SYNTHESIS AND CHARACTERIZATIONS OF BIOMASS-BASED GRAPHENE METAL OXIDE ANODES IN MICROBIAL FUEL CELLS (MFCs) APPLICATIONS

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by

ASIM ALI YAQOOB

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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 PhD 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 75 %) was already published in various journals by us (Asim Ali Yaqoob and main supervisor Associate Prof. Dr. Mohamad Nasir Mohamad Ibrahim). The list of publications was given at the end of the thesis.

Best regards,

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TABLE OF CONTENTS

LIST OF PUBLICATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBERIVATIONS

3D	Three dimensional
A	Cross-sectional area
AAS	Atomic adsorption spectroscopy
AFM	Atomic force microscopy
Ag	Silver
BET	Brunauer-Emmett-Teller
CCB	Centre for Chemical Biology
CD	Current density
CB	Carbon brushes
CNTs	Carbon nanotubes
COD	Chemical oxygen demand
C_p	Specific capacitance
CV	Cyclic voltammetry
DI	Deionized
DMFCs	Double-chamber microbial fuel cells
DNA	Deoxyribonucleic acid
DSSC	Dye-sensitized solar cells
EAS	Electrochemically active surface area
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy Dispersive X-Ray
EET	Extracellular electron transfer
EFB	Empty fruit bunches
EIS	Electrochemical impedance spectroscopy
ETR	Electron transfer rate
FTIR	Fourier Transform Infrared

GAC	Granular activated carbon
GO	Graphene oxide
GO/ TiO_2	Graphene oxide/Titanium oxide
GO/ZnO	Graphene oxide/Zinc oxide
Gr`	Graphene
LCC	Layered corrugated carbon
MFCs	Microbial fuel cells
NH_3	Ammonia
nm	Nanometre
NPs	Nanoparticles
OPEFB	Oil palm empty fruit bunch
PANI	Polyaniline
PCR	Polymerase chain reaction
PD	Power density
PEDOT	Poly (3,4-ethylenedioxythiophene)
PEMs	Proton-exchange membranes
Ppy	Polypyrrole
PSF	Polysulfone
r	internal resistance
R	External resistance
R_{ct}	Charge transfer resistance
RE %	Remediation efficiency
rGO	Reduced graphene oxide
R_s	Electrolyte resistance
SEM	Scanning electron microscope
SERC	Science and Engineering Research Centre
SS	Stainless steel

TEM	Transmission electron microscope
TGA	Thermal gravimetric analysis
TiO_2	Titanium oxide
UV	UV-Visible
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
ZnO	Zinc oxide
Z_w	Warburg element
Ω	Resistance

LIST OF SYMBOLS

%	Percentage
$^{\circ}C$	Celsius
µL	Microlitre
A/m^2	Ampere per square metre
Cd	Cadmium
cm	Centimetre
cm^{-1}	Wavenumber
cm^{-1}	Reciprocal centimetres
Co	Cobalt
CO_2	Carbon dioxide
Cr	Chromium
e^-	Electron
Emf	Electromotive force
F/g	Specific capacitance
g	Garam
g/L	Garam per litre
H^+	Proton
Hg	Mercury
I	Current
kHz	kilohertz
m^2/g	Meter square per gram
mA	Milliampere
mA/m^2	Milliampere per square metre
mg	Milligram
mg/L	Milligrams per litre

mHz	Millihertz
Minute, hr	Time
ml	Millilitre
mm	Millimetre
mV	Millivolt
mW/cm^2	Milliwatts per square centimetre
mW/m^2	Milliwatt per square metre
Ni	Nickel
nm	Wavelength
Pb	Lead
ppm	Parts per million
psi	Pound per square inch
rpm	Revolutions per minute
S/m	Siemens per meter
W/m^2	Watt per square metre
W/m^2	Watt per cubic metre
μg/L	Micrograms per litre
μS/cm	Microsiemens Per Centimetre
$\mu W/cm^2$	microwatt-seconds per square centimetre

SINTESIS DAN PENCIRIAN ANOD OKSIDA LOGAM GRAFENA BERASASKAN BIOJISIM DALAM APLIKASI SEL BAHAN BAKAR MIKROB (MFCs)

ABSTRAK

Sel bahan bakar mikrob (MFCs) merupakan antara pendekatan bioelektrokimia yang paling menjanjikan untuk menjana tenaga di samping menyingkirkan bahan pencemar daripada air buangan, namun prestasinya yang agak lemah, telah mengehadkan usaha pengkomersialannya. Pembuatan bahan elektrod canggih bagi MFC adalah topik yang paling berpotensi pada masa ini untuk mengatasi masalah kadar pemindahan elektron yang rendah dalam operasi MFC. Dalam kajian ini, nanopartikel logam oksida (ZnO dan TiO_2) yang disintesis secara hijau telah diperkenalkan kepada oksida grafena (GO) yang berasal daripada lignoselulosa untuk dijadikan anod nanokomposit. Anod nanokomposit GO/logam oksida memberi pemindahan tenaga dan penyingkiran ion logam yang cekap. Beberapa logam toksik seperti plumbum, kadmium, kromium, nikel, kobalt, dan merkuri dijadikan sasaran dalam kajian ini. Kajian menyeluruh dilakukan terhadap penyingkiran logam tunggal yang disasarkan menggunakan MFCs serta logam dalam bentuk pukal di dalam sampel air kolam yang tercemar. Elektrod anod daripada grafit komersial berfungsi sebagai elektrod kawalan terhadap rekaan terbaharu anod yang berasal daripada biojisim (GO, nanokomposit GO/ZnO dan nanokomposit GO/TiO_2). Dalam suatu siri eksperimen, setiap anod telah memberikan kecekapan tenaga dan kecekapan penyingkiran logam yang berbeza. Hal ini menunjukkan bahawa sifat sesuatu logam mempengaruhi keseluruhan prestasi MFCs. Penyingkiran ion merkuri didapati sehingga 80% bagi anod nanokomposit GO/ZnO. Seterusnya, dapatan hasil kajian menunjukkan bahawa elektrod komersial memberikan nilai kepadatan arus (CD) sebanyak 17.543 mA/m^2 dan nilai ketumpatan kuasa (PD) sebanyak 0.0588 mW/m^2 bagi kes ion logam berbentuk pukal di dalam sampel air kolam yang tercemar. Sementara itu, GO berasaskan biojisim menunjukkan nilai CD dan PD yang masing-masing 1.7 dan 3.3 kali lebih tinggi berbanding dengan grafit komersial. GO/ZnO menunjukkan nilai CD 6 kali lebih tinggi (105.263 mA / m^2) dan nilai PD 34.3 kali lebih tinggi (2.021 mW/m^2) daripada anod grafit komersial. Begitu juga dengan anod GO/TiO_2 yang memberikan nilai CD sebanyak 3.9 kali lebih tinggi (68.421 mA/m^2) dan PD 17.17 kali lebih tinggi (1.01 mW/m^2) daripada anod grafit komersial. Di samping itu, kecekapan penyingkiran semua logam didapati lebih tinggi pada anod nanokomposit GO/ZnO berbanding dengan yang lain. Susunan mengikut kecekapan penyingkiran logam adalah nanokomposit GO/ZnO> nanokomposit GO/TiO_2> GO> grafit komersial. Kajian ini telah membuktikan bahawa penambahan nanopartikel oksida logam yang disintesis secara hijau (ZnO dan TiO_2) dapat meningkatkan kekonduksian sesuatu elektrod. Beberapa kajian elektrokimia, pencirian bahan dan analisis biologi telah dijalankan untuk membuktikan kecekapan anod yang terubahsuai daripada biojisim dalam operasi MFCs.

SYNTHESIS AND CHARACTERIZATIONS OF BIOMASS-BASED GRAPHENE METAL OXIDE ANODES IN MICROBIAL FUEL CELLS (MFCs) APPLICATIONS

ABSTRACT

Microbial fuel cells (MFCs) ranked among the most promising bioelectrochemical approaches for generating electrical energy while removing pollutants from wastewater, however, their relatively poor performance, has limited their commercial viability. The fabrication of advanced electrode material for MFCs is the most potential topic at present to address the issue of low electron transfer rates in the MFCs operation. In the present work, green synthesized metal oxides (ZnO and TiO_2) nanoparticles (NPs) were introduced to biomass-derived graphene oxide (GO) as nanocomposite anodes. The GO/metal oxide nanocomposite anodes provide efficient energy transfer and metal ions remediation. Several toxic metals such as lead, cadmium, chromium, nickel, cobalt, and mercury were targeted in the current study. Thorough investigations were conducted on targeted single metal remediation using MFCs as well as metals in bulk form in the polluted pond water samples. A commercial graphite anode electrode served as a control against the newly fabricated biomass-derived anodes (GO, GO/ZnO nanocomposite and GO/TiO_2 nanocomposite). In a series of experiments, each anode delivered different energy efficiency and metal remediation efficiency. It indicated that the nature of metal affects the overall performance of the MFCs. The mercury ions remediation was up to 80 % for the GO/ZnO nanocomposite anode. Further, the results indicated that the commercial electrode delivered 17.543 mA/m^2 CD (current density) with 0.0588 mW/m^2 PD (power density) in the presence of a bulk form of metal ions in the polluted pond water

sample. The biomass-derived GO showed 1.7 and 3.3-times higher CD and PD, respectively than the commercial graphite. The GO/ZnO nanocomposite anode showed 6-times higher CD (105.263 mA/m^2) and 34.3-times higher PD (2.021 mW/m^2) than the commercial graphite anode. Similarly, the GO/TiO_2 nanocomposite anode delivered 3.9-times higher CD (68.421 mA/m^2) and 17.17-times higher PD (1.01 mW/m^2) than the commercial graphite anode. The remediation efficiency of all metals was found the highest in the GO/ZnO nanocomposite anode as compared to others. The sequence of remediation efficiency order is GO/ZnO nanocomposite > GO/TiO_2 nanocomposite > GO > commercial graphite. The study proved that the addition of green synthesized metal oxide nanoparticles (ZnO and TiO_2) enhanced the conductivity of the electrodes. Several electrochemical studies, material characterizations, and biological analyses were carried out to prove the efficiency of the biomass-derived modified anodes in the MFCs operation.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Two of today's most alarming environmental issues are water pollution and energy crisis and they stand at the crux of wastewater treatment. Despite several types of biological, chemical and physical approaches to treat pollutants in wastewater resources, they all pose various drawbacks including high energy requirements as well as high operating costs, the huge consumption of chemicals and the generation of waste as by-products [1]. Among those said disadvantages, the high amount of energy needed to treat wastewater coincides with the worldwide energy crisis that we are facing now. As a result, much effort has gone into developing cost-effective and low-energy methods of removing toxic pollutants from wastewater [2]. Owing to their sustainable nature, microbial fuel cells (MFCs) have been recognized as a viable technology for producing energy and simultaneously remediating toxic pollutants from wastewater. Its emergence as a promising approach to convert chemical energy into electricity in the presence of biocatalysts and at the same time remediating toxic pollutants from water [3]. The electroactive bacteria species degraded the organic waste and the released electrons from the degradation process were passed from organic waste to electrode for energy generation. The MFCs have proton exchange membranes (PEMs). Along with the PEMs, MFCs consist of two electrodes—an anode and a cathode—where the former is responsible for providing sufficient space for bacterial growth and respiration to oxidize organic matter in the wastewater. Protons and electrons will then be generated out from the reduction and oxidation reactions taken place in the MFCs cells. Despite of that, MFCs have lacked commercial viability due to their low efficiency [4-6]. Several parameters directly affect the performance of MFCs such as their scale and design, the PEM's efficiency, cell resistance, organic substrate and the materials used as the electrodes.

Of those said parameters, the material of the anode is critically affecting the performances of MFCs. This is because anode facilitates microbial adhesion on its surface and therefore improve the transferability of electrons [7]. To date, several types of electrode materials have been investigated, including carbon (e.g., cloth, rod, sheet, foam, carbon nanotubes and sponges), graphite (e.g., rod, sheet, and cloth), metal-based electrodes and graphene-derived electrodes. In addition, several type of carbon nanomaterials such as carbon nanotubes (CNTs), carbon black and graphene derivatives were explored to improve the electrical conductivity of the anodes. Further, in literature there are several materials reported which were used to prepare the anodes such as natural biomass resource, conventional carbon-based, metal/metal oxides as well as conducting polymer composites [8-10]. A conventional carbon-based anode was commonly used as the anode but still fail to transport electrons effectively [11]. On the other hand, metal-based or polymeric composites showed good electric conductivity, but metal corrosion issue, toxicity and hazard of polymer shedding decreased the durability of the electrodes. Although all the above said materials have drawbacks (high cost, low conductivity and limited biocompatibility), graphene derivatives have demonstrated excellent properties, including large surface area, high electron mobility, robust mechanical strength, high thermal stability, and good chemical stability with reasonable electrical conductivity [12]. Moreover, by minimizing their limitations such as electrical conductivity and preparation cost, graphene-derived electrodes can be promising idea for commercial scale applications. Modifications on the waste-derived material are needed to provide effective a platform for bacterial growth and thus will improve the transportation of electrons from bacteria cell to anode. In this study, lignin (as a starting material to produce graphene oxide (GO)) was extracted from oil palm *(Elaeis quineensis)* empty fruit bunches (EFB), as palm oil industry is the main generator of biomass in Malaysia [13]. In addition, oil palm biomass was also used as an organic substrate since oil palm trunk sap is a perfect diet for bacterial species [14]. Considering all the above, composite-based electrodes may enhance the performance of anodes, improve the electrical conductivity of MFCs, and thus make their industrial-scale use viable.

Whereas metal-based materials also demonstrate properties beneficial to anodes, their corrodibility curtails the performances as anodes [15]. Metal oxide-based nanoparticles (NPs) such as cobalt oxide, iron oxide, zinc/cobalt oxides etc. were recently used as modifiers for anode electrodes in MFCs [16, 17]. As compared with metal oxide and carbon-based materials, pure NPs metal showed lower biocompatibility and corrosion resistance, which hampered the durability of the electrode [18]. Hence, it is essential to prepare a highly durable, stable, and low-cost anode for improving the electron transportation and bacterial biofilm formation on the surface of anode. Therefore, a graphene–metal oxide composite seemed to be a novel and bioinspired material suitable for these purposes. Added to the fact that graphene cost can be reduced by using waste material to prepare GO. Indeed, this approach has recently attracted considerable attention from various fields. Thus far, several waste materials have been used as electrodes, including compressed milling residue, loofah sponges, coconut shells, corn straw, mushrooms, silk cocoons, onion peels, cocklebur fruit, and even pinecones [19-20]. Additionally, to improve the conductivity of waste-derived GO, the metal oxides as a modifier is an ideal option. Among all metal oxides, zinc oxide (ZnO), and titanium oxide (TiO_2) are considered as promising materials due to their unique electrical, high electrocatalytic ability, semiconductor, and optical activities [17,21]. These metal oxides are synthesized by using the waste material through green synthesis method. The GO-green synthesized metal oxide composite improved the biocompatibility of the anode electrode. The green synthesis method promoted the compatibility toward the bacterial species. For example, Shakeel et al.[22] studied the green synthesis of ZnO on polyindole functionalized multi-walled CNTs and its usage as anode for biofuel cell. Furthermore, the authors highlighted the enhancement about the biocompatibility, chemical stability, high electron transportation and high surface area of the green synthesized ZnO based composite. Therefore, the utilization of ZnO can be helpful to increase the electrocatalytic activities and electron transportation rate. Furthermore, several other studies also reported that ZnO showed significant activities in generation of green energy through dye-sensitized solar cells (DSSC) as a photoanode [23-25]. DSSC is a technology which can be used to convert the solar energy into electric energy in which photoanode was considered as the most important part of the DSSC, due to its function to transfer the electrons [25]. Similarly, Kilibarda et al. [26] studied the introduction of TiO_2 particle as anode in DSSC to facilitate the charges and observed the good efficiency of electron transportation. The composite of GO with TiO_2 material as anode seems to be highly conductive, biocompatible, cost effective and stable. Kazmi et al. [27] studied the introduction of silver (Ag) NPs as modifier with TiO_2 and used the material as anode electrode in DSSC. They reported 6.95 to 12.58 mA/cm^2 improvement in the current efficiency due to the introduction of Ag NPs.

In this study, oil palm lignin is used to synthesize the GO. In an exceptionally cost-effective process, lignin can be converted into GO via simple carbonization and Hummers's method, after which the composite of lignin-based GO and metal oxides can serve as the anode material. The selected metal oxides are not only highly conductive, but also inexpensive. Additionally, the prepared anodes such as GO, GO/ZnO nanocomposite and GO/TiO_2 nanocomposites were also studied in terms of toxic metal remediation. According to literature, in the MFCs operations, anode plays a vital role in the treatment of the wastewater. Nowadays, metal pollution is one of the most troubling environmental concerns. Among the metals, lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), cobalt (Co) and nickel (Ni) are the most dangerous for human lives [28]. Mathuriya et al. [29] described that MFCs technology is more superior than other conventional wastewater treatment methods. The advantages of MFCs are, it produced stable sludge than aerobic treatment process, not generating more CO_2 than biological treatment methods and the conversion efficiency was also higher than the enzymatic fuel cells. Therefore, MFCs technology has emerged as a more promising method of remediating hazardous metals from an aqueous medium. Several previous studies indicated MFCs technology as a superior solution for metal ion removal from wastewater [30, 31]. Further, comparative studies were carried out to investigate the targeted anodes (commercial graphite, prepared GO, GO/ZnO nanocomposite and GO/TiO_2, nanocomposites) performances in terms of electron transportation and toxic metal remediation efficiency.

1.2 Problem Statement

To date, MFCs are still unpractical for commercial and industrial purposes due to insufficient energy production, wastewater remediation inefficiency and high cost. In Malaysia, people use almost 99% of surface water for different domestic purposes. In contrast, they use 1% from groundwater, and the total internal water resources in Malaysia are almost 580 km^3 per year [32]. Now, Malaysia is facing heavy metal contaminations (Hg, Cr, Ni, Co, Pb, Cd) in the surface water which is a critical challenge to solve with green and feasible approach as stated by Razak et al. [32]. MFCs are the most promising technique, although it does have several challenges that must be addressed. One of the key issues is the material and design of the anode electrode. Additionally, the transportation of electrons from bacteria cell to anode electrode surface was an essential step for energy transportation. In the last decade, several efforts were done to improve the electron transportation between bacteria cell and electrodes which ultimately affect the efficiency of the MFCs. A wide variety of materials to build the anode electrode for MFCs have already been studied such as carbon-based materials, metal/metal oxides and conducting polymers [33, 34]. However, conventional carbon-based materials, metal-based materials (Au, Cu, Al, Ag) and conducting polymers (CPs) (polypyrrole, polyaniline) failed to provide enough power generation and to treat wastewater efficiently [33]. The above-mentioned materials presented the following drawbacks: poor chemical stability, large pore size, poor mechanical stability, non-biocompatibility, corrosion and high cost [35]. Therefore, to overcome such drawbacks and to develop a stable anode, a high-quality material is required. Recently, Cai et al. [10] reviewed several natural wastes to be used as anode electrode materials in MFCs. Such natural waste-derived anode electrodes performed better than those built with conventional materials because they had larger surface areas and fine pore sizes. However, their electrochemical performance was still not satisfactory. Several biomasses do not offer fine and pure carbonized carbon due to direct biomass carbonization processes. To prepare fine and fully carbonized carbon, the biomass-derived fine powder is an essential step toward this challenge such as oil palm biomass derived lignin, cellulose waste etc. The most conductive and high-quality carbon-based material named graphene and its derivatives can also be synthesized from natural biomass wastes [36, 37]. In addition, the modification (e.g., with metal oxides) of highly conductive carbon-based materials such as graphene and its derivatives could provide a better outcome in MFCs. During the preparation of waste-derived modified anode, the composition optimization of waste material with modifier poses another challenge. An ideal composition of waste-derived material/metal oxide with binder can effectively bring major breakthrough in MFCs field. For example, ElMekawy et al. [38] reviewed the literature about graphene derivative-based electrodes for MFCs and indicated that the modified graphene-based anode electrode led to better results. Thus, the modified graphene-anode electrode enhanced the oxidation process of the substrate by promoting the healthy growth of bacteria on the surface of the anode electrode, thereby enhancing considerably the flow of electrons. Today, there is no work on waste-derived graphene anode electrodes in MFCs for energy generation and pollutant remediation has been conducted. The anode electrode built with waste-derived materials improved the electrochemical performance of MFCs as stated by Huggins et al. [39]. They studied the comparison of waste-derived anode with commercial anodes and found that the waste-derived material is still more satisfactory in terms of energy generation as well as metal remediation. Additionally, the modification of graphene with green synthesized metal oxides such as TiO_2, and ZnO NPs has a great impact on cost reduction and corrosion prevention [40]. The improved anode electrode can address the remediation performance issue in MFCs such as slow redox reaction to convert the soluble metal into insoluble state. The direct oxidation of organic substrate (such as oil palm trunk sap) at modified anode surface can bring major breakthrough in improving the remediation performance of MFCs. The green synthesized metal oxide as modifier may enhance the bacterial biocompatibility and boost the redox reaction which may lead to the high metal remediation performance.

1.3 Research Objective

1. To synthesize the biomass derived GO-based metal oxide nanocomposites anodes and investigate the prepared material through several physiochemical and morphological techniques.
2. To investigate the electrochemical and biochemical performances of the prepared anodes and commercial anode via MFCs.
3. To investigate the bacterial community of each MFCs operation by using the prepared anodes and commercial anode via MFCs.
4. To study the toxic metal remediation performances of the prepared anodes and commercial anode via MFCs.

1.4 Scope of Research

1. Biomass material was considered as primary source to synthesize the graphene derivatives such as GO. The targeted biomass was only oil palm empty fruit bunch (EFB). The EFB lignin and cellulose material is an eminent good feedstock to prepare the carbonized carbon for further applications. The extraction process of lignin is well discussed. The characterizations of the lignin were not considered because it was primary source, later it was converted into valuable product.
2. The synthesis of metal oxides is limited to ZnO, TiO_2, for the preparation of GO-metal oxide nanocomposites. The green synthesis method was used to prepare the metal oxides to promote

biocompatibility of the materials. Further, simple solvothermal method was used to prepare the nanocomposites.

3. The prepared GO, GO/ZnO nanocomposite and GO/TiO_2 nanocomposites are used to fabricate the electrode for MFCs applications. While commercial plain graphite electrode was used as cathodes in MFCs throughout the project. Double chamber MFCs were used which are separated by PEM (Nafion).
4. Different physiochemical characterizations of synthesized materials were performed by using several techniques. UV-Visible, Fourier Transform Infrared (FTIR), scanning electron microscope (SEM), Energy Dispersive X-Ray (EDX), transmission electron microscope (TEM), Brunauer-Emmett-Teller (BET), atomic force microscopy (AFM), thermal gravimetric analysis (TGA), Raman spectroscopy, and X-ray diffraction (XRD).
5. The prepared anodes performances were analyzed in terms of electron transportation and remediation efficiency with commercial plain graphite. The performance was investigated by different electrochemical measurements such as open circuit voltage trend, closed circuit voltage, cyclic voltammetry, electronic impendence spectroscopy and polarization behaviour. The biological characterizations were also performed such as bacteria identification process, SEM and EDX to examine the bacterial biocompatibility towards the anodes. Further, the deep study about the bacterial isolation and identification process is not the objective. The simple bacteria

identification process was considered to identify the listed bacterial species.

6. During the MFCs operation, the oil palm and glucose were supplied as organic substrates to enhance the rate of oxidation and reduction reaction.
7. The toxic metals i.e. Pb, Hg, Cr, Ni, Co and Cr are targeted. The metals were supplemented with local collected wastewater to use as inoculation source in double chamber MFCs.
8. The multiple parameter optimization (temperature, pH, etc.) and electrode fabrication composition optimizations are not included.

1.5 Organization of Thesis

Five chapters were organized in this thesis: Chapter 1 presents the background information and the purpose of this study. The problem description, goals, scope of study and contribution to research are clarified. Chapter 2 outlines an in-depth assessment of this study literature. The basic role of anode electrode with future perspective is described. Detailed information is available on biomass-derived anode electrode, its characteristics and its applicability as electrode in MFCs. Chapter 3 presents the methodology and the workflow process of synthesis of GO and its modification with metal oxide to fabricate the anode electrode. The chemicals and apparatus that were used for this research are specifically described. The performance of unmodified GO anode as well as modified GO anode as efficient source of electron transfer and remediation support through MFCs are discussed in detail. Chapter 4 showing the results and detail discussion on synthesis of material as well as performance in MFCs. The results included SEM, TEM, EDX, Raman, XPS, TGA, XRD, FTIR, UV, BET, PL, CV, EIS and general procedures of microbiological analysis. The findings and results of this research are summarized in Chapter 5. The proposal for improving future research and activities is also included in this Chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The increasing world population, urbanization and industrialization have led to high energy demand. Currently, the world is depending largely on different fossil fuel sources for energy. However, fossil fuel is no longer an ideal energy source in terms of security, efficiency, and environmental impact. For example, in 2011, Japan displayed efforts to make a nuclear power as an alternative energy source to meet the energy crisis. Despite the efforts, the operational reliability, security and practical implementation aspects of the nuclear power still needs to be scrutinized for a safely and sustainable solution [41]. Increasing energy consumption and managing water pollution are two of the most emerging concerns in the modern world, both of which are critical to maintaining a stable green environment for a healthy lifestyle. A large number of scientific literature describes innovative ways for producing energy and treating wastewater, but they are limited by a number of factors, including high costs, production by-product sludge, energy consumption, and cost [42]. Therefore, development and advancement in green energy technologies along with environmental protection are essential elements which currently attracts a wide interest of researchers. To address these two challenges, one of the first alternatives is MFCs, which generate successfully green energy by treating different kind of pollutants from wastewater. The production of green energy from waste materials by using available electroactive bacteria (such as *E. coli*, *Geobacter* spp. etc.). It is a cost effective and relatively simple process in which carbon is converted to energy by means of bioelectrochemical processes [43]. This process has received a wide interest in the remediation of pollutants from water systems. There are several factors to take into consideration for the MFCs performances, such as anode, cathode, organic substrate, and PEM [44, 45]. Among all factors, one of the most important factors is the performance of anode and the reactions and interactions that take place in the anode [46]. Anode electrode is a component of the MFCs in which the bacteria grow to produce a biofilm around the anode surface. This bacterial growth encourages the decomposition of substrate to generate protons and electrons. The generated electrons are pushed towards the cathodic chamber by using a provided circuit, whereas the generated protons are transferred directly into the cathodic chamber through a PEM [47]. The transferred protons and electrons from the anode are consumed in the cathode chamber. The combination of protons and oxygen in the cathode chamber, results in the formation of water molecules. The generation of energy through MFCs depends on the oxidation of organic substrate on the surface of anode. While the reduction reaction occurs on the surface of cathode electrode. Several factors such as extracellular electron transfer (EET), bacterial attachment on anode, substrate decomposition and biofilm growth are directly linked to the anode electrode [48]. Despite all efforts, the quality of the anode material, cost and free availability issues do not allow the utilization of MFCs at large scale. The most utilized materials reported for the anode have been carbon-based, metal/metal oxides and conducting polymer composites. In early time, the precious metals such as gold received significant interest due to their high conductivity and excellent mechanical strength. Later, to minimize the cost of the anode material, the transitional metals like copper, nickel, titanium, aluminum, and stainless steel have also been utilized [11]. Similarly, the carbon-based material such as carbon rod, felt, fiber, graphite etc. are still extensively studied. These are all conventional materials that have shown several drawbacks such as for example: the metal-based materials showed corrosion under long term operation, and high cost, similar conventional carbon material showed low efficiency due to a low electric conductivity of the material. These conventional materials require extensive surface modification to enhance their quality and thus minimize their limitations. Recently, Aziz et al., [49] studied the paper waste-derived conventional three‑dimensional carbon aerogel which was further modified with nitrogen‑doped reduced graphene oxide (rGO) to enhance the anodic performance in MFCs. They achieved power density of 1468 mW/m^2. They found that the improvement in conventional carbon-based material is preferable due to cost effectiveness as well as energy performance. Wang et al., [50] studied the conventional carbon foam and derived from the corncob wastes. They modified the conventional carbon foam to fabricate the 3D N-doped carbon foam anode. The prepared 3D N-doped macroporous carbon foam delivered 4.99 ± 0.02 W/m^2. Therefore, modification of a conventional material is a priority for high performance of MFCs. It is a good idea to use waste material to produce highly conductive carbon-based material such as CNTs, carbon black and graphene (Gr`) derivatives. Later, among them the CNTs and carbon black-based anode showed cellular toxicity towards the bacterial community which may decrease the performance of microbial electrochemical technologies (such as MFCs) as explained by Hassan et al., [51]. Therefore, Gr` derivatives were introduced as materials that prevent the extensive limitations that have risen by the other carbon-based materials. Gr` is a 2D allotrope of carbon which is considered suitable for the anode due to its high conductivity, high chemical, thermal, mechanical stability, and biocompatibility towards bacterial growth [10]. The commercial Gr` derivatives are very expensive, therefore, carbon derived from waste biomass material for anode preparation may serve as a low-cost preparation approach. Several recent studies showed that natural waste derived carbon (which may be converted into precious carbon form such as Gr` derivatives, CNTs etc.) can serve as anode and the reports indicated that significant improvement were achieved in MFCs applications [38]. Additionally, doping or composite of natural derived precious carbon with metal oxides can lead to a breakthrough in the field of MFCs. The metal oxides such as ZnO, TiO_2, CuO, AgO, Al_2O_3 etc. have been extensively studied as cost-effective materials. This is because they can be produced through utilizing natural waste sources. The composite of these two or more different natural waste materials can serve as an ideal anode in MFCs. Additionally, the modified waste-derived Gr` derivatives anodes has the capacity to improve bacteria-anode interaction which increase the formation and strength of biofilm which may lead to high electrons transfer from bacterial cell to anode electrode. Kumar et al., [52] also considered the preparation of the Gr`/poly (3, 4 ethylenedioxythiophene)/Fe_3O_4 nanocomposite as cost-effective oxygen reduction catalyst for high energy generation and wastewater treatment. The achieved maximum power density was 3525 mW/m^2. However, the aim of the present literature is to discuss the efficiency of energy generation and MFCs performance by utilization of the modified anode materials.

2.2 MFCs: Mechanisms of Energy Generation and Pollutant Remediation

A fuel cell is usually defined as a conversion of a chemical energy into an electrical energy without using any kind of combustion. MFCs approach is also a form of electrochemical fuel cell. MFCs approach promotes the growth of bacteria to oxidize the organic substrate in wastewater to generate electrical energy. However, prior to knowing the mechanisms of pollutant biodegradation or biotransformation through the utilization of MFCs, it is necessary to know the proper mechanisms of energy generation through MFCs. In the MFCs chambers several strains of bacteria may present the capability of transferring the electrons and protons through electrodes. It has been found that there are five dominant groups of microorganisms such as *Firmicutes, Proteobacteria, Acidobacteria, fungi* and *algae* that are present the characteristic of electricity generation associated to their respiration process in the MFCs chamber [52]. Some of the bacteria that have been reported earlier, which acts as electron exchanger with electrodes are *Clostridium butyricum*, *Rhodoferax ferrireducens*, *Shewanella* sp., *Geobacter* sp. and *Aeromonas hydrophila* [53-55]. Further, there are some bacterial species which can transfer the electrons directly to the anode. These types of species are recognized as electrogenesis. The bacterial species which carry out this process are known as exoelectrogens [56]. During the MFCs operation, the bacterial species oxidize the various organic substrates to generate electrons and protons.

However, microorganisms can transfer electrons to electron acceptors that are present in an insoluble state. For example, some *Geobacter* species have pili (pili is body part of bacteria), which are as conductive as metal and are actively expressed. Microorganisms grow on the surface of the electrodes and develop a biofilm to transfer electrons more efficiently [11]. The formation of biofilm on the surface of anode is the most significant mode of interaction to pass the electrons. To reduce the competition between electron carriers' mediators and oxygen, anaerobic conditions must be applied. The oxygen supply (oxygen is as electron acceptor) in the anode chamber lead to poor energy generation [57].

In biofilm, both forms of bacterial culture, such as mixed bacterial culture and pure bacteria culture, have been previously documented in the literature. For example, according to Jadhav et al., [58] the mixed bacterial culture offered higher power efficiency than pure culture. The key advantage of the mix bacterial consortia is that they can oxidize the organic substrate more effectively. This is due to the syntrophic relations between exoelectrogens and fermentative bacteria; such interaction may improve the exoelectrogenic activities. On other hand, the pure single bacterial colonies are easy to investigate in biofilm for electron transfer mechanisms study. Meanwhile, the anaerobic condition of anode chamber is an essential metabolic pathway for the oxidation of the complex organic substrate by bacterial species. During oxidation process, any complex organic substrate first hydrolyzed into simple compounds like aromatic compounds, monosaccharides, fatty acid, and amino acid as shown in Figure 2.1. Next, the hydrolysed simple compounds are fermented or oxidized to CO_2 which may generate and transfer the electrons to the anode electrode. The maximum generation of electrons and complete oxidation of simple compounds are the ideal conditions in MFCs.

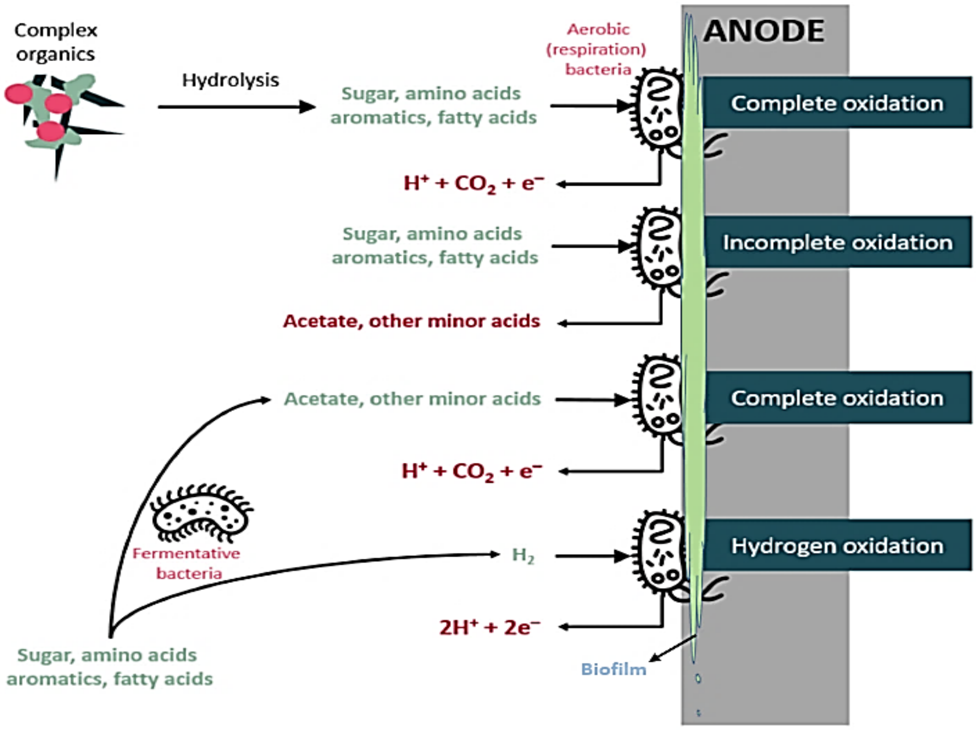

Figure 2.1 Oxidation process of organic substrate on the surface of anode in presence of biofilm to generate the electron and proton (Adapted from [57] with MDPI permission).

2.3 Mechanism of Electron Transfer from Bacteria to Anode Electrode

Microorganisms that transfer the electrons extracellularly are classified as exoelectrogens and some species that have the ability to transfer electrons in this way are: *Geobacter lovleyi*, *Geothrix fermentans*, *Thermincola carboxydophila*, *Geobacter sulfurreducens*, *Shewanella oneidensis*, *Rhodopseudomonas palustris*, *Thermincola potens*, *E. coli*, and *Shewanella putrefaciens* [59, 60]. For energy generation, exoelectrogens can transfer electrons from electrodes through some mechanisms that have been clearly stated as shown in Figure 2.2.

i. In brief, the short-range transfer of electrons towards the anode electrode is carried out via electron shuttles molecules such as in the case of *G. fermentans*. Both, gram positive and negative bacteria, can transfer electrons through using self-producing shuttle molecules. *Desulfuromonadaceae* and *Geobacteraceae* are two families of bacteria that produce self-electron shuttles, for example, c-type cytochromes are a group of these electron shuttles that include MtrC, MtrD, MtrF, OmcA and MtrE components [61].

ii. *Geobacter sulfurreducens* is a good example of microorganism that can assist the electrons transformation towards the anode via anaerobic enzymatic-based metabolism activities. The bacteria *Geobacter sulfurreducens* facilitates the electron transformation to several types of acceptors such as fumarates etc. It has been reported that several redox active proteins such as OmcT, OmcZ, OmcS, OmcE and OmcB are available in exoelectrogens [62].

iii. The long-range transfer of electrons through conductive pili attracts great interest. A little filamentous projection in bacteria body which promotes surface adhesion and is not used for mobility is known as pili. These pili are usually conductive and help to transfer the electrons from the biofilm to the anode surface. The conductive pili are generally available in the strains of *Shewanella oneidensis, Pelotomaculum thermopropionicum, G. sulfurreducens*, and *Methanothermobacter thermautotrophicus* [63]. The direct interspecies electron transfer approach was studied mostly in *Geobacter metallireducens* and *Geobacter sulfurreducens.* In this approach, the microorganism serves to transfer the electron directly and enhances the mutual growth. The *Pelotomaculum thermopropionicum* and *Synechocystis* are also examples of this kind of transformation in MFCs [64].

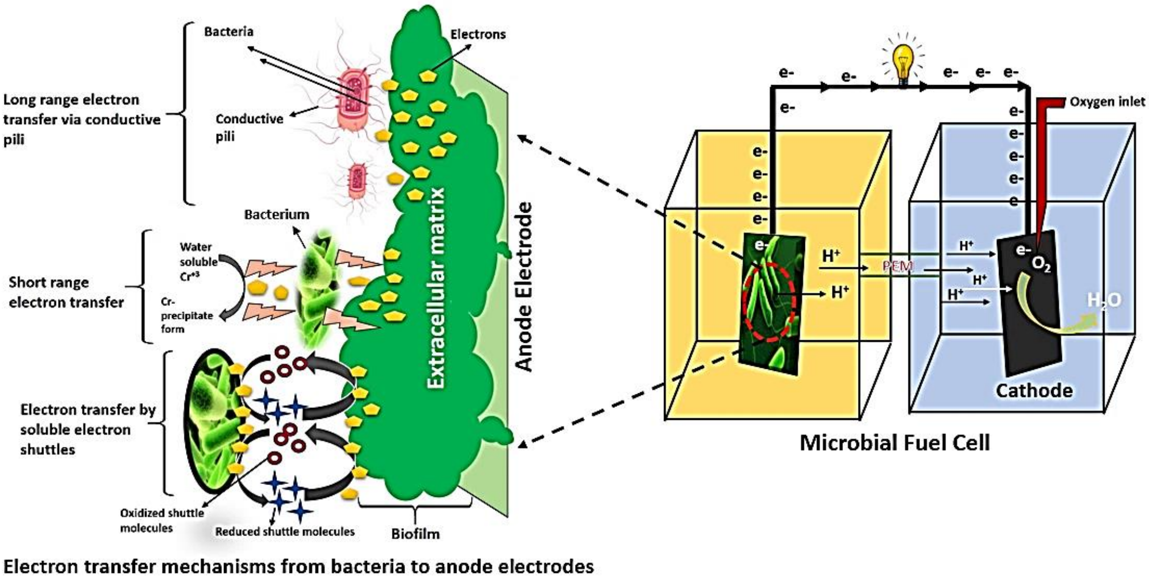

Figure 2.2 Schematic presentation of MFCs and electron transfer mechanisms from exoelectrogens to electrodes.

2.4 Reduction Mechanism of Heavy Metals

To date, several chemical, physical, analytical, and biological approaches have been introduced for the remediation of heavy metals. Among all, MFCs is the feasible, cost-effective, and eco-friendly approach which can reduce heavy metals and simultaneously generate energy. The microorganism that can accept electrons from (anode and cathode) electrodes is known as electrotrophs [65]. This fact opens a new direction for treatment of heavy metals via reduction reaction. The heavy metals are removed through the reduction reaction at cathode while organic substrates are oxidized at anode [66].

There are many types of bacteria (such as *Geobacter* species) that produces electrons from organic substrates [61]. There are many toxic heavy metals like Cr, Ni, Zn, Hg, Pb, Cu, V ions etc. that are reduced by different microorganisms. The reduction process followed the same mechanisms, i.e., *G. sulfurreducens* produce electrons and simultaneously reduces the U^{6+} to U^{4+} form (soluble to insoluble). The U^{4+} is insoluble, and it is deposited on the electrodes [67]. Watts et al., [68] studied that *G. sulfurreducens* has the capability of reducing Cr^{6+} to Cr^{3+}, where it converted the highly toxic oxidation state of chromium to the less toxic form. The reduction of Cr^{4+} depends on the oxidation of substrate (acetate) at the anode electrode. Later the electrons were transferred to microorganisms and the reduction of chromium ions occurs at the cathode. The reduction reaction can be written as:

$Cr_2O_7^{-2} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$ -- (2.1)

Hao et al. [69] studied the removal of vanadium with microorganisms such as *Enterobacter, Macellibacteroides* and *Lactococcu*s. The authors recorded vanadium removal efficiency of 93.6% and a high current density of 543.4 mW/m^2. Similarly, the most toxic heavy metal which is mercury (Hg^{2+}) can also be reduced via MFCs. The Hg^{2+} redox potential was recorded as -320 mV. The removal mechanism of Hg^{2+} in a precipitate form occur in the presence of chloride (Cl^-) ions and the reduction through electrons occur at cathode electrode as shown below:

$Hg_2^{2+} + 2Cl^- \rightarrow Hg_2Cl_{2(s)}$ -- (2.2)

$Hg_2Cl_2(s) + 2e^- \rightarrow 2Hg_{(l)} + 2Cl^-$ --- (2.3)

The final product was Hg_2Cl_2 and deposited at the bottom of the cathode while the elemental Hg^{2+} was found on the surface of cathode. During this process, the maximum power density achieved was 433.1 mW/m^2 as stated by Wang et al., [70].

Similarly, the reduction mechanism of Cu^{2+} to Cu can be written as:

$Cu^{2+} + 2e^- \rightarrow Cu$ -- (2.3)

$2Cu^{2+} + H_2O + 2e^- \rightarrow Cu_2O + 2H^+$ --- (2.4)

$Cu_2O + 2e^- + 2H^+ \rightarrow 2Cu + H_2O$ --- (2.5)

During reduction of Cu^{2+} in MFCs operation, two major products may produce which are Cu_2O or Cu at cathode. The electrons originated from the oxidation process of organic substrate which occur in anode compartment [71]. Anode has received significant attention in MFCs research because the working mechanism of MFCs directly depends on the performance of anode. Therefore, the improvement of the anodic component of MFCs systems is of upmost importance over others.

2.5 Biochemical Cell Reactions and Electrochemical Measurements

During the MFCs operation, organic substrate such as glucose, acetate sucrose etc. are oxidized by bacteria to generate the electrons. The oxidation reaction at anode and reduction reaction at cathode can be written as follow with different organic substrate [72, 73].

(a) If glucose is used as organic substrate

Oxidation reaction at anode: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$ ------------ (2.6)

Reduction reaction at cathode: $24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$ --------------------- (2.7)

Overall reaction: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Electricity + Biomass ------ (2.8)

(b) If acetate is used as organic substrate

Oxidation reaction at anode: $CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$ ------------ (2.9)

Reduction reaction at cathode: $8H^+ + 8e^- + 2O_2 \rightarrow 4H_2O$ ------------------- ------ (2.10)

Overall reaction: $CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$ + Electricity + Biomass - (2.11)

(c) If sucrose is used as organic substrate

Oxidation reaction at anode: $C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$ ------ (2.12)

Reduction reaction at cathode: $4H^+ + 4e^- + O_2 \rightarrow 2H_2O$ -------------------------- (2.13)

Overall reaction: $C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O$ + Electricity + Biomass… (2.14)

Further, the produced electrons move from anode to cathode electrode while different electrochemical measurements are carried out to measure the performance of the electrode

2.6 Essential Properties of Anode Electrode Materials

The selection of an anode electrode material in MFCs is still a critical challenge for researchers to achieve desired results in terms of electrochemical efficiency, electron transformation and bacterial adhesion [74]. Some essential properties which must be present in an ideal anode electrode are listed in Figure 2.3a.

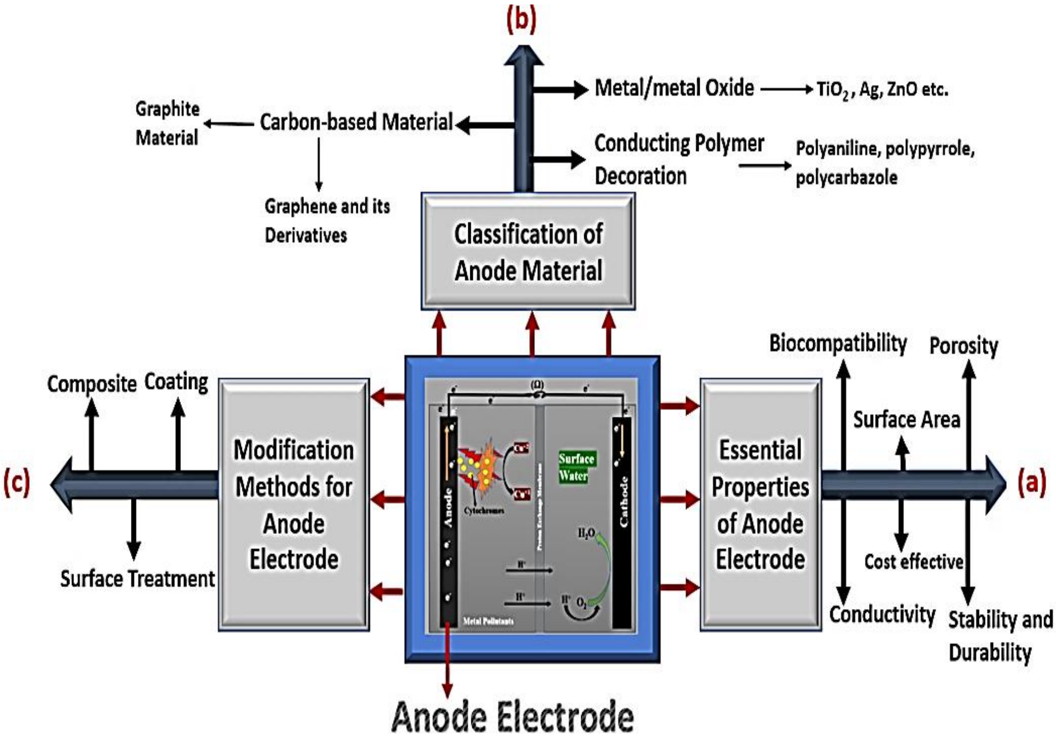

Figure 2.3 Schematic presentation of an anode electrode. (a) Essential properties of anode electrodes; (b) Classification of anode electrode materials; (c) Modification strategies for anode electrodes.

2.6.1 Conductivity

Conductivity is an important property of anode material because the electrons released by bacteria are transferred to anode electrode which further travel to cathode via the outer circuit. Thus, the anode material is responsible for enabling the flow of electrons and increasing their speed [75, 76]. Highly conductive materials help to reduce the bulk solution resistance and increase the transfer of electrons. The interfacial impedance between the substrate and the electrode must be low to boost the transfer of electrons [77, 78]. The electrical conductivity of materials is usually studied before building the anode electrodes for MFCs.

2.6.2 Surface Area and Porosity

Energy generation in MFCs is seriously affected by the surface area of the anode electrode [79]. The anode electrode resistance is directly proportional to the ohmic losses of fuel cell and, thus, the best way to reduce the resistance power is by increasing its surface area. Additionally, a large surface area offers more active sites for bacterial growth and increases the efficiency of the electrode kinetics. Microorganisms such as *Escherichia coli, Geobacter* species, *Pseudomonas* species, etc. were efficiently and actively immobilized on the surface of anode electrodes ensuring the effective and direct electron transfer to the anode electrode [34]. Biological reactions occur on the surface of the anode electrodes and, therefore, the surface area extremely influences MFCs performances [80]. As can be seen in Table 2.1, conventional carbon materials have a smaller surface area than Gr` derivatives [44, 81, 82].

2.6.3 Biocompatibility

Biocompatibility of the anode electrode has a significant importance in MFCs operations because it is directly in contact with bacteria and their respiration process. In MFCs, several materials such as copper, silver and gold are not considered biocompatible to be used as anode electrodes because they are prone to corrosion [44]. The toxicity of the said metals can inhibit the bacterial growth during MFCs operation and thereby decrease the generation of energy.

2.6.4 Stability and Durability

The long-term contact of conventional anode electrodes with substrate and inoculated microorganisms in MFCs generally produces swelling due to non-