Chapter

Electrode Material as Anode for Improving the Electrochemical Performance of Microbial Fuel Cells

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Abstract

The energy generation without causing environmental pollution is a unique idea to make a better survival for human beings. In this regard, microbial fuel cells (MFCs) have been considered to be eco-friendly and efficient technology to produce renewable energy. The operations and functioning of MFCs technology were affected by many factors but the electrodes are the most essential and significant aspects in MFCs. Moreover, a wide variety of electrodes and MFCs configurations have been developed to enhance the electrochemical performance of MFCs. The carbon materials (graphite, graphene etc.) were commonly used for the electrode fabrication, due to some unique properties such as high conductivity, good thermal stability, high surface area, good mechanical power etc. In this chapter, different electrode materials, used for anode fabrication were summarized to reveal the performance/efficiency toward the generation of electricity. Finally, the electrochemical characterizations tool, current challenges, and future perspectives of the electrode in MFCs were discussed briefly.

Keywords: Microbial fuel cells, Anode electrodes, Energy, Electrochemical characterizations, Anode oxidation

1. Introduction

Due to the increasing worldwide population, industries, etc. the commercial, and domestic demands of energy are on their rising trends. The rapidly growing population and industrialization have carried out a vast influence on environment contamination such as water, air pollution [1]. Therefore, it has been essential to overcome the energy demand without environmental pollution. In past decades, wastewater was considered as an efficient source for generation of energy. Hence, there is a crucial need to introduce a method by improving energy recovery from water resources without high expenditure. To respond to this inefficiency, the scientific community introduced a new method called microbial fuel cells (MFCs), considered as the most economical and environmentally stable process. Logan et al. [2] defined the MFCs as a most prospective approach to generate energy along with treatment of wastewater by using microbes as catalysts. Similarly, Nikhil et al. [3]

studied that the MFCs have the capability to generate electricity along with the treatment of wastewater and raised it to be an ideal solution for energy and water issues. Usually, MFCs presented extensive advantages as compared to other conventional techniques such as less activated sludge produced from wastewater, energy input is not present for aeration and the system works at various pH, temperature and substrate to generate electricity through using microorganism electro-catalyst. Despite all developments in MFCs, even an ideal improvement in power density was observed earlier but still practical application has not been implemented in the market due to the less electric efficiency. There are several factors such as electrodes (anode & cathode), incolumn source, proton exchange membrane, MFCs scale, design and organic substrate etc. which directly affect the operation of MFCs [4]. Moreover, the MFCs have two basic types of electrodes in the system such as anode and cathode system. In all factors, one of the most important aspects is the electrode material, particularly anode electrode and its working efficiency. The lower transportation rate of electrons becomes a major problem which decreases the electric performance of the MFCs. In early stage, a large variety of electrodes (activated carbon cloth, carbon felt, cloth, rod, mesh, fiber, paper, brushes, 3D graphite, glassy carbon, granular graphite, graphite block, graphite felt, reticulated vitreous carbon and graphite oxide) have been widely studied which were made using different materials [5]. Both types of electrodes play a significant role in the performance of the system but regarding in terms of functionality, anode attracted a lot of attention by researchers [6].

Furthermore, the organic substrate is oxidized by electrochemical active microbes usually known as exoelectrogens which are present on the surface of anode. The exoelectrogens produce the electrons/protons and significantly reduce the toxic effect of pollutants from wastewater. The redox reaction is thermodynamically significant, releasing electrons by microbes' stream from anode toward the cathode electrode through the provided outside circuit, and thus producing electricity. Therefore, it is not difficult to say that the MFCs performance depends on efficiency of the electrode material (anode and cathode material) [7, 8]. Furthermore, to promote the rate of organic substrate oxidation at the surface of anode electrode, a significant development is required for anode material to increase the electron transportation. The high oxidation rate led to the high generation of electrons which can travel toward the cathode so an efficient medium (anode material) can effectively transfer the electrons from anode to cathode chamber. Recently, Yaqoob et al. [7] reported the modified graphene/polyaniline nanocomposite as anode vs. graphite as cathode in MFCs to enhance electron transportation. A.A.Y. et al. reported the 87.71 mA m^{-2} current density which was higher as compared to simple graphite anode. In another study, Yaqoob et al. [8] also proved that the modification of anode electrodes can bring high electron transfer which led to high performance of MFCs. A.A.Y. et al. also used the graphene/ZnO composite as anode and reported 142.98 mA m^{-2} current density. In the previous literature, ElMekawy et al. [9] studied the graphene-based electrodes (anode & cathode) and compared the performance in terms of electricity. The highest achieved power density was reported as 4 W m^{-2} by using graphene-based anode electrodes vs. carbon brushes as cathode. Similarly, modified cathode (graphene-based) was offered with a power density as 3.5 W m^{-2}. Therefore, this comparatively higher power density by graphene modified anode, was considered more efficient as compared to graphene-modified cathode. The reason for higher power density in case of graphene modified anode is due to the high electron transportation toward cathode. While in graphene-based cathode the energy was low due to poor rate of electron transportation from anode. Therefore, the generated electron required an effective anode material to transfer the electrons rapidly toward the cathode. Moreover, the authors also proposed that graphene anode modification is more important than the cathode electrode to get better performance. The anode material braked the progress of MFCs at practical application due to low conductive material, bacterial toxicity, corrosive and cost issues. The significant value, role of anode and above-mentioned issues urge the utilization of highly conductive material as anode material to overcome these problems. Generally, some properties such as high surface area, high conductivity, biocompatibility, cost effectiveness, high mechanical and thermal stability are essential required parameters for high performance of anode electrode [9]. To overcome these current challenges, the utilization of high-performance material will be considered as a great idea. Although the materials used mostly are carbon-based, less effort seems to develop graphene and its derivatives-based electrodes. Therefore, the present chapter describes the role and working mechanism of anode oxidation in MFCs. Presently, several efforts are still ongoing to improve the anode material as electrodes for high performance related to MFCs. Moreover, this chapter will be proved very useful for the researcher to develop an idea for further improvement regarding anode electrodes. However, the basic required properties and future recommendations for high performance anode are briefly summarized in this article.

2. Working mechanism of microbial fuel cells via anode oxidation

Many microbial species are well known exoelectrogens in MFCs and they can oxidize the organic substrate to produce the electron and proton. The *Aeromonas hydrophila, Shewanella spp. Geobacter spp., Clostridium butyricum Enterococcus gallinarum* and *Rhodoferax ferrireducens* are mostly reported exoelectrogens in MFCs studies [10, 11]. Some bacterial species such as *Geobacter species* also have the ability for electron generation properties [10–12]. For example, *Geobacter* based biofilms can work as transistors and supercapacitors due to the conductive phenomenon. The exoelectrogens can also pass and produce electrons, and this production of current is generated because of their respiration phenomenon. Usually, the anode is not part of the ordinary aquatic environment; so, microbes typically act as a transference medium for electrons to insoluble natural electron acceptors [13]. Throughout the reduction of pollutants, the pili of *Geobacter* are vigorously voiced. The microbes are ascribed to anode electrodes and produce the biofilms. The microbes are responsible to transfer the electrons toward the anode surface as compared to natural insoluble mediators as electron acceptors. In microbial metabolism and bioelectrogenesis, organic substrates comprising carbohydrates are generally used in MFCs. These carbohydrate-based organic substrate molecules generate the acetyl coenzyme A by entering into the glycolysis process and further respective ways. This acetyl coenzyme A enters the tricarboxylic acid cycle (Kreb's cycle). All these processes take place in the cytoplasm and one complete round of Krebs cycle generate three molecules of reduced nicotinamide adenine dinucleotide (NADH) and one molecule of reduced flavin adenine dinucleotide ($FADH_2$) with CO_2 as a by-product [14–16]. To generate the adenosine triphosphate (an energy carrier molecule), the NADH and $FADH_2$ act as electron transporters, and then they permit their electrons toward the electron transport chain (ETC). The ETC operates in the cell membrane (inner and outer membranes and periplasm) and these electrons move through consequent protein channels (NADH dehydrogenase, ubiquinone, coenzyme Q, and cytochromes) of the ETC and lastly captured by electron acceptors [17]. Later, they were transferred to the cathode electrode from the anode via an external circuit.

This entire cycle produces approximately 34 adenosine triphosphate molecules and H_2O from the carrier molecules as shown in **Figure 1**.

In this process, the electrons are transferred from bacteria to anode electrode through following mechanisms; (i) through redox-active proteins (ii) through soluble electron shuttling molecules (iii) through conductive pili and, (iv) through direct interspecies transfer mechanism [10]. However, the most dominant mechanism is assigned for electron transfer via conductive pili because they behave and show metal-like conductivity as shown in **Figure 2.**

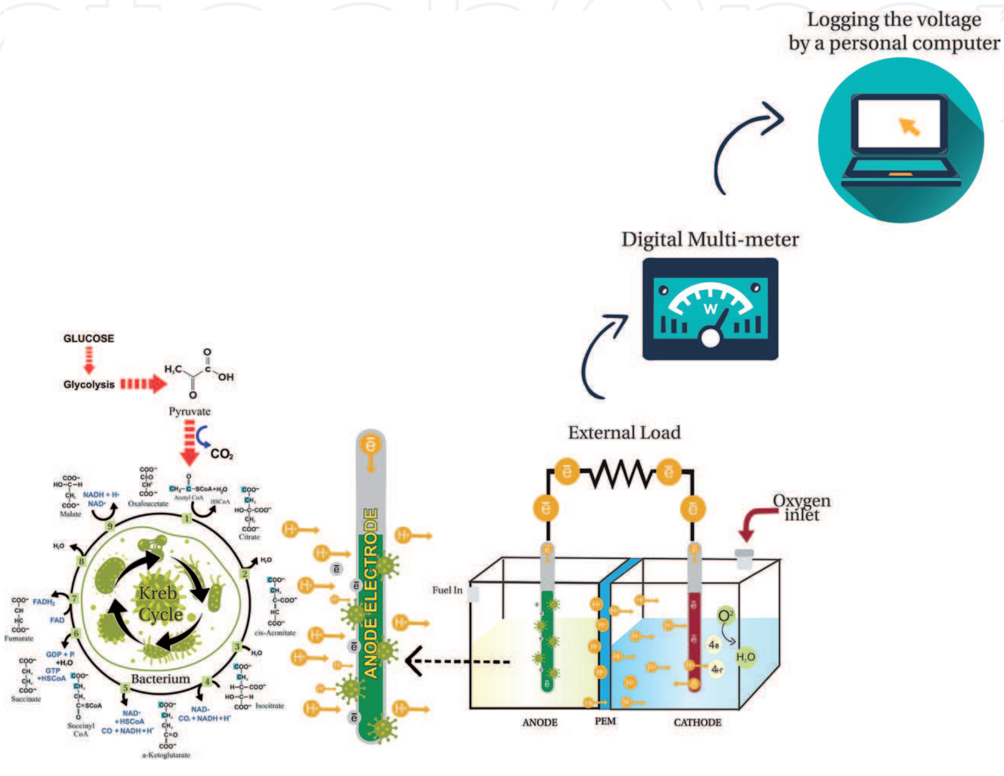

Figure 1.
Mechanism of electron transfer from bacterial cell to anode. (Adapted from Yaqoob et al. [8] reference with Elsevier permission.)

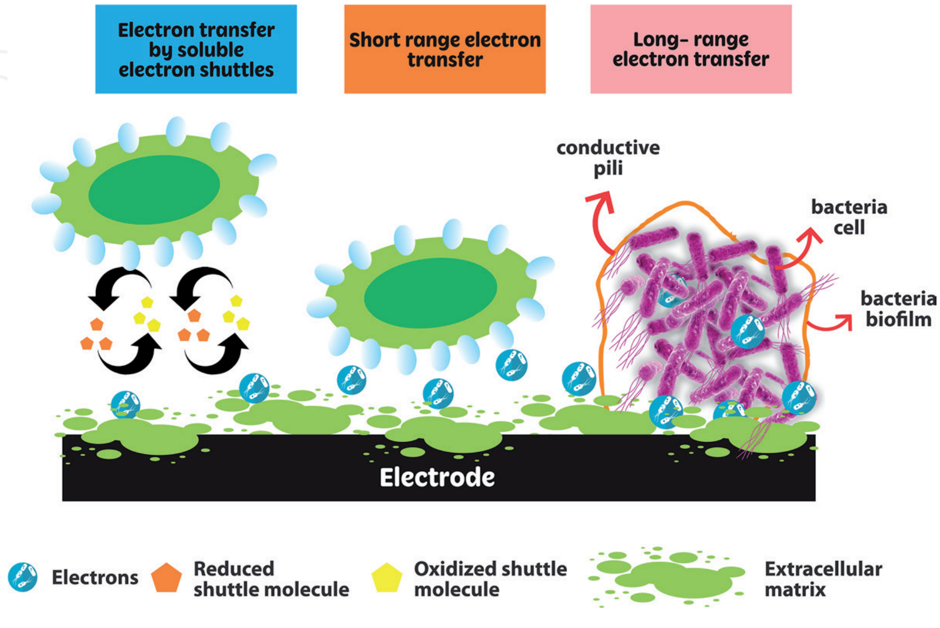

Figure 2.
Mechanism of electron transfer from microbes to anode electrode. (Adapted from Yaqoob et al. [8] reference with Elsevier permission.)

3. Required properties of anode

One part which could be significantly studied to analyze the energy generation output from MFCs operation, is the anode material. The selection of material for an ideal anode is also a promising challenge in terms of electron generation, transformation, and bacterial adhesion. However, for an ideal anode, it must have few significant properties. Many studies are present to increase the energy production by using several types of material such as conventional carbon-based materials, metal-based and conducting polymer or composite etc. [18, 19]. Although the selection criteria for the anode are different from cathode electrodes because anode electrodes are in direct contact with microbes. Therefore, some important parameters are summarized here as follows.

3.1 Electrical conductivity

The electrical conductivity of the material is considered as the most essential factor to serve as anode in MFCs operation. During operation, the electrons and protons were released by microbes which resulted as a consequence of respiration. Different mechanisms were employed to transfer the electrons from microbes to anode as shown in **Figure 2.** Three methods are essentially employed to transfer the electrons from microbes to anode (i) direct electron transfer via conductive pili (ii) electron transfer through redox-active proteins molecules (iii) use of electron shuttles to transfer electrons. However, released electrons moved from anode to cathode via an external circuit. The high conductive material helps to increase the flow of electrons and exhibit less resistance. The highly electrically conductive material reduces the resistance, which is according to Ohm's law, current is inversely proportional to resistance. The rapid flow of electrons is, because high conductive material provides a chance to release electrons to remain closer to the nucleus which generates a band where material acts as an open highway. While simultaneously, the interfacial impedance must be less between analyte and electrode to enhance the electron transfer during the process [14, 20].

3.2 Porosity and surface area

The surface area of the anode electrode carried out a direct effect on the performance of MFCs. The large surface area can offer better opportunities to microbial species for their growth and respiration effectively at the surface of anode. The anode's material porosity effects the catalytic activities of the microbes. The microbes were successfully immobilized on the surface of the anode material and produce the electron via oxidation of organic substrate. The electron harvesting was carried out in presence of ohmic losses. The internal resistance and ohmic losses of the system could be reduced by improving the anode surface area. The internal resistance is directly proportional to the ohmic losses of MFCs, so by increasing the surface area, the resistance will be reduced as stated by Chuo et al. [10]. The larger surface area of material providing a smooth space for microbes to grow and respiration occurred effectively [21]. The electrodes porosity must be sustained during operation of MFCs. However, the power generation greatly depends on the surface area of anode although high porosity in anodic material reduces the conductivity of anode material [21]. Additionally, a high surface area offers a more active site, which increases the electrode kinetics. Although many conventional carbon-based materials show better surface area, in modern trend graphene and its derivatives exhibit higher surface area than conventional carbon-based material.

3.3 Biocompatibility

The biocompatibility of anode is also very important in MFCs operation for better outcomes in terms of energy production. The produced microbes are in direct contact with the surface of anode. If the anodic material is not biocompatible with microbial growth, then the generation of electrons will be decreased. Moreover, there are several anode materials which show cytotoxicity and might reduce the microbial healthy growth on the surface of the material. The substantial potential and energy losses carried out due to absence of healthy compatibility of microbes with the anodic electrodes [22, 23].

3.4 Stability of electrode

The oxidizing and reducing atmosphere may lead to decomposition and distend of anode in MFCs. Although, the excellent roughness of surface increases durability while it might enhance the probabilities of fouling, thus may reduce the long-term stability of material. Therefore, both types of electrodes (anode & cathode) must highly show durability and stability in both the basic and acidic nature of the environment. In comprehensive, anode material always has direct contact with inoculated microbes and organic substrates which might lead to higher damage of the anode. It entirely disturbs the physical integrity of anode. Therefore, the hydrophobic-based material is preferable to minimize this mentioned effect. The electrode's stability is constrained through moieties which are engaged in material pores and reduce the space for microbial growth. The rough surface of electrodes is preferred for detaching the H_2O molecules and giving more area to microbes for their sustainability. However, optimized surface roughness and toughness of electrodes are required to enhance the performance of MFCs and minimize the adverse fouling effect [21, 24]. Furthermore, chemical, thermal, and physical stability are also important parameters for an ideal anode to work more effectively.

3.5 Availability and cost of electrode

The cost and easy availability of material for electrodes are also very important parameters and play an important role in selection of anode material. The availability with lower cost along with all above mentioned properties are considered as an ideal material for electrodes. The cost and availability of materials make the MFCs unsuitable at large scale. For practical applications of MFCs, low cost, highly stable and easily accessible materials are required. For example, nanocomposites consisting of Pt, Au etc. are highly expensive and non-sustainable materials. However, metal oxide composite with other materials such as carbon-based polymers might be a substitute to decrease the cost of material. The availability issue can be overcome by synthesizing the metal oxide and carbon-based material through green synthesis methods by using waste material. Moreover, green synthesized composites also exhibit good biocompatible, better stability and easy availability which helps to enhance the life of MFCs operation [25].

4. Material for anode electrode

The performance of MFCs and economic possibility are vitally related with progresses in anode materials. The anode electrodes have expressively impacted on formation of biofilm and transfer of electrons between electron acceptor and microbes. Several types of materials are reported earlier for the fabrication of anode for MFCs and achieved significant results, due to having the excellent properties as mentioned-above [4, 5, 9, 11]. Most familiar and used material is carbon-based, metal/metal oxide-based and conducting polymers which carry substantial outcomes, and some recent data are summarized in **Table 1.**

4.1 Carbon-based anode material

The carbon derivatives are most often used as anode electrode due to their high conductivity, excellent electrons transfer kinetics, good biocompatibility, reasonable chemical activity, mechanical and thermal stability. The carbon derivatives offer a good prospect to enhance the performance of MFCs with minimum/economic cost. Mostly used carbon derivatives are reticulated vitreous carbon, carbon paper, glassy carbon, carbon rod, carbon felt, carbon fiber, carbon cloth, activated carbon cloth, carbon brushes, graphite felt, graphite paper, 3D graphite, graphitic granular and graphene oxide powder [45]. Recently, graphite and its derivatives such as graphite rod, plate, sheet, cloth, or paper are commonly used because graphite material is more valuable than simple carbon types. The graphite material is very rigid, brittle, thin and so far, non-toxic material. The graphite material got much attention before, but later the scientific community found some drawback of this material which makes the MFCs unsuitable at higher scale i.e., low surface area and high porosity. These factors inhibit the healthier microbial growth on the surface of anode to generate the electron more rapidly [46]. Similarly, the same disadvantage is reported in case of simple carbon sheets and carbon cloth-based anodes. However, the activated carbon cloth and carbon fiber offer much better results than other conventional carbons due to reasonable surface area and better adsorption at anode [47]. Wang et al. [48] studied the carbon mesh as anode in MFCs which is cheaper than commercial carbons and exhibited reasonable current density. Zhu et al. [49] studied the graphite felt as anode and achieved the 385 W m^{-3} power density which was considered much better due to the effect of organic substrate. Therefore, there is still not enough energy efficiency to take the MFCs to industrial scale. Furthermore, carbon was employed in packing form to increase the electrode surface area for better growth of microbes [50]. The graphitic material also available in packing form and graphite packed granules showed low porosity in material but suffered from clogging which may be projected as another problem in this material [51]. Furthermore, the results were also based on charge storage efficiency and energy generation by using a single carbon-based granule. For example, the activated carbon granule stored the charges in electric double layer form which usually corresponds to enhancing the anode performance. The activated carbon-based granule produces 0.6 mA current by acting as anode. The discharge/charge mechanism shows that the activated carbon granules produce 1.3–2 times extra charge than graphitic granules. Furthermore, Zhang et al. [52] also studied graphite brushes as an anode with specific 5cm^2 diameter. The achieved power density was 1430 mW/m^2 which is better than conventional carbons anodes. Yazdi et al. [53] extensively reviewed the literature of the carbon-based material as anode electrode for MFCs. The literature showed that the modern carbon material known as carbon nanotubes (CNTs) and its modification showed cellular toxicity against the microbial community during operation. On the other hand, the carbon allotrope called graphene and its derivatives have attracted much attention recently as electrode material in MFCs. The graphene derivatives showed excellent biocompatibility, flexibility, conductivity, mechanical robustness, specific surface area, chemical inertness and stability. The commercial graphene derivatives such as graphene oxide is expensive to use as anode but according to Huggins et al. [54] it was less expensive comparatively to waste-derived graphene derivatives.

Type of Material	Electrodes		Size of Anode	Surface area of Anode (cm^2)	Power Density ($mW\ m^{-2}$)	Reference
	Anode	Cathode				
Carbon-based Anode	Plain Carbon paper	Carbon paper	2.5 × 4.5 cm	—	33	[26]
	Graphene oxide	Carbon paper	2 cm × 1 cm	—	102	[27]
	Graphene oxide wit CNT	Carbon cloth	—	—	434	[28]
	Graphene nanosheet coating on carbon paper	Carbon cloth	—	5.4	610	[29]
	Carbon cloth	Carbon cloth	2 cm × 2 cm	4	679.7	[30]
	Carbon felt	Carbon fiber felt	2.5 × 2.5 cm	2.5	784	[31]
	Graphite brush	Carbon cloth	3 cm × 2 cm	8	1280	[21]
	3D Graphene	Carbon cloth	Anode: 30 mm × 5 mm (diameter × thickness)	9.41	1516 ± 87	[32]
	Graphene	Carbon cloth	2 cm × 2 cm	4	2850	[33]
Metal and metal oxide Anode	Titanium rod	graphite felt	20 mm	20 ± 1	—	[34]
	Titanium	—	—	4	700	[35]
	Ti/TiO_2	Pt meshes	—	—	2317	[36]

Type of Material	Electrodes		Size of Anode	Surface area of Anode (cm^2)	Power Density (mW m^{-2})	Reference
	Anode	Cathode				
Conducting Polymer & Composite-based Anode	Graphene powder/ Polytetrafluoroethylene on Carbon cloth	Carbon cloth	4 × 4 cm^2	—	0.329	[37]
	Zero-dimension nitrogen-doped carbon dots modification with carbon paper	Carbon paper	2.5 cm × 2.5 cm^2	—	0.329	[38]
	Nickel foam/CNTs/PANI	carbon cloth	—	1 cm^2 of anode surface-area	113	[39]
	Graphene/Au composite	Carbon paper	—	6	508	[40]
	PANI networks onto GNRs-coated on carbon paper (CP/GNRs/PANI)	Carbon paper	2 cm × 2 cm	4	856	[29]
	rGO/PPy	Carbon paper	1 cm × 1.5 cm	—	1068	[41]
	PPy/graphene oxide	Carbon felt	3.0 cm × 2.0 cm × 0.5 cm	—	1326	[42]
	rGO/ Carbon cloth-PANI	Carbon felt	1.8 cm × 1.8 cm	—	1390	[43]
	rGO/SnO_2/Carbon cloth composite	Pt rode	3 cm × 2 cm	6	1624	[44]
	TiO_2 and r GO composite	Carbon fiber/brush	1 cm × 1 cm	1	3169	[21]

Table 1.
Summary of anode material with different size and surface.

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The conclusion demonstrated that the biomass-derived material as anode can offer efficient performance in MFCs at reasonable cost. At the moment, to synthesize the graphene derivatives from waste materials, one of the most commonly used methods was Hummer methods [55]. However, so far very little effort seems in this direction to use waste material as electrodes for MFCs as shown in **Figure 3.**

4.2 Metal/metal oxide-based anode

The metal/metal oxide can offer high electrochemical performance in MFCs as anode, but metal corrosion limits the applications to use as anode. Silver, nickel, titanium, gold, copper, copper, aluminum etc. all metallic strips can be serving as electrodes and offer better outcomes. Metallic material carries a higher rate of conduction than other materials and thus, metal-based materials flew the electron faster which help to enhance energy generation [56–58]. The metallic electrode also showed high electrical conductivity, good mechanical stability and so far, biocompatibility for a short time. For example, Yamashita and Yokoyama, [59] studied the effect of molybdenum as in case of anode and achieved 1296 W m^{-2} power density. Metal has exclusive and remarkable properties, but still it is not broadly employed as electrode due to corrosion effect and absence of strong bacterial adhesion. The metal-based electrodes showed very less biocompatibility toward microbes, after some time of operation the metal started getting corrosion which is a serious threat to microbe's life. Nitisoravut et al. [60] studied the stainless steel as anode to enhance the energy out but after some time the achieved power density was 23 mW m^{-2} due to presence of poor microbial adhesion. Moreover, diverse dimensional biocompatible aspects of metal oxides such as vertical 3D porous structure of metal oxide (TiO_2) sheets, can offer a great active surface area, which increases the electron movement and molecular diffusion during MFCs operation. To some extent, the metal oxide offers a better electron transfer rate and good microbial adhesion. Firdous et al. [61] studied the TiO_2 based anode rod to treat the vegetable oil industries wastes and produced 5839 mV maximum voltage with 90% removal of chemical oxygen demand. The metal or metal oxide composite with other materials is a promising technique to overcome many types of issues such as cost, biocompatibility, stability, and conductivity. There are several methods to synthesize

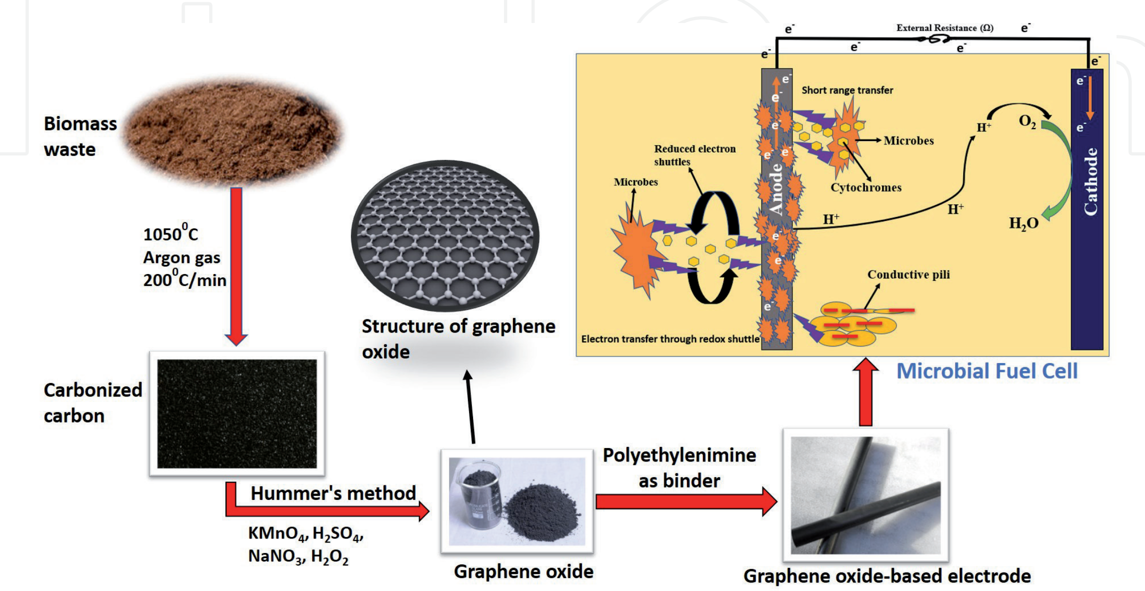

Figure 3.
Systematic demonstration of graphene-based anode fabrication for MFCs by using waste. (Reproduced from Yaqoob et al. [18] with Elsevier permission.)

the metal oxide by using the green synthesis method without any environmental hazard [21, 24]. So, a better idea would be to synthesize the metal oxide by using waste material for fabrication of anode and preferably to prepare composite with carbon-based material to enhance the performance of MFCs.

4.3 Conducting polymer material

The conductive polymer such as polycarbazole, polyaniline, poly-co-o-aminophenol, polypyrrole, polythiophene, etc. also one of the sources to fabricate the anode. Can act as anode due to having excellent electrical conductivity properties. The conductive polymers are providing significant outcomes through modification processes with other materials such as carbon-based, metal/metal oxide. For example, the polyaniline modification with carbon cloth showed better energy output as compared to unmodified material [62]. Similarly, Pandit et al. [63] studied the polyaniline coating on graphite felt and employed as anode to achieve 2.9 W m^{-3} power density, the obtained result was not good due to lack of other parameters such as organic substrate, concentration etc. The polypyrrole was considered as a potential material which exhibited 452 mW m^{-2} power density when modified on the surface of carbon paper [64]. The polypyrrole can enter a microbe cell membrane and transfer the electron by employing a metabolic pathway. Therefore, conductive polymer composites can bring a great revolution to increase the efficiency of anode. Dumitru et al. [65] studied the polyaniline/CNTs and polypyrrole/CNTs nanocomposites as anode. The CNTs/polypyrrole and CNTs/polyaniline nanocomposite showed 167.8 mW m^{-2} and 202.3 mW m^{-2} which is higher than unmodified CNTs. The observed pure CNT power density was 145.2 mW m^{-2}. The conclusion showed that the polymer modification with CNTs decreased the cellular toxicity effect of CNTs toward the microbial community. This reason led to higher energy efficiency of MFCs. The synergistic effect of CNT/conducting polymers shows high electrochemical applications. This is an emerging and promising research direction to fabricate the polymeric-based composite as anode in MFCs. Some mostly utilized metal-based, carbon-based, and conductive polymer-based materials are shown in **Figure 4.**

3D, Three dimensional; CNT, Carbon nanotubes; r GO, Reduced graphene oxide; PANI, Polyaniline; PPy, Polypyrrole; Pt, Platinum; GNRs, Graphene nanoribbons; CP, carbon paper; Ti, Tanium; TiO_2, Titanium oxide; SnO_2, Tin oxide.

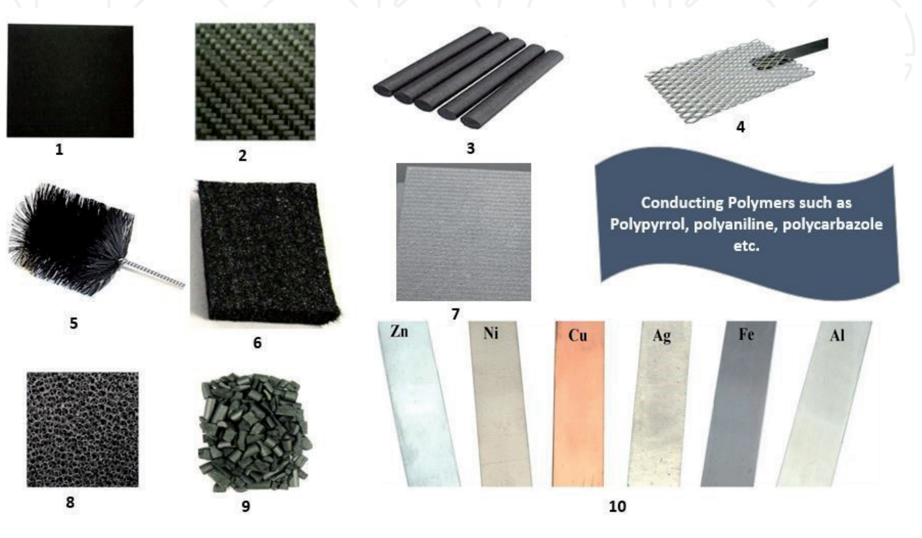

Figure 4.
Mostly used anode electrodes (1) carbon paper (2) carbon mesh (3) graphite rod (4) platinum mesh (5) carbon brushes (6) carbon felt (7) carbon fiber (8) reticulated vitrified carbon (9) graphitic granular (10) different metal electrode strips. (Adapted from Yaqoob et al. [17] with MDPI permission).

5. Characterization tools

The study of MFCs can be performed by using different types of analytical, surface, biochemical, spectroscopic, and bioelectrochemical characterization methods [66]. The morphological (surface) properties analyzed characterization methods, for example, fluorescent microscopy, scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) are very convenient in investigation of the microbial biofilm growth around the surface of anode. To understand the biofilm density, growth patterns, thickness, size and heterogeneity of the biofilm, electrochemical cauterizations are required. They are also suitable in reviewing the electrode porosity and membrane-based materials which were used in MFCs. The organic substrate application, product configuration and extracellular-based mediators in the MFCs and anolyte can be sensed and studied by a diversity of spectroscopic, biochemical, and electrochemical approaches [67]. The biochemical approaches e.g., redox mediator assesses are measurable and assistance in measurement the redox mediator's concentration, while spectroscopic systems such as HPLC, LC–MS and UV–VIS, are also vastly valuable in classifying and then computing the chemical classes. On the other hand, the electrochemical methods like differential pulse voltammetry (DPV), linear sweep voltammetry (LSV), cyclic voltammetry (CV), are qualitative techniques. They help in classifying the mechanisms which are liable for electron shuttling in current peaks form on their changeable electrochemical actions. The CV has been widely used in biological-fuel cells characterization where the mediated electron transference is the main mechanism of electron transportation. The *Shewanella sp.* secretion (flavins) and the extracellular electron transference have been acknowledged via CV and their meditations were then calculated by means of chromatographic techniques. Gradually, study based on alternative electrochemical methods, specifically electrochemical impedance spectroscopy (EIS), is today being employed. In voltammetry, through imposing possible phases or curves, the electrode is determined to a disorder from symmetry and the answer will be detected which a fleeting signal is frequently. Though, in EIS, the scheme is disconcerted with an irregular current of minor magnitude and the method follows the steady state. Additionally, the important benefit of impedance analysis is that the method is non-destructive or non-intrusive [68]. It can be achieved in the working MFCs without troubling the arrangement, while other characterization apparatuses deliberated above need the MFCs to be concerned and the products are being calm for succeeding analyses. Later, EIS is investigated in a stable state of bioelectrochemical method wherever the analyses are done without changing the current–voltage characters of the fuel cell system [69]. EIS has also been extensively studied in different zones of bioelectrochemical investigation such as fuel cells and corrosion. Moreover, EIS is a valuable tool to examine the influence of diverse internal resistances to the total impedance of the MFCs.

6. Current challenges and future perspectives

The MFCs received a lot of attention in the modern era and offered significant results. There are some modern challenges which still need attention for high electrochemical performance. The utilization of conventional electrodes has failed to improve the electric efficiency in terms of MFCs. There are various challenges related with anodes which create a hurdle in the case of MFCs applications at a commercial scale:

i. Anode materials should be economically stable for MFCs practical applications. So, making a cost-effective anode material is considered a modern challenge. For example, the high conductive graphene can offer significant results, but the cost of graphene exceed the economic feasibility of the approach. To address this problem, the effort should be on waste derived highly conductive materials (such as graphene, its composite with metal oxides/polymers) and formulate it according to the required anode design/architecture. The waste-derived resources are a virtuous source for fabricating the modified updated carbon-based anode [70].

ii. The anode development also faced another issue which is related to the binder material. It is very significant for material fabrication in any required shape. The binder selection is an important step for a researcher because this material is used to improve the properties of material, as better cohesive and firm. It is necessary to find further appropriate and economically favorable binders for development of the anode electrodes [71]. The binder played a vital role in composite anode because it was the capability of binder which binds the two materials very effectively and as a result enhanced the electron transportation rate. According to our knowledge, there is no wide-range study on the material as binders for fabrication of electrodes.

iii. The design (foam, sheet, brushes, rod, cloth,), size (length and width) of anode electrodes are very significant parts in the fabrication process. The enough surface area, conductivity, electrode spacing, of anode electrode are answerable for electron transportation from the anode in MFCs. The proper designing according to the MFCs setup drawing is very critical. The proper and feasible size and design can bring to the effective outcomes [72]. The high surface area can offer better opportunities to microbes for their respiration.

iv. Furthermore, to enhance the conductivity for the anode electrodes, the modification steps are very important in MFCs particularly in improving the power generation. Though, the appropriate mechanisms are still indistinguishable. Researchers must find a further appropriate mechanism for alterations in anodes which can be carried out more professionally. The modification refers to the composite-based anodes i.e., graphene/metal oxide, graphene/polymers, metal oxide/polymers, CNTs/metal oxide, CNTs/polymer [73]. The modern composite anode can be ideal to overcome the electron transportation issue and can bring the MFCs to the commercial status.

v. Additionally, the anode long-term stability is one of the important aspects which decreases the chances of MFCs implementation. Usually, some MFCs setup are taking a very long operation such as 4–6 month where the anode should be stable to work properly until the end of operation. The stability losses might be due to the less stable anode material or utilization of less efficiency binder materials [73–76]. Many researchers already reported that the stability of anode is a challenging step but still so far there is no proper mechanism studied to overcome this issue.

7. Conclusion

This chapter highlights the importance of anode electrode for MFCs. The anode electrodes are considered as a main fragment of MFCs approach and for survival of the biocatalyst such as microbial communities. For anode electrode development, the important growth is microbial attachment which is usually referred to as biofilm development. Substantial energies have been engrossed to raise the electrode surface area and conductivity. The high-tech electrode materials lead to the high biofilm densities. Additionally, there is still a lack of mechanism for proper development of the advanced anodes. Electrode materials are essentially very constant in the wastewater atmosphere in the MFCs process for long-term. The highly conductive materials such as graphene derivatives, metal oxides composite which can be used as anode electrode for better electrochemical performance of MFCs. Currently, the anode materials cost problem and indistinct mechanism of modification delays the modification approach in MFCs. Hence, cost effective, proper availability of materials and effective approaches for metal, or polymers nanocomposite or carbon-based type anode electrodes should be familiarized to implement MFCs applications.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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