m³ NAC d. The organic removal rate appeared less affected by shock loading. This system presents a promising approach to enhance the economic viability of MFC-based technology for wastewater treatment applications, showcasing a significant improvement in current generation compared to several studies utilizing low-surface-area plain graphite electrodes (Call and Logan 2008).

Figure 2.7: Schematic diagram of the microbial fuel cell used in this study.
Source: Han *et al.*, (2023)

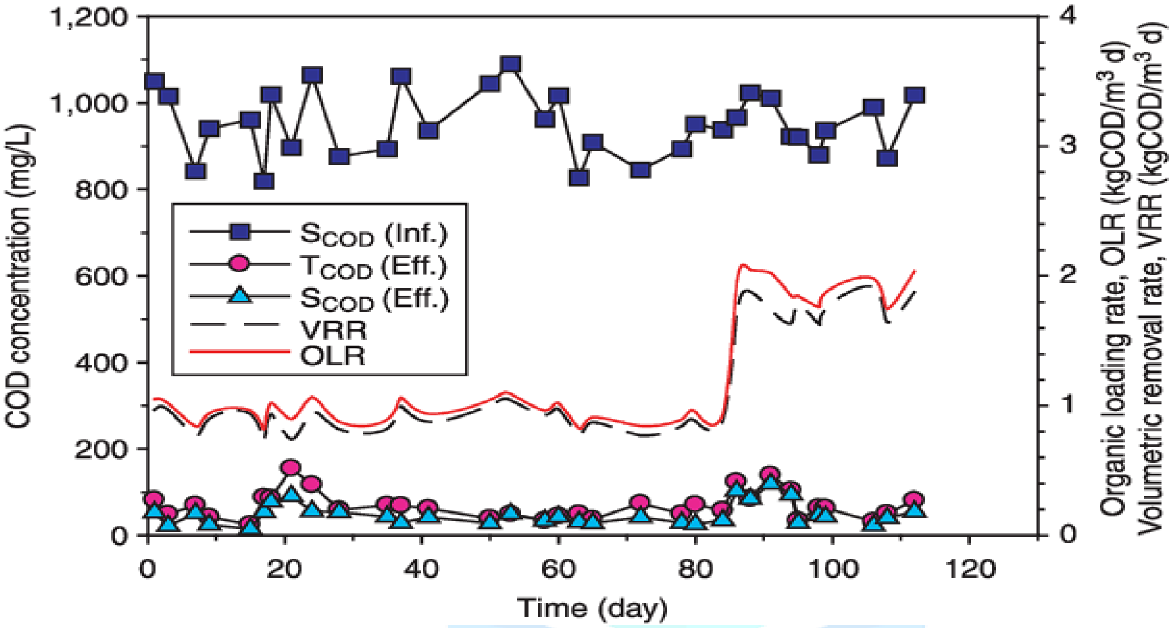


Figure 2.8: Time course of COD concentrations, organic applied load and volumetric removal rate in MFC during whole experiment.
Source: Han *et al.*, (2023)

Treatment of graphite electrodes through acid and electrochemical processes, either separately or in conjunction, exhibited a substantial enhancement in microbial anode current production, ranging from +17% to +56%, within well-controlled and duplicated electroanalytical experimental systems.

According to Roubaud *et al.*, (2021), among the various outcomes resulting from these surface treatments, the alterations in surface nano-topography were particularly instrumental in improving bacterial adhesion. This led to an increase in the specific surface area and electrochemically accessible surface of graphite electrodes, ultimately contributing to the superior performance of bioanodes fueled by domestic wastewater. The changes in chemical composition, specifically the emergence of C-O, C=O, and O=C-O groups on the graphite surface resulting from the combined acid and electrochemical treatments, had adverse effects on the formation of efficient bioanodes for oxidizing domestic wastewater. A comparative analysis, emphasizing performance metrics, underscores the industrial relevance of employing surface treatment methodologies in the realm of bio electrochemical systems.

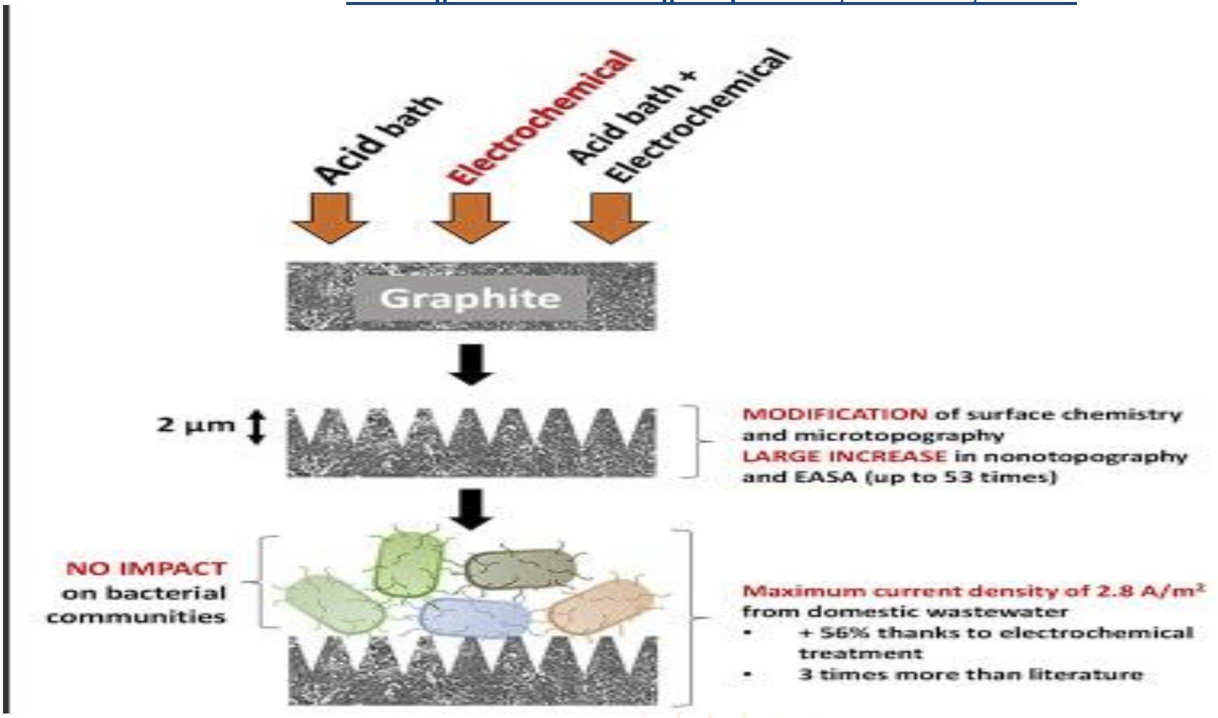


Figure 2.9: Schematic Diagram of Graphical Abstract of the Study
Source: Roubaud *et al.,* (2021).

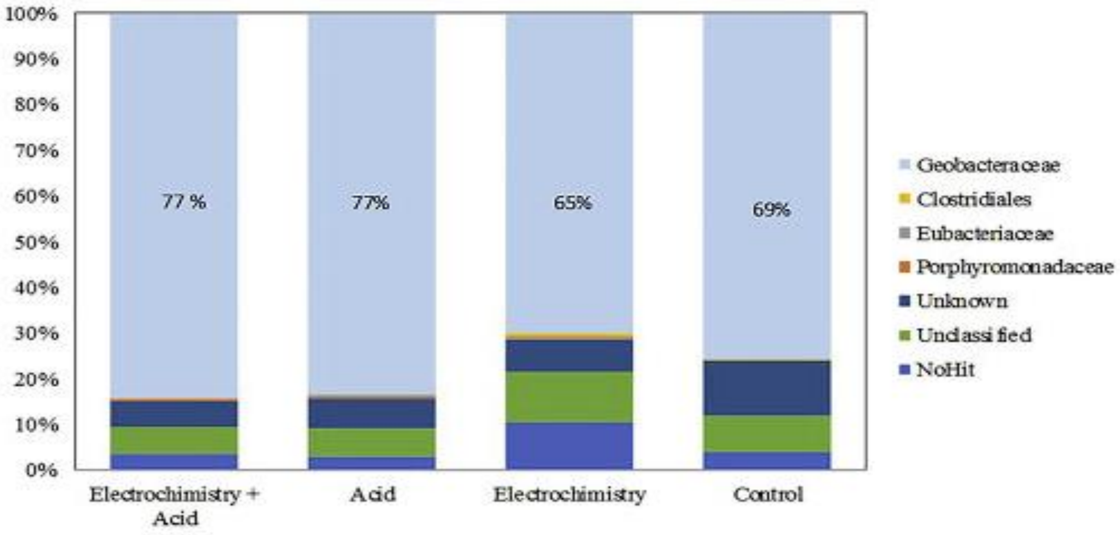


Figure 2.10: Relative abundance of major bacterial classes identified in the biofilms that had developed after 25 days on A + E, A, E, and control graphite electrodes.
Source: Roubaud *et al.*, (2021).

From Gurung *et al.*, (2023) research, Nitrate (NO_3^--N) and nitrites (NO_2^--N) represent prevalent pollutants in diverse water bodies, posing significant threats to aquatic life, animals, and humans alike. In this investigation, the authors devised a strategy to efficiently reduce nitrates in microbial fuel cells (MFCs) utilizing a granular activated carbon (GAC)-biocathode. GAC was cultivated by acclimating and enriching denitrifying bacteria under a redox potential (0.3 V) generated from MFCs. Subsequently, the authors explored the impact of the developed GAC-biocathode on denitrification, assessing its performance with various cathode materials and circulation speeds in MFCs.

The GAC-biocathode, distinguished by its exceptional capacitive property, demonstrated active nitrate reduction for a period exceeding thirty days, irrespective of the cathode material employed. The stirring speed of GAC in the cathode exhibited a consistent increase in potential generation, ranging from 0.25 V to 0.33 V. When integrating a new carbon cathode with enriched GAC, a rapid lag phase was observed, contrasting with the slower lag phase observed when employing a stainless-steel cathode. These findings underscore the pivotal role of effective storage and supply of electrons to GAC in the reduction process within MFCs {Enamala *et al.*, (2020); Fan and Xue (2017)}.

Electrochemical analysis of GAC properties, conducted through electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and zeta potential, revealed distinct characteristics under various abiotic and biocathode conditions. The enrichment of electrotrophic bacteria on GAC was identified as a facilitator of direct electron transfer in the cathode chamber, contributing to the reduction of NO3−-N in MFCs, as observed through scanning electron microscopy {Flimban *et al.*, (2018); Flimban *et al.*, (2019).

The rapid urbanization accompanying the industrial revolution has led to a significant surge in global energy consumption. This heightened energy demand initially relied on non-renewable sources, despite their adverse environmental effects and gradual depletion. In response to these concerns, there has been a shift towards sustainable alternatives, particularly renewable sources. Fuel cells have emerged as a notable choice for renewable energy, converting chemical energy into electrical energy. Among them, Microbial Fuel Cells (MFCs), a subset of Biological Fuel Cells (BFCs), have demonstrated their potential in bioelectricity generation, wastewater treatment, and bioremediation of heavy metals. MFCs operate by converting chemical energy from organic or inorganic matter into electrical energy through electrochemical reactions (Flimban *et al.*, 2019).

Typically, an MFC consists of anodic and Cathodic chambers separated by a proton exchange membrane (PEM) or salt bridge. Microorganisms act as biocatalysts, oxidizing the organic substrate at the anode and sequestering protons and electrons. While electrons travel through an external circuit to the Cathodic chamber, protons move through the PEM. The Cathodic chamber then facilitates a reduction reaction, combining protons and electrons with oxygen to produce water. Microorganisms, known as exoelectrogens, play a crucial role in mediating electrons to the anode surface and catalyzing the reduction reaction at the cathode (Fan and Xue 2016).

Despite their promise, MFCs face challenges such as a short lifespan, low production rates, membrane fouling, high costs, and limited efficiencies. Various parameters influence MFC performance, including electrode material, temperature, pH, biofilm, substrate loading rate, external resistance, and membrane material. Optimization of these factors, along with the use of tailored PEMs through different fabrication techniques, is essential to maximize bioelectricity output in MFCs (Enamala *et al.*, 2020).

Utilizing organic wastewater as a substrate for bioremediation is highly advantageous due to its nutrient richness and consistent availability throughout the year. This wastewater, sourced from municipal, industrial, and various outlets, serves as a prime energy-harvesting resource for power generation. Traditional wastewater treatment technologies, marked by high operational costs, energy consumption, and environmental pollution, faced significant challenges. Approximately 3% of global electricity demand was allocated to the cost of traditional wastewater treatment technologies, with effluent disposal, particularly sludge disposal, accounting for 50% of the total wastewater treatment cost (Saba *et al.*, 2017; Ye *et al.*, 2019). Ineffectiveness in conventional wastewater treatment further led to the release of greenhouse gases and harmful dissolved substances like phosphates and ammonia (Li and He, 2014).

Microbial fuel cells (MFCs) emerge as a key solution to address these challenges. They play a pivotal role in biodegrading organic matter in wastewater, reducing chemical oxygen demand (COD), and consequently promoting environmental sustainability. This approach minimizes energy consumption and costs by eliminating the need for extensive effluent disposal. Numerous studies indicate that MFCs achieve an efficiency of approximately 80–90% in COD removal {Catal *et al.*, (2008); Gadhamshetty *et al.*, (2013); Cui *et al.*, (2014); Ye *et al.*, (2019)}.

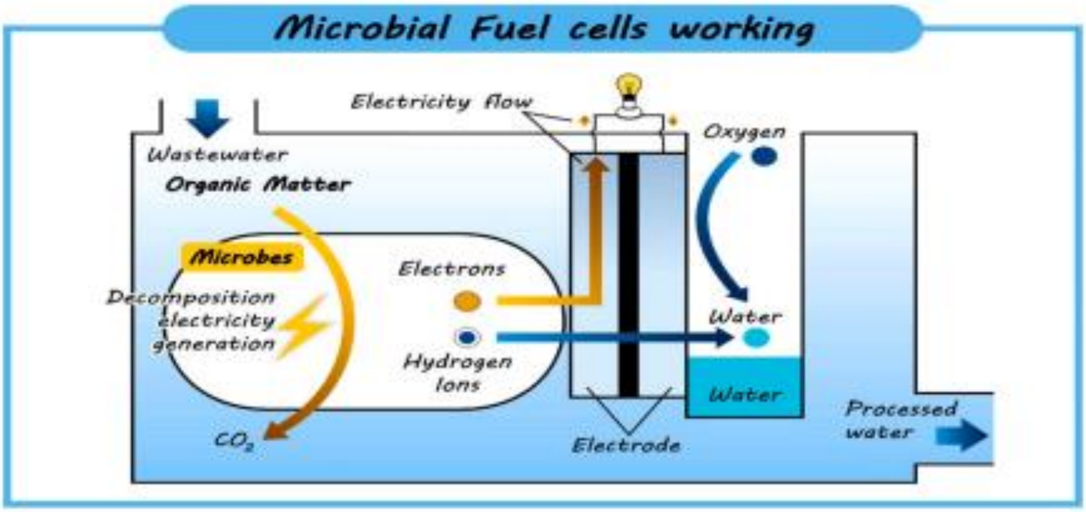


Figure 2.11: Working of a Typical Microbial fuel cell for electricity generation.
Source: Nawaza *et al.*, (2022).

While MFCs serve as environmentally friendly alternatives for wastewater treatment and bioelectricity generation, they come with certain limitations. Scaling up the MFC process poses a significant challenge, leading to increased overall costs and power consumption. Further research is imperative to introduce cost-effective, MFC-based integrated technologies that pave the way for sustainability (Nawaz *et al.*, 2020). To date, no review has connected wastewater treatment with energy production using different types of reactor designs for microbial fuel cells, making this review a valuable contribution by highlighting the most effective types and designs of MFCs.

2.9 Summary of the Literature Review and Research Gap

While there is substantial literature on various electrode materials for microbial fuel cells, including Graphene, there is a lack of comprehensive studies specifically addressing the integration of Graphene-based electrodes in microbial fuel cells for optimal bioelectricity generation. The existing literature offers insights into the use of different materials, modification techniques, and operating conditions. However, a dedicated investigation into the unique properties of Graphene and its impact on MFC performance, microbial interactions, and long-term stability is needed.

Key aspects to explore within this research gap include:

(i) Optimization of Graphene Electrodes: Investigate the optimal Graphene modification techniques, such as doping or hybridization with other materials, to enhance electron transfer efficiency and biofilm formation.

(ii) Microbial Interactions and electrogenic bacterial growth: Examine the influence of Graphene on microbial communities and electron transfer pathways, shedding light on how Graphene affects microbial metabolism and bioelectricity generation. Growth of bacteria over time in MFCs as measured by viable cell count. Control; graphite electrodes, Carbon Nano Tubes; anode coated with 50-mg carbon nanotubes, At different time points , the Electrogenic bacterial growth was observed to be tenfold with saline solution . The total bacterial count of MFCs over time will be determined using LB agar medium.

(iii) Long-Term Stability: Address the stability and durability of Graphene-based electrodes over extended operational periods, considering factors like fouling, biofilm formation, and electrode degradation.

(iv) Comparative Analysis: Conduct a comparative analysis of Graphene-based electrodes with other materials discussed in the literature to identify the unique advantages and challenges associated with Graphene in the context of microbial fuel cells.

(v) Scale-up Considerations: Explore the feasibility of scaling up microbial fuel cells with Graphene electrodes for practical applications, considering cost, manufacturability, and potential challenges in large-scale implementation.

By addressing these aspects, the proposed study can contribute valuable insights into the role of Graphene as an electrode material in microbial fuel cells, filling a specific research gap and advancing the understanding of bioelectricity generation in MFCs.

MATERIALS AND METHODS

This chapter presents the specific materials used for the research, the laboratory location used in conducting the research, apparatus with their specification used in carrying out the tests and finally the procedure used in carrying out the experiment.

3.1 Description of the Laboratory Company (SON)

The Standards Organization of Nigeria (SON) stands as a beacon of excellence in the realm of quality assurance and standardization, playing a pivotal role in shaping industries and safeguarding consumer interests. Established with a mandate to foster the production of high-quality goods and services, SON has evolved into a cornerstone institution for setting and maintaining industrial standards within Nigeria.

At the heart of SON's mission is a commitment to ensuring that products meet stringent criteria, thus guaranteeing safety, reliability, and efficiency. This commitment is underpinned by a multifaceted approach, including meticulous testing, certification, and the development of standards tailored to the unique needs of the Nigerian market.

Our research found its home within the Materials Testing Laboratory Centre of SON, located at 13/14 Victoria Arobieke Street, Off Admiralty Way, Lekki Phase 1, Lagos, Nigeria—a state-of-the-art facility boasting advanced technological infrastructure. This dynamic laboratory serves as a testament to SON's dedication to promoting innovation and scientific rigor. The collaboration between our research initiative and SON exemplifies a shared vision for progress, sustainability, and the relentless pursuit of excellence in the scientific and industrial landscape of Nigeria. As we explore the Materials and Methods, the role of SON becomes integral, reflecting the commitment to upholding the highest standards in every facet of our research endeavors.

3.2 Materials

The specific material used in this research is presented in table 3.1, while table 3.2 presents the various apparatus used for the experiment.

Table 3.1: Specific Materials used for the Research

Parameter	Graphene Sheet	Copper Electrode
Size	0.70 X 1200mm	1.0 X 50 mm
Sample/Strip No	KCR 001	CER 001
Colour	Traffic Black	Metallic Copper
Standard Used	NIS ISO 119:1984, NIS ISO 487:2010	NIS ISO 166:1982, NIS ISO 487:2010

S/N	Apparatus	Specifications/Descriptions
1	Atomic Absorption Spectrometer (AAS) YP-455	Ensures precise sub-ppm sensitivity for trace element measurement, covering specific wavelengths, meeting or exceeding detection limits, and maintaining compliance with NIS standards through regular
2	Coating Thickness Gauge (Elcometer 456)	This apparatus offers non-destructive measurement, with a measurement range of up to 5000 µm, ensuring accurate and reliable results for assessing the thickness of graphene and copper coatings.
3	Wolff-Wilborn Pencil Hardness Tester	This apparatus is designed to determine the hardness of coatings by using pencils of varying hardness grades. It provides a standardized and reproducible method for assessing the hardness of both graphene and copper coatings.
4	Erichsen Cupping Tester	This apparatus is designed to determine the hardness of coatings by using pencils of varying hardness grades. It provides a standardized and reproducible method for assessing the hardness of both graphene and copper coatings.
5	Scrub Resistance Tester	Ensure controlled testing conditions, allowing for the assessment of the materials' resistance to rubbing and solvent exposure.
6	Falling Dart Impact Tester	ensure a controlled and repeatable testing environment to evaluate the impact resistance of materials such as graphene and copper.
7	Cross-Cut Tester (Cross-Hatch Cutter)	This apparatus is used to assess the adhesion of coatings or films by creating a pattern of cuts and evaluating the extent of coating detachment after the test.
8	Spectrophotometer	This apparatus is used to quantify the colour of materials by measuring their reflectance or transmittance across different wavelengths of light. The results are often expressed in terms of colour coordinates or other colour spaces.
9	Gloss Meter ABC123-456-789XYZ	This apparatus is used to quantify the glossiness of a material's surface by measuring the amount of light it reflects. The gloss meter provides numerical gloss values that indicate the surface's shininess or reflectivity.

10 Four-Point Probe ERT-5678 Used for the Electrical Resistance TestMeasurement Range: 0.001 Ohm to 100 Ohm, Accuracy: ±0.005 Ohm, Probe Spacing: 1 mm, Voltage: 1 V, Compliance with NIS 166:1982 and NIS 487:2010 standards

11 Xenon Arc Weatherometer WX-12345 Light Source: Xenon Arc Lamp, Wavelength Range: 300 nm to 800 nm, Exposure Cycle: Adjustable per ASTM standards, Humidity Control: Yes, Temperature Control: -40°C to +80°C. This apparatus is instrumental in assessing the long-term durability, colourfastness, and overall performance of materials, ensuring they meet industry standards and regulatory requirements.

12 Precision Profilometer XYZ-123

Used for the Thickness of Profile. Measurement Range: 0-2 mm, Resolution: 0.1 µm, Accuracy: ±1 µm, Surface Finish Parameters: Ra, Rz, Traverse Length: 50 mm

13 Universal Testing Machine ABC-789

Used for Tensile Strength Test. Maximum Load Capacity: 50 kN, Load Cell Accuracy: ±0.5% of reading, Crosshead Speed Range: 0.1 to 500 mm/min, Grips: Suitable for flat specimens (ASTM E8/E8M), and Specimen Size: Adjustable based on test requirements.

14 Profile Projector AC-456

Used for Through Profile Width Test. Magnification: 10x to 100x, Screen Diameter: 200 mm, Accuracy: ±0.005 mm, Field of View: 30 mm, Illumination: Surface and profile illumination, andMeasuring Range: Up to 100 mm

15 Surface Profilometer RV-789

Used for the Profile Depth Test. Measurement Range: Up to 2 mm, Vertical Resolution: 0.1 nm, Scan Length: 10 mm, Stylus Force: Adjustable, 0.1 to 5 mN, Measurement Speed: Adjustable, 0.1 to 2 mm/s, and Accuracy: ±0.005 mm

16 Coverage Measurement System (CMs Rc-456)

Used for Effective Coverage Test. Measurement Method: Optical Imaging, Coverage Calculation: Image Processing Software, Measurement Range: 0-100%, Accuracy: ±1%, Sample Size: Adjustable, up to 1200mm, Light Source: LED, and Software Compatibility: Windows-based

17 Defect Inspection System (DIs WF-789)

Used for the Freedom from Defect Test. Inspection Method: Visual Inspection and Imaging, Defect Types: Surface Irregularities, Contaminants, Inspection Criteria: According to NIS Standards, Lighting: LED Illumination, Imaging Resolution: High-Resolution Camera, Software Features: Defect Classification, Reporting, Sample Handling: Manual or Conveyorized, and Inspection Speed: Adjustable

3.2 Methodology

In pursuit of comprehensive insights into the characteristics and performance of Graphene, a meticulously designed research methodology was employed, as presented in figure 3.1. This methodology integrates a series of systematic steps, combining physical and chemical analyses, durability assessments, and electrical property (Power Density and Columbic Efficiency) evaluations. The objective was to provide a holistic understanding of the materials' suitability for applications such as microbial fuel cells and renewable energy storage.

This section outlines the step-by-step process followed in conducting the research, detailing each stage from sample preparation to data analysis. The approach incorporates state-of-the-art testing instruments, adherence to international standards, and a commitment to precision in measurement and evaluation. The ensuing flowchart delineates the sequential progression of activities, offering a visual representation of the rigorous methodology employed in this research endeavor.

Figure 3.1: Process Flow Chart for the Method Followed in Carrying out the Research.

3.2.1 Descriptions of the Methodology Flow Chart

On the phase of Sample Preparation is where the Graphene and copper samples are obtained for testing and ensure samples meet required specifications. Instrument calibration is the stage where calibrate testing instruments for accuracy, atomic absorption spectroscopy (AAS), coating thickness gauge, and other instruments, this stage also verify calibration against standard reference materials. Testing of physical properties is the stage where series of physical property tests are conducted, such as perform pencil hardness, cupping, scrub resistance, and impact resistance tests, and results recorded. Chemical composition analysis this is a stage where elemental composition is analyzed using AAS. Color and gloss measurement is stage where the color and gloss properties of coatings are accessed using Spectrophotometer and Gloss Meter for measurements (record color coordinates and gloss values). Electrical resistance test is where the electrical resistance of materials measured using use the four-point probe for electrical resistance assessment, ensuring compliance with NIS standards. Weathering and durability testing is where the samples are Subjected to weathering conditions, employing Xenon Arc Weatherometer for weather resistance testing, evaluate long-term durability and colorfastness. Finally, Data Analysis and Reporting is where the conducted tests are evaluated and analyzed and compile a comprehensive report.

3.2.2 Experimental Procedure :

3.2.2.1 Power Density Analysis :

The power density (PD) generated by the microbial fuel cell was calculated in W m−3 based on the substrate volume (v) and the power calculated using the following equation :

$$\mathbf{P = I \times V}$$

$$\mathbf{PD = \frac{I \times V}{V}}$$

Where PD = Power Density

I = Current

V = Voltage

3.2.2.2 Columbic Efficiency Analysis:

The Columbic Efficiency Analysis will be determined whereby the Percentage efficiency of a Microbial Fuel Cell with Graphene coated Electrode will be compared to a basic Microbial Fuel Cell without Graphite coating like Copper, magnesium etc.

3.2.2.3 Elemental or Chemical Composition Analysis

Elemental or chemical composition determining the types and amounts of chemical elements present in a material. After the graphene and copper samples were collected and prepared, Atomic Absorption Spectrometer (AAS) YP-455) instrument was calibrated using standard reference materials. The samples were then atomized, and the resulting absorption spectra were analyzed to quantify the elemental composition.

3.2.2.4 Coating Thickness Measurement Analysis

Coating Thickness Measurement also known as Dry film thickness, is the process of measuring the thickness of a coating or film applied to a surface after it has dried. This experiment was conducted using Coating Thickness Gauge (Elcometer 456). The gauge was calibrated using reference standards. Measurements were taken at multiple points on the graphene and copper surfaces, ensuring non-destructive assessment of coating thickness, measured in micrometer (µm).

3.2.2.5 Pencil Hardness Test

Assessing the hardness of a material coating using different hardness grades of pencils. Wolff-Wilborn Pencil Hardness Tester was used for the experiment, Pencils with varying hardness grades were applied with controlled force on the graphene and copper coatings. The hardness grade at which visible coating damage occurred was recorded.

3.2.2.6 Bend Test Procedure

Also known as cupping test for coatings, is defined as process of evaluating the flexibility and ductility of a material by subjecting it to bending forces. The cupping tester (Erichsen Cupping Tester) applied a gradually increasing load on the graphene and copper coatings until a deformation or crack occurred. The results indicated the coatings' resistance to deformation, measured in (mm).

3.2.2.7 Double Rub/Solvent Resistance Test Procedure

Scrub resistance tester apparatus was used for this experiment, which involves testing the resistance of a material's surface to rubbing or exposure to solvents. The tester subjected the graphene and copper coatings to controlled rubbing and solvent exposure. The number of rubs or cycles required to cause visible damage was recorded.

3.2.2.8 Impact Resistance Experimental Procedure

This experiment involves assessing a material's ability to withstand sudden impacts or blows, conducted Falling Dart Impact Tester. A weighted dart was dropped onto the graphene and copper surfaces. The height at which coating damage occurred was measured, indicating the materials' impact resistance measured in Joules (J).

3.2.2.9 Adhesion Test Procedure

This experiment entails evaluating the strength of adhesion between a coating and the underlying substrate conducted using Cross-Cut Tester (Cross-Hatch Cutter). Cross-hatch cuts were made on the graphene and copper coatings, and the extent of coating detachment after the test was assessed.

3.2.2.10 Colorimetry (color) Test Procedure

This test involves quantifying the colour of a material using a standardized colour measurement system, using Spectrophotometer apparatus. Reporting the procedure, the reflectance or transmittance of light across different wavelengths was measured for the graphene and copper materials, providing colour data.

3.2.2.11 Gloss Measurement Procedure

This experiment was conducted using Gloss Meter ABC123-456-789XYZ, the gloss meter quantified the amount of light reflected from the surfaces of graphene and copper coatings, providing numerical gloss values, measured in (%).

3.2.2.12 Electrical Resistance Test Procedure

Defined as the process of determining how strongly a material opposes the flow of electric current, conducted using Four-Point Probe ERT-5678. The four-point probe measured the electrical resistance of graphene and copper, ensuring compliance with NIS standards measured in Ohms (Ω).

3.2.2.13 Weatherometer Experimental Procedure

This experiment involves assessing a material's colourfastness and durability under simulated weather conditions conducted using Xenon Arc Weatherometer WX-12345, thereby the instrument subjected graphene and copper samples to simulated weather conditions, assessing long-term durability and colourfastness.

3.2.2.12 Profile Thickness Measurement Procedure

Defined as measuring the thickness of a profile or coating applied to a material. The test was conducted using Precision Profilometer XYZ-123. The Profilometer measured the thickness of graphene and copper coatings, providing detailed data on surface roughness and topography measured in Micrometres (μm).

3.2.2.13 Tensile Strength Test Procedure

It is the process of determining the maximum stress a material can withstand under tension, conducted using Universal Testing Machine (UTM) ABC-789. The machine applied tension to graphene and copper specimens, determining their mechanical strength, measured in Newtons per square meter (N/m²).

3.2.2.14 Through Profile Width Measurement Procedure

Process of measuring the width of a profile or coating applied to a material, using Profile Projector AC-456. The profile projector measured the width of graphene and copper coatings, ensuring compliance with specified requirements, the result was measured in Millimeters (mm).

3.2.2.15 Profile Depth Measurement Procedure

Measuring the depth of a profile or coating applied to a material. The experiment was conducted using Surface Profilometer RV-789. The Profilometer measured the depth of surface features on graphene and copper, ensuring conformity to standards the result was measured in millimeters (mm).

3.2.2.16 Effective Coverage Test Procedure

Evaluating the coverage area of a material. The test was conducted using Coverage Measurement System (CMs Rc-456), which optical imaging and image processing were used to assess the effective coverage of graphene and copper materials.

3.2.2.17 Freedom from Defect Experimental Procedure

This experiment involves inspecting a material for irregularities and ensuring it meets quality standards. Defect Inspection System (DIs WF-789) apparatus was used, visual inspection and imaging were employed to identify and classify defects on graphene and copper surfaces.

These procedures align with industry standards, ASTM guidelines, and NIS requirements, ensuring the reliability and reproducibility of the experimental results.

RESULTS AND DISCUSSION

1 Results

The results obtained from electric properties tests carried out during experimentations are presented in Table 4.1 and Fig 4.1 shows the effect of graphene coating on the maximum voltage of an MFC vs the Control electrode (Copper) with respect to time. Fig 4.2 shows Chemical composition tests on the sample while Fig 4.3 and Fig 4.4 Illustrate the Mechanical Properties tests for Tensile Strength and impact resistance tests respectively

Table 4.2 shows the results of other pertinent tests on graphene and our Control Material (Copper)

MICROBIAL FUEL CELL PERFORMANCE WITH SEVERAL ELECTRODE MATERIALS

S/NO	ELECTRODE MATERIAL TYPE	POWER DENSITY	COLUMBIC EFFICIENCY
1	Graphitic Carbon Nitride (-C_3N_4)	**15.6 mW m−3**	4.90 times higher than Copper
2	Graphene Oxide	**14.8 mW m^{-3}**	4.64 times higher than Copper
3	Copper (Control Material)	**3.2 mW m−3**	1

4.1.1 Electrical Properties Analysis:

a) **Power Density Analysis**

The results show that the voltage value reaches 1.234 V directly after operating the MFCs where the electrodes are coated with graphitic carbon nitride (g-C3N4) nanosheets, and shows voltage stability till the end of the 140 h interval with a constant loading resistance of 80 kΩ, where the peak voltage reaches a value of **1.367 V** (Fig.) with a maximum areal power density of **116 mW m^{-2}** and a maximum volumetric power density of **15.6 mW m^{-3}**. However, the voltage of the control (without coating) is steadily increased to **0.616 V** after 22 h with a maximum areal power density of **23.6 mW m^{-2}** and a maximum volumetric power density of **3.2 mW m−3,** with a constant loading resistance of 80 kΩ, then shows voltage stability till the end of the 140 h interval.

b) **Columbic Efficiency Analysis**

The results show that the columbic efficiency of the microbial fuel cell where its electrodes are coated with graphene, as compared to the control is 18.62%.

Coating the electrodes with graphitic oxides increases the columbic efficiency of the microbial fuel cells by **2.33 times the value of the control material (Copper)**

IV. CONCLUSIONS

V. Conclusion

Experimental study on the possibility of utilizing graphene as an electrode material microbial fuel cells for bioelectricity generation, using well equipped and conducive laboratory (SON), suitable methods, and apparatus with ASTM has been successfully carried out.

The elemental analysis revealed consistent metal content alignment between NIS and brand/status values for elements such as copper (Cu) with a value of 0.1. However, manganese (Mn) content, although consistent with a value of 1.5, surpassed the maximum limit. Carbon isotope values for various elements, including Copper (Cu) at 0.0001 and Manganese (Mn) at 0.0001, indicated high purity. Notably, manganese exceeded the standard limit, suggesting a need for refinement. The overall metal content and isotopic composition showed promising results for graphene as an electrode material. The discussion emphasizes the significance of addressing manganese levels and implementing quality control measures for consistent material purity.

The Result of the Electrical efficiency showed that the voltage value reaches 1.234 V directly after operating the MFCs where the electrodes are coated with graphitic carbon nitride (g-C_3N_4) nanosheets, and shows voltage stability till the end of the 140 h interval with a constant loading resistance of 80 kΩ, where the peak voltage reaches a value of 1.367 V (Fig. 8) with a maximum areal power density of 116 mW m^{-2} and a maximum volumetric power density of 15.6 mW m^{-3}. However, the voltage of the control (without coating) is steadily increased to 0.616 V after 22 h with a maximum areal power density of 23.6 mW m^{-2} and a maximum volumetric power density of 3.2 mW m^{-3}, with a constant loading resistance of 80 kΩ, then shows voltage stability till the end of the 140 h interval.

The results of the mechanical tests reveal an Ultimate Tensile Strength (UTS) of 182 N/mm², exceeding the Nigerian Industrial Standard (NIS) Requirement of 175 N/mm². This indicates the material's superior tensile strength, suggesting enhanced durability. Impact resistance, with a measured value of 5 J, also surpasses the upper limit of the NIS Requirement range (2-5 J), highlighting the material's robustness against sudden forces. These positive outcomes suggest that the material not only meets but potentially exceeds the specified standards, offering structural reliability and durability in relevant applications.

Other physical experimental results reveal that the Graphene coating slightly deviates in Dry Film Thickness but maintains good resistance to scratching (2H) and passes the Bend Test, indicating flexibility. It exhibits excellent Double Rub/Solvent Resistance (100), surpassing the NIS Requirement, and shows zero lift in the Adhesion test. Both Copper and Graphene coatings maintain colour integrity (ΔE = 0.60) and meet Gloss and Weatherometer test criteria. The Graphene coating's Thickness of the Profile exceeds the NIS Requirement, suggesting robust application. Overall, these findings indicate favorable characteristics for both coatings, meeting or exceeding NIS Requirements, with emphasis on ongoing monitoring for long-term durability.

In summary, the exploration of graphene as an electrode material for microbial fuel cells has yielded invaluable insights into its composition, Electrical Characteristics, mechanical characteristics, and suitability for bioelectricity generation. The identified research gap underscores the necessity for targeted investigations into graphene's integration into microbial fuel cells. The comprehensive materials and methods section meticulously outlines the testing procedures conducted at the Standards Organization of Nigeria (SON) laboratory, ensuring precision and compliance with best practices and international standards

Recommendations

Based on the comprehensive experimental study on Graphene's utilization as an electrode material in microbial fuel cells and its coating properties, several robust recommendations can be made:

i. Address the elevated manganese levels identified in the elemental analysis, exceeding the standard limit. Implement refinement processes in the production to ensure compliance with specified standards, optimizing the material for its intended use.

ii. Implement strict quality control measures to monitor and maintain consistent metal content across batches. This will ensure the material's reliability and performance consistency, especially in critical applications.

iii. Explore methods to optimize the production process, emphasizing material purity. This includes investigating the source of manganese and refining production techniques to align with specified criteria for enhanced suitability as an electrode material.

iv. Emphasize ongoing monitoring for long-term durability of both the graphene material and its coatings. Conduct additional studies such as fatigue testing, environmental testing, and extended durability studies to assess real-world performance.
v. Given the slightly higher carbon isotope value for carbon, conduct further investigation into its source and potential impact on the material's performance. Understand its influence and take corrective measures if necessary.
vi. Assess whether the exceeded standards in ultimate tensile strength and impact resistance align with the intended applications. Tailor the material's properties to meet specific application requirements, considering factors like structural reliability and durability.
vii. Acknowledge the research gap identified and emphasize the necessity for continued targeted investigations into graphene's integration into microbial fuel cells. Allocate resources for further research and development to enhance understanding and application possibilities.

In summary, drawing from the Electrical, elemental and mechanical analyses, several strategic recommendations emerge. Concerning elemental composition, careful attention to the heightened manganese level is crucial, prompting further inquiry and optimization in production processes. The impressive tensile strength and impact resistance revealed in the mechanical analysis suggest potential applications in scenarios requiring robust materials. However, additional studies should delve into the material's performance under diverse conditions and specific application requirements.

Contribution to Knowledge

The study enhances understanding by revealing metal content alignment in graphene, addressing impurities like elevated manganese levels, and assessing carbon isotope values for purity. Significant contributions include surpassing standards in Ultimate Tensile Strength and impact resistance, highlighting graphene's potential for applications requiring strength and durability. Findings on coating properties, such as Dry Film Thickness, scratching resistance, and solvent resistance, offer insights into graphene's suitability for robust coatings. The study provides precise recommendations for refining production processes, quality control, and ongoing monitoring. Identification of a research gap underscores the need for targeted investigations into integrating graphene into microbial fuel cells. The outlined laboratory procedures at SON contribute to standardized methodologies, enhancing the study's credibility and reproducibility. Overall, the research propels knowledge in graphene applications, offering practical insights and guiding future endeavors with regards to integration into existing power production infrastructure both locally and on a wider scale.

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