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Review

Microbial Fuel Cell, Their Type, Working Principle and Different Factors Affecting Their Performance: A Review

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Abstract: Microbial Fuel Cells (MFCs) are a green technology and an alternative energy resource to fossil fuels. MFCs are a class of bio-electrochemical systems with novel properties, like wastewater treatment, electricity generation, and biosensor operation. MFCs are ingenious devices that harness the power of bio-electrochemical processes to generate electric current by breaking down organic waste found in wastewater. These systems establish a fascinating connection between microbial metabolism and electricity production. The microbes within MFCs thrive on the nutrients present in their environment and convert the energy stored in organic matter into usable electricity. This electrical energy can be effectively utilized to power various essential portable electronic devices such as mobile phones, laptops, TVs, air dryers, threading machines, chargeable torches, as well as devices used in the air force, outer space, and weather stations. The maximum power produced by an MFC using an iron anode is 170 $mW \cdot m^{-2}$ at 0.645 V, while MFCs with mixed culture microbes show better power efficiency at 30 mA and 3,600 $mW \cdot m^{-2}$. It has been observed that MFCs equipped with carbon-based electrodes tend to have a longer lifespan compared to those using metal-based electrodes. However, one drawback of carbon-based electrode MFCs is that they generally exhibit lower power output. In recent years, increased investment in MFC research has significantly improved the analysis of their chemical, microbiological, and electrochemical aspects. These advancements have led to notable enhancements in the sensing capabilities of MFCs. In this review, we summarize MFC technology, including working principles, types, composition, and the various factors that affect MFC performance.

***Keywords*:** microbial fuel cell, green energy, fossil fuels, greenhouse gasses, wastewater

1. Introduction

Due to the fast growth of the global population coupled with extensive increases in the world's GDP, the domestic and industrial energy demand has risen to 30%. According to International Energy Agency (IEA) report. About 70% of the world`s total energy is derived from fossil fuels which are about 12 billion tons per year and this amount is expected

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to rise to 18 billion tons by 2035.[1] The environmental effects of fossil fuels hold it back from being a favored energy resource in recent future.[2] Reliable and clean energy sources are required for sustaining world energy demand, currently nuclear power plants are in operation and are meant to be alternative resources of energy.[3] The risk associated with nuclear plants is enormous and the destructive effect of radioactive elements ruins the safe and dependable application of nuclear plants.[4]

In the future, the approach to clean water will be a big issue in various regions of the earth which will have a bad influence on agriculture growth and portable water. Recycling of wastewater become compulsory for clean drinking water and agriculture purposes. Current wastewater purification plants consume quite a lot of energy. Rendering to fresh reports, in the USA, wastewater recycling plants consume about 5% of the total energy consumed in the country, that meant to be a vast load on the energy era.[5] While microbial fuel cells (MFCs) can be accessed to treat wastewater in economical and maintainable systems. Microbial Fuel Cell is a kind of device that produces electricity by chemical reactions. It consists of two chambers namely a cathode and anode.[6] In an anode oxidation reaction occurs the result electrons are generated that travels throw an external circuit to the cathode where a reduction reaction takes place that results in the production of current.

In this review, we have summarized the MFCs, their working principle, types, composition and the various factors which affect the performance of these MFCs.

2. Wastewater as a renewable source of bio-energy

The wastewater which is discharged from sewage, industries and food processing factories contains a high amount of organic substrates, like feces or urine, carbohydrates, protein, vitamins and lipids.[7] The bio-degradable organic waste present in wastewater is decomposed by microbes and converts the chemical energy into electrical energy. The phenomena can be achieved by using microbial fuel cells.

In this review paper the microbial fuel, types of microbial fuel cells (Single chamber, double chamber, mediator and mediator less microbial fuel cell, its working principle, type of losses and factors affecting their performance of microbial fuel cell will be discussed briefly.

3. Microbial fuel cells

Microbial fuel cell is a green technology that produces current by degradation of organic waste present in wastewater through a bio-electrochemical process which is connected to the metabolism of microbes to generate electrical energy.[8] MFC contains microbes that utilized the nutrients in their surrounding environment and relishes energy present in the food in the shape of electricity.

3.1 *Early discoveries*

• In 1911, M. C. Potter conducted an experiment that showcased the generation of electricity through microbial activity using a plant-based microbial fuel cell.

• Ludwig and Charles Langer build the first MFC device by consuming air and industrial coal gas and they titled the device as fuel cell.[9]

• In 1931 Branet Cohen made a microbial half-power module that generated 35 millivolts and 2 milliampere current just when associated in continuous arrangements pattern.[10]

3.2 *Improving performance*

During the late 1990s to early 2000s, notable progress was made in the field of microbial fuel cells (MFCs), primarily driven by advancements in materials and design, resulting in significant improvements in power generation capabilities.

One breakthrough was the adoption of carbon-based materials as electrodes, which significantly enhanced the conductivity within MFCs.

Additionally, the introduction of proton exchange membranes played a crucial role in improving the separation between the anode and cathode, leading to more efficient MFC performance.

3.2.1 *Introduction of mediator-based MFCs*

In the early 2000s, a pivotal development in microbial fuel cell (MFC) technology was the introduction of mediators, also known as electron shuttles. These mediators facilitated the transfer of electrons between microorganisms and electrodes, resulting in two significant advancements.

Firstly, the incorporation of mediators led to enhanced power output in MFCs, significantly increasing their electricity generation capabilities.

Secondly, this breakthrough broadened the range of microorganisms capable of generating electricity, unlocking new possibilities and potential applications for MFC technology.[11]

3.2.2 *Mediator-less MFCs*

In the mid-2000s, a groundbreaking discovery was made in the field of MFCs with the identification and application of electroactive microorganisms capable of direct electron transfer. This advancement eliminated the need for mediators in MFCs, simplifying the design and making the technology more sustainable. These electroactive microorganisms could directly transfer electrons to the electrodes, streamlining the MFC setup and paving the way for more efficient and environmentally friendly designs.[12]

3.3 *Scaling up and practical applications of MFCs*

The 2010s: Increasing interest in large-scale applications, such as wastewater treatment and energy production, result in the exploration of hybrid systems combining microbial fuel cells with other renewable energy technologies.[13]

3.4 *Advanced materials and engineering*

Ongoing research and development focusing on improving electrode materials, such as nanomaterials and bio-inspired designs and Integration of MFCs with other technologies, such as microbial electrolysis cells (MECs) for hydrogen production.

3.5 *MFC structure*

A typical double-compartment MFC consists of two compartments namely anode and cathode chambers which are connected by an external circuit and a proton exchange membrane (Figure 1). In the case of a Single-compartment MFC the cathode is directly exposed to the air which offers a simpler and budget saving design while various MFCs structures have been planned and distinctive materials for the development have been tested but it always been quite difficult to calculate the ideal performance of MFC, which mainly depends upon the nature of biochemical reactions, organic substrates and population of microbes.[13] A polarization bend curve of an MFC could show to which degree the different variables add to the general potential drop. Subsequently, extraordinary measures may be taken to enhance the MFC execution, including MFC setup, terminal structure, arrangement and sort of microbial population.[14] Organization and structure of terminals are two components worth being examined keeping in mind the end goal to enhance the effectiveness of MFCs.

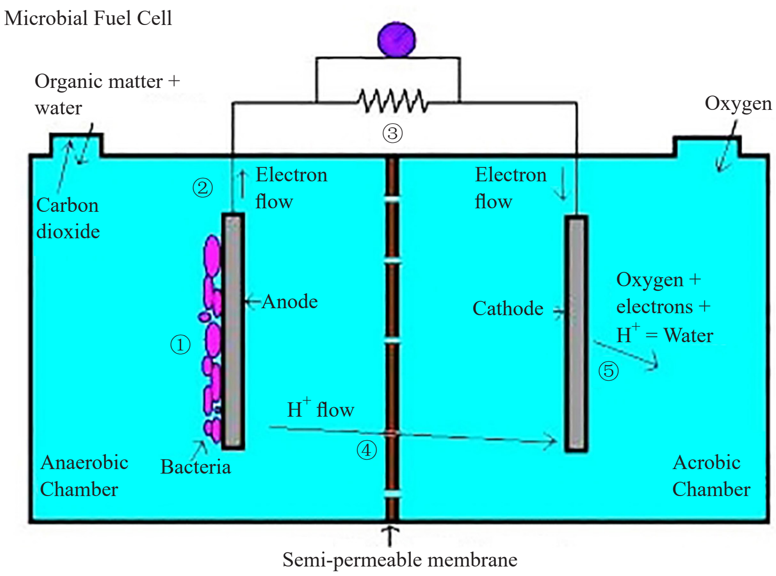

Figure 1. A typical working microbial fuel cell[15]

3.6 *Working principle of MFC*

Bioenergy is observed as an efficient technique to lessen the present global warming. Renewable resources are greatly, anticipated for the production of electricity without net carbon dioxide emission.[16] Microbial fuel cells provide a source of electrical energy by breaking chemical bonds of organic compounds and relishing electrons the process is achieved through catalytic reaction of microorganisms.[17] For biodegradation of organic waste present in wastewater while bacteria are used that relies on electron which travels through the electrode surface. At anode, microorganisms(bacteria) oxidize the substrates as a result it generates free electrons and protons while in the process CO_2 is formed as the oxidation product.[18] In the process, net gain of carbon dioxide is zero because the CO_2 initially comes from the atmosphere through the photosynthesis process. While electrons are transported to the cathode by an external conductor and protons travel throw a salt bridge, enter the cathodic chamber and react with atmospheric oxygen resulting in water.[19] In a dissimilar process of oxidizing microbes of anodic chamber produces protons and electrons for degradation of substrate in anaerobic conditions it is necessary to keep microbes away from the atmosphere or another incurable acceptor. Cell reaction continues until degradation of organic compound is completed.

4. Overall cell reaction

Overall cell reaction containing carboxylic acid.

$$\text{Anodic reaction: } CH_3COOH + 2H_2O \longrightarrow 2CO_2 + 6H^+ + 6e \quad (1)$$

$$\text{Cathodic reaction: } O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O \quad (2)$$

The general reaction is the breakdown of the organic substrate into carbon dioxide and water while electric current is produced as by-product. MFC bioreactor produces power from the electron spill out of the anode to the cathode in the outer circuit.

Currently, there are two types of MFCs that are in operation namely mediator and mediator-less MFCs. A mediator-less MFC requires no mediator to transfer an electron from the bulk of the solution to the electrode surface but uses electrochemically active bacteria to transfer the electrons to the surface of the electrode.[20] These MFCs show vital potential for generating bioenergy from waste organic matter present in water. Carbohydrates and other bio-wastes also can be used to generate electricity in MFC. On the other hand, in the case of mediator-MFCs, the direct transfer of electrons from the bulk of the solution to the electrode surface occurs at a low rate. To enhance the electron transfer rate an electron transfer mediator is employed such as thiamine and methyl red etc.[21]

In microbial fuel cells, microorganisms oxidize organic substrates and produce free electrons and H^+ ions in the anodic chamber. The H^+ ions are transported to the cathode through the hydrogen transfer membrane or the filter media (in the case of membrane-less MFCs). However, the electrons are transported to the anode through various routes depending on the nature of the microorganisms used. Two major routes, that are, direct and indirect routes, for the electron transfer from the bulk phase to the anode surface in MFC have been reported.[22] In the direct route, microorganisms themselves transfer electrons to the anode through physical contact between the bacterial cell membrane and the electrode surface via cytochrome or putative nanowire structures.[23] However, a soluble redox active compound (mediator) is responsible for electron transfer in the indirect route. Some microorganisms can release redox-active compounds that are responsible for indirect electron transfer to electrodes. Mediators are added to promote energy production in an MFC when bacteria cannot transfer electrons alone.[24] The reduced mediator is readily oxidized at the anode, and the oxidized form of the mediator shows favorable kinetics for reduction by the microbes.[25] The addition of mediators may influence the metabolic properties of organisms. In a mediator, MFC commonly used electron shuttles or exogenous mediators are various dyes such as neutral red, methylene blue, thionine, azure A, and anthraquinone-2,6-disulfate.[26] Mediators are generally expensive and toxic compounds have short lifetimes. Potassium ferricyanide and humid acids have also been used as a mediator. The high consumption rate and the risk associated with these compounds make their use practically not viable.

5. Types of microbial fuel cells

There are several kinds of microbial fuel cells currently operating which are mediator MFC, meditator less MFC, single chamber MFC and double chamber MFC.

5.1 *Mediator microbial fuel cell*

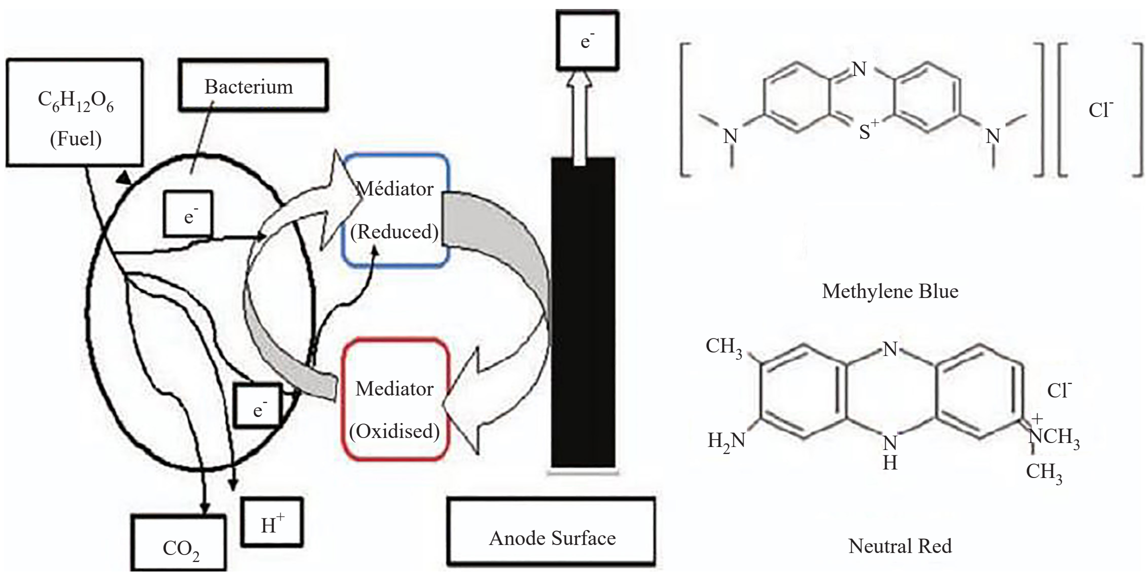

Figure 2. Mediator microbial fuel cell diagram[28]

A microbial fuel cell requires mediators for the transportation of electrons from the fermentation center into the electrode surface. Thionine, methyl, viologen, methyl blue, humic acid, and neutral red are mediator compounds used in mediators MFC they work as a redox reactions, mediators are reduced at bacterial surface gain electrons and are oxidized at the electrode surface releasing electron.[27] Figure 2 shows the transfer of electrons from the bacterial surface into the electrode surface by methylene blue and neutral red. This procedure is frequently poisonous and more expensive due to the materials required.

5.2 *Mediator less microbial fuel cell*

In mediator-less MFC electrochemically active bacteria are used to transfer the electrons which are produced in the bulk of solution to the surface of the electrodes (Figure 3). The most common bacteria which are used are Shewane*lla* putrefaciens and Aeromonas hydrophilia.[29] The factors which prevent the best performance of mediator-less MFCs are the type of bacterial strain used, the type of ion exchange membrane and the situation of the system.

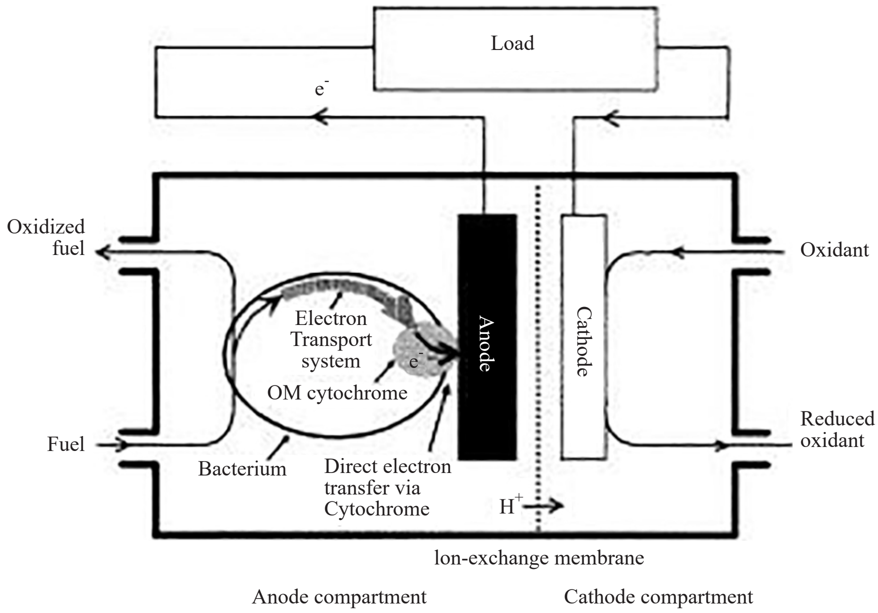

Figure 3. A mediator less MFC scheme[30]

5.3 *Phototropic Biofilm fuel cell*

These MFC contain a Biofilm of photosynthetic bacteria at the anode which are providers of organic metabolic and electron contributors (Figure 4). The biofilm and electrochemical study genetic factor profiling of bio-cathodes of seawater constructed microbial solar cells (MSC) injected from bio-cathode of already presented seawater created microbial solar cells, the second generation of microbial solar cell catalytic activity, bio-cathode reduced with time if lighting is given in process of growth, in this process the growth is done in the dark.

5.4 *Solid based microbial fuel cell*

The solid-based microbial fuel cells are made up of soil anodic media and proton exchange membrane (Figure 5) The electrodes or anode is fixed in the soil at a definite distance and the cathode rests on the upper part of the soil visible to the air oxygen.[31] As it is known that soil contains the he MFCs. Soil contains some aerobic microorganism which means that these decompose oxygen while working as a filter which is compared to the PEM components used in the laboratory.

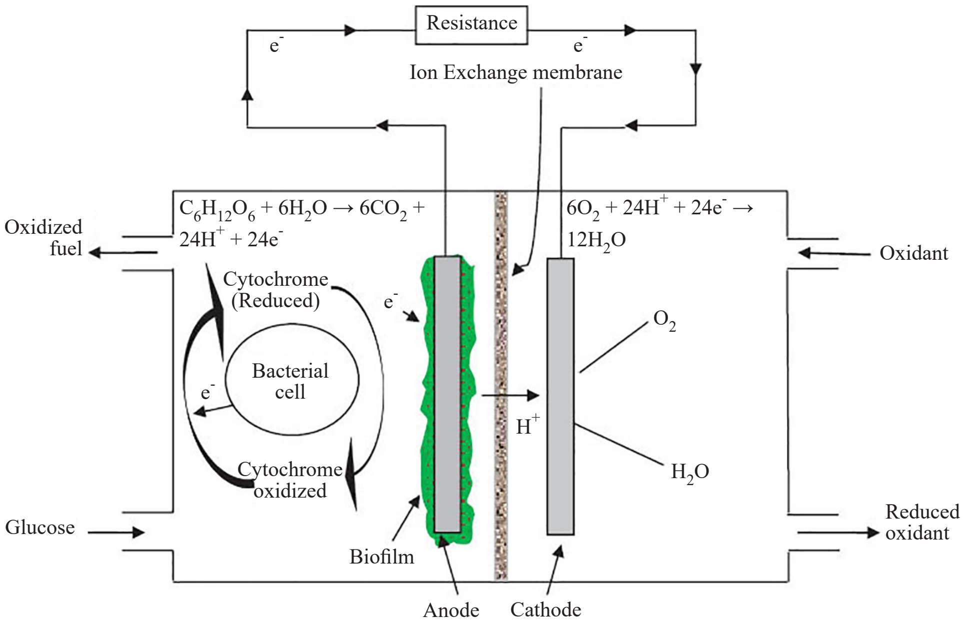

Figure 4. Phototrophic biofilm microbial fuel cell[1]

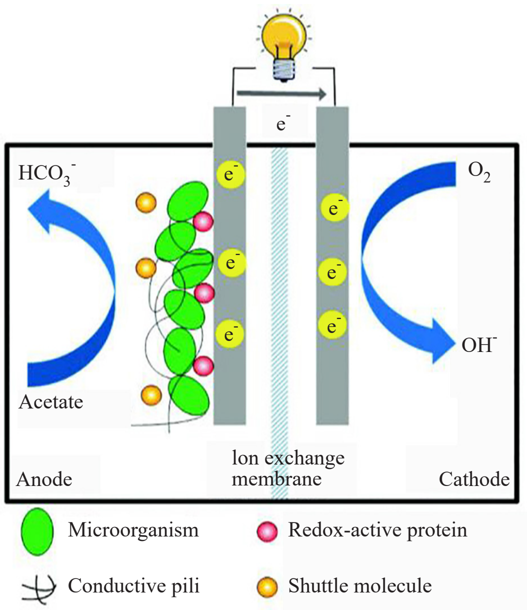

Figure 5. Solid based microbial fuel cell[32]

5.5 *Nano-porous membrane microbial fuel cell*

Are the type of MFC that are capable to work in acquis aerobic environmental conditions? Nano-porous membrane microbial fuel cells are charged by nutrient distribution the other MFCs are charged by the energy-demanding pumps.

Nano-porous membrane microbial fuel cells (NMMFCS) extract the electrochemically active microorganism in the device to depend on those electrochemically active microorganisms which are already present in the surrounding to permit it to be utilized in the aerobic aqueous medium.[28] The plus point of this type is that it is made of a non-expensive Nano-porous membrane which is composed of polycarbonates or other materials. These designs can generate currents from microwatts to mile-watts. The materials used for the construction of Nano-porous membrane microbial fuel cells are given in Table 1.

Table 1. Materials that used for the construction

Items	Materials
Anode	Graphite, graphite felt, carbon paper, carbon cloth, Pt, zinc, Cu.
Cathode	Graphite, graphite felt, carbon paper, carbon cloth, Pt, zinc, Cu.
Proton exchange membrane	Salt bridge, porcelain septum, semipermeable membrane
Electrode catalyst	Polyaniline, Microbes and electron mediators

6. Design and component of microbial fuel cell

There are fundamental parts of MFCs that are critical in development. Electrodes, wirings, and salt bridges have a critical part. A salt bridge is supplanted with Proton exchange membrane in PEM power device there are several components of microbial fuel cells which are extremely essential for the development. Electrode materials, salt-bridge and wirings are critical for the better efficiency of cells. The anode carbon-based electrode is commonly used, and the proton exchange membrane is mostly used as a separate for better efficiency of MFCs.

7. Electrode material used in microbial fuel cell

The assortment of the correct electrode material is critical for the performance of microbial fuel cell in the case of bacterial union, electron transference and electrochemical efficiency. There are many studies to scale up the power production using different carbon-based materials such as carbon paper, carbon felt, carbon fiber as well as carbon nanotube-based composites.

7.1 *Anode material*

The electrode used in the anode is a conductor that facilitates electrons to travel from anode to cathode and low resistance, chemically stable and suitable mechanical strength dictate the choice of electrode.[33] The electrode in an anode chamber must have a large surface area for better efficiency that provides decent attachment of bacteria to the electrode surface. Platinum, graphite and carbon electrodes are mostly used as anode, due to their greater surface area high conductivity and mechanical strength, carbon materials with nanotechnology have attracted the researcher to be used more frequently as anode.[34] Also, their dimensional electrodes are being utilized like graphite particles and granular activated carbon is being utilized for the larger surface area for the attachment of bacteria and the work of MFCs.

7.2 *Carbon-based electrodes*

Carbon-based electrodes are frequently employed in microbial fuel cells owing to their biocompatibility with high toughness, excellent conductivity, and affordable cost, carbon electrode exhibit excellent morphological and structural features for scheming attractive and effective electrodes. These electrodes enhance the accessory of bacteria by quickening. Electrochemically Active Bacteria (EAB) formation and gives an efficient conductance path for the Extracellular Electron Transfer (EET) of electron.[34]

7.3 *Graphite*

Graphite is an allotropic form of carbon with high constancy and conductivity which is considered as useful stuff for bio-electrodes in microbial fuel cells.[35] Different types of graphite electrodes has been used in MFC like brushes, rods, foams and felts. Graphite anodes in a mixed culture of Rhodoferax and Ferrireducens microbes and glucose as substrate yield a greater ampere than graphite rod, while graphite felt to have high porosity which causes dense biofilm increase on the anode, which enhance oxidation of glucose substrate at anode.[36]

7.4 *Mesh, carbon cloth, foam, felt and paper*

Other normally utilized carbon substances in MFCs incorporate carbon paper, mesh, and felt. Carbon froth got from a sponger's normal item Pomelo strip created higher current thickness over graphite felt.[37] Carbon froth got from regular natural items demonstrated wrinkled surface and high porosity for productive biofilm development (Figure 6). As of late, sticky carbon paper cathodes covered with conductive polymers showed preferable bio anode execution over carbon felt anodes by giving vast pore size to the bacterial attachment[37]. It is realized that the virgin types of carbon fabric and felt counteract thick biofilm arrangement at the bio-anode because of their hydrophobicity and less permeable structures. Thus, the surface of bio-anodes ought to be pretreated before their applications in MFCs for better biofilm development. Utilization of an incompletely oxidized carbon felt gotten by UV/O_3 treatment as the bio-anode incredibly enhanced the execution of MFC when contrasted with untreated carbon felt through improved biofilm development.[38] The partial oxidation of carbon felt created oxygen-containing functional groups at the bio-anode surface that caused an increase in hydrophilicity, a favorable property for bacterial attachment.[37] The incomplete oxidation of carbon felt made oxygen-accommodating practical gatherings at the bio-anode surface that provoke an expansion in hydrophilicity, an ideal property for bacterial attachment, activation of carbon felt through nitric acid reaction additionally indicated better execution over other commercial carbon felt in MFCs.[39] The enhanced execution of MFC with nitric corrosive treated bio-anode was potential because of an impressive decrease in the anodic obstruction and an increase in surface harshness for bacterial connection. Basic warm treatment of carbon work and paper can essentially build the power age of MFCs as the warmth treatment can evacuate polluting influences in the bio-anode that impede the conductivity of anode.[40] A remarkable improvement in the MFC performance was observed by employing plasma-modified carbon paper as the bio-anode. The plasma presentation modified the surface premises, of carbon paper, for example, hydrophobicity by presenting H^+ particles, which promoted electron exchange to the bio-anode treatment of carbon mesh with NH_3 expanded the power yield of MFCs because of nitrogen dipping. Liu et al. revealed that basic drenching of carbon cloth in HCOOH could support the execution of bio-anode by making a cleaner surface and lessening oxygen contented identically, treatment of carbon fabric with H_2O_2 and HCl enhanced the property of microbial fuel cell MFCs. Generally, bacterial cell surfaces are negatively charged and hydrophilic. Hence, the introduction of positive charges and hydrophilicity on the bio-anode surface is a good strategy to develop efficient biofilm for better MFC performances. Chodkowski et al. systematically studied the influence of surface charge and hydrophilicity on the MFC performance by generating various functional groups on carbon-based bio-anodes.[41] Positively charged bio-anodes with hydrophilic nature showed excellent bio-electrocatalytic performance. In addition, this study provided explanations on why acid, thermal, ammonia, and plasma pretreatment improved the bio-anode performances. Normally, bacterial cell membrane bears a negative charge and can dissolve in water. Hence, the presentation of +ve charges and hydrophilicity on the bio-anode surface is a decent methodology to create a productive biofilm for better MFC performance. Kalathil et al. methodically examined the impact of surface charge and hydrophilicity on the MFC performance by producing different functional groups on carbon-based bio-anodes, the examination inferred that emphatically accused bio-anodes of hydrophilic nature demonstrated fantastic bio electrocatalytic performance. Also, this investigation gave clarifications on why acid, NH_3 and plasma treatments enhanced the bio-anode performance.[42]

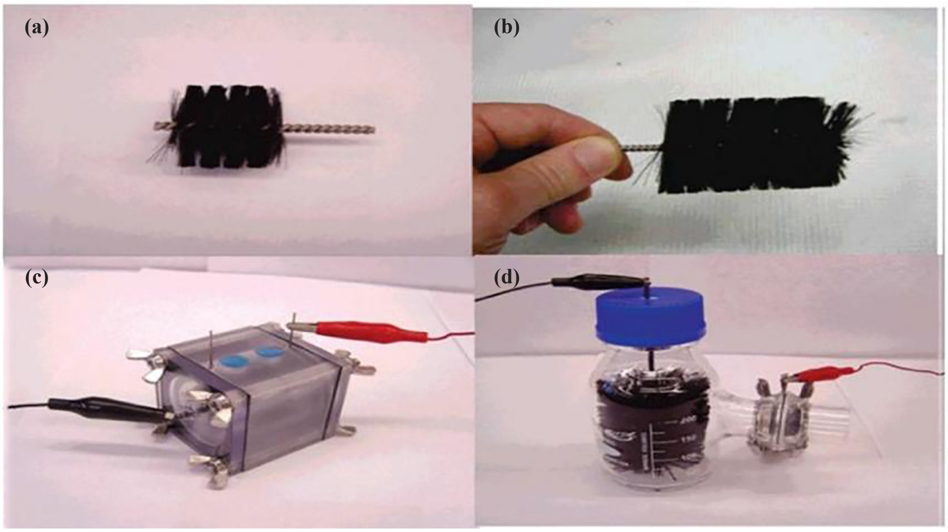

Figure 6. Cubic-MFC in which Graphite fiber brush anode electrode is used. (c-MFC)-Bottle-MFCs (b-MFC) (a) and (b) contain brush electrodes, both (c) and (d) MFCs contain brush anode[42]

7.5 *Stainless steel (SS)*

Treated steel (SS) is a phenomenal metal-based material option in contrast to carbon-based materials as the MFC bio-anode because of its extraordinary mechanical properties, electrical conductivity, and stainless property (Figure 7) (e.g., of high review SS materials).[43] It is quite easy to scale up and offers strength for long-haul activity of MFCs. As of late, a few examinations have detailed SS as proficient bio-anodes for delivering stable current densities. A plain SS bio-anode beat level graphite cathode by creating a higher current under indistinguishable test conditions, the plain SS isn't enough to grow thick and strong biofilm, which may stop generating summit current from the MFC. To handle this subject, Roubaud et al. initiated a three-dimensional SS froth with fitting porosity as the bio-anode for MFCs.[44] The 3D SS froth delivered higher current than plat SS (multiple times) and carbon cloth (multiple times). Surface change of the SS additionally ended up being a variable methodology to enhance the performance of SS-based anodes.[45]

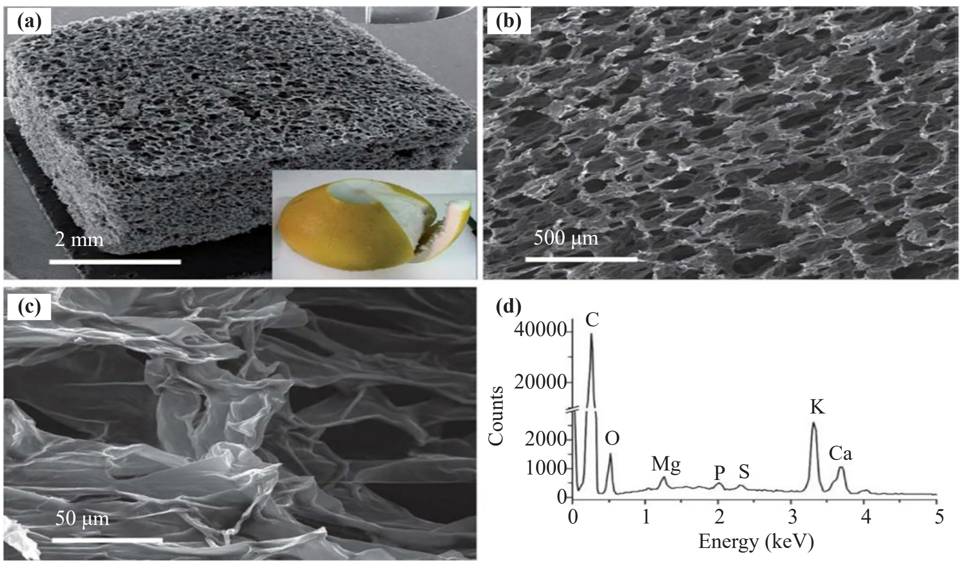

Figure 7. (a), (b) and (c) SEM of reticulated carbon foam-pomelopeel (RCF-PP) at different magnifications, (d) energy vs count compression of carbon foam in different metal ions[46]

7.6 *Ceramic electrodes*

ceramic-based bio-anodes appear to guarantee anode materials for pragmatic uses of MFCs. There are numerous investigations accessible on earthenware MFCs with exceptional current outputs and ceramic gives a helpful domain to bacterial attachment forming a thick bio-film that show fantastic dependability under extreme wastewater conditions. For instance, ice-templated titanium-based ceramics (ITTC) bio-anode yield current of 128.7 A/m^2 (Figure 8).[46]

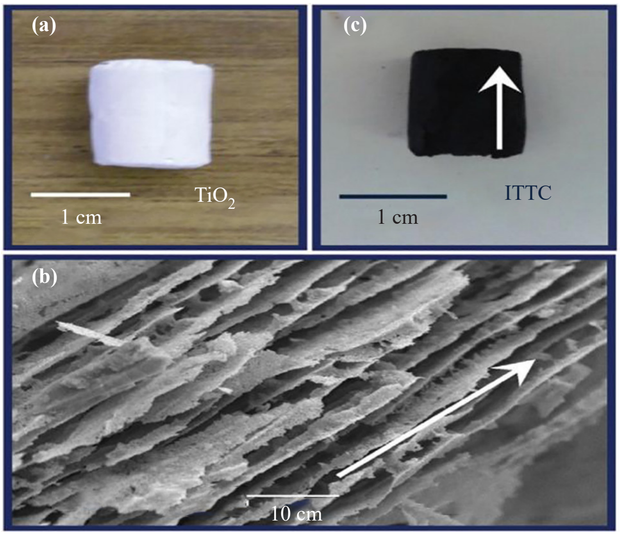

Figure 8. (a) 3DTiO_2 porous scaffold, precursor of ITTC electrodes, (b) SEM image of the internal architecture of theTiO_2 scaffold and (c) ITTC electrode obtained by reduction of TiO_2 porous scaffold[46]

8. Cathode materials

Like the anode, the cathode is additionally a vital piece of MFCs. Normally, apathetic substrate reduction reactions on the surface of the cathode attribute underprivileged catalytic actions of substance primarily limit MFCs performance.

The advancement of better working cathodes has an enormous need to enhance the performance of MFCs. The majority of the anode materials utilized as anodes have additionally been utilized for the cathode advancement. Two classes of cathodes are named abiotic and biotic cathodes.

8.1 *Abiotic cathodes*

These electrodes normally perform reeducation of oxygen resulting in the production of water abiotic cathode are more favored in the occurrence of catalyst. Platinized carbon electrodes is normally used as a reducing agent for oxygen at cathodes in MFCs.[1] But the Pt-based cathodes are costly and catalyst-destroying character disabled them in commercial uses likewise Pt electrodes are substituted by the least expensive electrodes such as heteroatom doped carbon, Fe_2O_3 and Mn_2O_3.[47]

8.2 *Biotic cathodes*

The most fascinating characteristics of bio-cathode are self-manageability and high steadiness in harsh wastewater states. Kalathil et al. revealed that the present age of a MFC with the biotic cathode expanded with time and balanced out following two months of constant MFC task, which was higher than an abiotic cathode.[42] Activated Carbon bio-cathode proved better performance in the cause of wastewater treatment, producing higher ampere and stability. Some chemical species such as oxygen, nitrate and sulfate have the ability to act as electron acceptors at the cathode with denitrifying bacteria in these because the anaerobic condition is must.[42] The primary preferred standpoint of anaerobic bio-cathode over aerobic bio-cathode is that it can anticipate oxygen dispersion to the anode that can generally unfavorably influence the anodic biofilm. Table 2 summarizes earlier used cathode and anodic materials.

Table 2. Some cathode and anode materials been used before

Anode	Cathode	Power (mW·m^{-2})	Open circuit voltage (V)	References
Graphite anode	Graphite fiber	7.5	0.400	48
Graphite fiber	Graphite fiber	87.85	0.664	49
Carbon felt	Carbon felt	56.4	0.158	50
Graphite felt	Iron	63	0.431	51
Iron	Graphite	170	0.645	51

9. Separating system in double chamber MFC

Microbes in the anode chamber oxidize organic compounds and electrons flow to the external circuit through the anode but the protons formed at the anode compartment travel throw the separator to the cathode while the separator can be a proton exchange membrane or a salt bridge.[52]

The proton exchange membrane is one of the necessary components for double chamber MFC for the transfer of protons, PEM has a big effect on the performance of MFC and it is mostly used separator in double chamber MFCs because of its great conductance of positive ions and low internal resistance related to other separators.

Others separators like Cation exchange membranes are also frequently employed in most commercial MFC for shifting protons from anode to cathode.

The performance of the separator is mediated according to its capability to transport of protons from anode to cathode. The separator must prevent flow or transfer. Moreover, they must prevent the transfer of oxygen from the aerobic cathode to the anaerobic anode and materials or minerals from the anode to cathode chambers. furthermore, the membranous separator has a big effect on the MFC current output because it has a poor proton transfer rate, but this issue is yet not been researched in depth.[1]

10. Microorganisms

Some microorganisms are proficient in removing electrons coming from the metabolism of organic matter while Shewan Ella, Rhodoferax and Geobacter are Iron-reducing bacteria such species are believed to be the most effective in transferring electrons.[53] They form a biofilm on the anode surface and transfer electrons directly to the anode by conductance through their membrane. The above-stated species belong to dissimilar metal-reducing microorganisms that produce energy in the form of ATP during the reduction of metal oxides (i.e. Fe (III) and Mn (IV) oxides) under anaerobic conditions in soils and sediments, where electrons are transferred to Fe_2O_3 mainly by direct contact. An MFC operated with these species is very similar to this natural process, with the anode acting as the final electron acceptor substituting Fe (III) and Mn (IV) oxides.[54]

To acknowledge, how MFC work, it is essential to have some data about some basic functions of the bacteria. In basic principle, the bacteria degrade organic compounds and reveal energy in the process. Those bacteria which have the strength to generate electricity and to transfer electrons in the anode will be in focus. These types of bacteria are named Exoelectrogens. Some anaerobic bacteria just permit the electron to soluble compounds such as nitrate or sulfate that can prolix across the cell membrane and into the cell. The Exoelectrogenic bacteria are much favored for the function of

MFC because they transfer electrons outside of the cell easily.[55]

This group of bacteria is useful in mediator-less MFC, which do not require a mediator for electron transfer. The Exoelectrogens can be situated in different areas while they are obtained in soil, marine sediment, wastewater, fresh water sediment and activated sludge.[56] Such are rich in these microorganisms. A fascinating association is found between fungi and Exoelectrogens in current research that increase the strength of electron transfer as fungi act as a natural organic mediator. This can be a significant step toward scaling up MFC systems as fungi and bacteria can be found naturally. Exoelectrogens have the capability to produce electricity in mediator-less microbial fuel cells (MFCs) by extracellular electron transmission to the anode.[57]

10.1 *Pure bacterial culture*

These bacteria generally show high electron transfer efficiency but a slow growth rate, a high substrate specificity and low energy transfer efficiency.

The use of pure culture implies a continuous risk of contamination of the cell with undesired bacteria.

10.2 *Mix bacteria culture*

- Higher resistance against process disturbance.
- Higher substrate consumption rate.
- Smaller substrate specificity.
- Higher power as compared to pure culture.
- Low risk of cell contamination.

Table 3 summarizes the microbial fuel cell experiments with different microorganisms, substrates, anode materials, current values and power density.

Table 3. comprehensive table summarizing the microbial fuel cell experiments with different microorganisms, substrates, anode materials, current values, power density

Microbe	Substrate	Anode	Current (mA)	Power (mW/m²)	References
Shewanella putrefaciens	Lactate	Woven graphite	0.031	0.19	58
Geobacter sulfurreducens	Acetate	Graphite	0.40	13	59
Rhodoferax Ferrireducens	Glucose	Graphite	0.2	8	60
Rhodoferax ferrireducens	Glucose	Woven graphite	0.57	17.4	60
Rhodoferax ferrireducens	Glucose	Porous graphite	74	33	61
Mixed seawater culture	Acetate	Graphite	0.23	10	62
Mixed seawater culture	Sulphide/Acetate	Graphite	60	32	63
Mixed active sludgy culture	Acetate	Graphite	5	-	64
Mixed active sludgy culture	Glucose	Graphite	30	3,600	65

11. Extracellular electron transfer mechanisms between microbes and electrodes

The important constituents of the respiration process and metabolic pathways are electron transmissions (TET), in numerous living organisms, as well as microbes. The terminal electron acceptors which have the maximum potential are used by microorganisms to improve their achievement of energy of the electrons gained from the electron presenter are transferred by this presented potential and their TET passages are adjusted in this way. The soluble electron acceptors adopt fermentation metabolism or start using insoluble compact electron acceptors as they have restricted accessibility in the environment, then the achievement of the final reduction reaction of the so-called extracellular electron transfer (EET) process is performed, which causes the shifting of the electrons by the microbes outward of the cells (Figure 9). Correspondingly, insoluble electron donors could be oxidized by microbes through Electron transfer in soluble electron donor exhausted atmospheres. Electron acceptors or contributors are hard minerals or huge organics in natural surroundings that are used by microbes. Such as MFCs, the anode and cathode act as electron acceptors and donors in engineered surroundings, correspondingly. In microbial ET to the anodes as part of the main process as well as recognized and acknowledged thus far are shown in Sharma et al.[66]

Indirect and direct Electron transfer mechanisms as a result of the microbial reagents shift the formed electron of microbial metabolism to the anode. Electron transfer follows through self-secreted or exogenous redox mediators in an indirect Electron transfer mechanism and by means of the oxidation of condensed primary metabolites for example format and H_2. Close corporal interaction of the microbial cell to the anode is essential in the direct Electron transfer mechanism. Cellular membrane-spanning (periplasmic and outer-membrane) enzymes and cytochromes, and electrically conductive pili or nanowires assist Electron transfer to the anode in this case. Some Exoelectrogens such as Shewanella oneidensis and Geobacter sulfurreducens MR-1 use such a direct Electron transfer mechanism to interconnect electrochemically with the anodes. Some microbes can consume the indirect Electron transfer mechanism which is assisted by electron passages because they are not able to consume a direct Electron transfer path or are not in corporal interaction with the anode. Electron transfer from the cathode to microbes is apprehensive and it is well known. Most of the MFC studies favored abiotic air cathodes, for assisting the oxygen-decreasing reaction O_2-reducing microbes can also be applied at the cathode.[66] The cathode is mentioned as an O_2-reducing bio-cathode in this situation. According to some studies, it is suggested that mechanisms of Electron transfer are similar in anode and in bio-cathode but only direction is changed.

Though, significantly, the bacterial constituents work at different potentials involved in Electron transfer at the cathode.

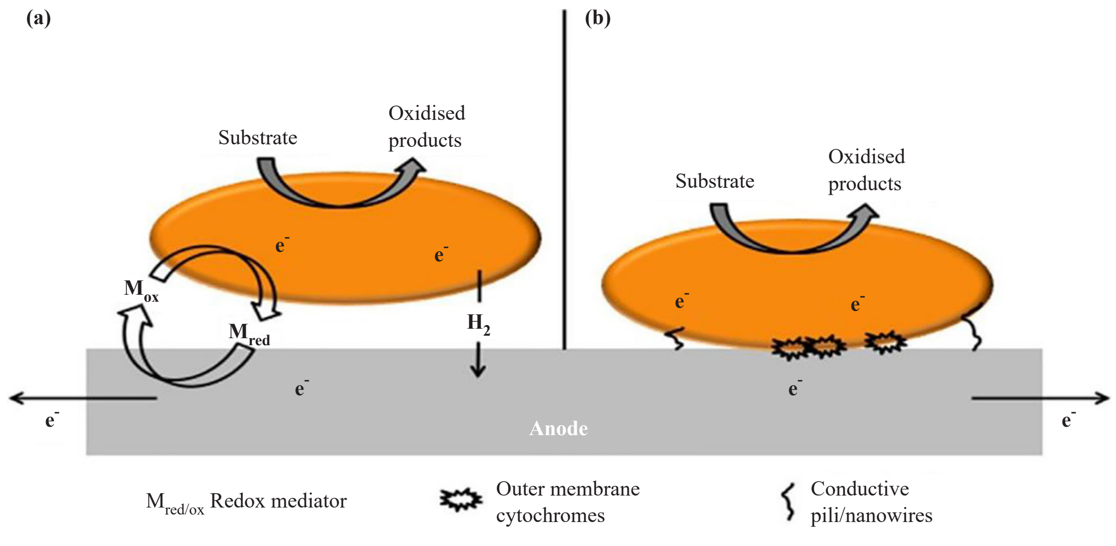

Figure 9. Electron transfer mechanisms from microorganisms to the anode in microbial fuel cells. (a) Indirect electron transfer via redox mediators and primary metabolites such as H_2, and (b) direct electron transfer via outer-membrane cytochromes, proteins

12. Factors affecting the performance of MFCs

For the development of the function and reduction of construction and maintenance costs of MFCs, some factors need to be discussed. These points are classified into three groups; system design, operating condition and biological factors.

12.1 *Design of system*

The performance of an MFC system is heavily influenced by its design and material choices. Several critical construction factors play a key role in determining the efficiency of the system. These factors include the type of reactor used, the electrode spacing, the presence and type of separator, as well as its size, the surface area and volume of the electrodes, the materials used for the electrodes, and the selection of catalysts. All these aspects are crucial in optimizing the MFC's performance and ensuring its successful operation.[67] Table 4 highlights the design aspects of a microbial fuel cell.

Table 4. Outlining the design aspects of a microbial fuel cell system

Design Aspect	Description
System Configuration	Single-chamber or dual-chamber microbial fuel cell (MFC)
Anode Material	Carbon cloth, graphite, porous carbon, or other conductive materials
Cathode Material	Platinum, carbon cloth, air cathode, or other suitable materials
Separator	Proton exchange membrane (PEM) or ion exchange membrane
Microbial Culture	Specific microorganisms or mixed microbial consortia
Substrate	Organic compounds (glucose, acetate, lactate, etc.)
Reactor Volume	Size of the MFC system, typically measured in liters
Electrode Surface Area	Surface area of the anode and cathode electrodes
Electrode Configuration	Flat plate, tubular, or other specialized designs
External Load	Electrical load or resistance connected to the MFC
Monitoring and Control	Sensors for pH, temperature, flow rate, and voltage
Recirculation or Flow Control	Recirculation of media or continuous flow system

12.2 *Effect of reactor design*

The reactor design significantly influences the performance, operation, and construction cost of MFCs. Among the various types of reactor designs discussed earlier, the double-chamber MFCs are currently receiving considerable attention. However, it's worth noting that these reactors tend to have higher internal resistance due to their large electrode spacing. Moreover, the double-chamber design can increase the overall construction cost, making it less suitable for field applications where cost-effectiveness is a priority.[68] Table 5 highlights the effects of reactor design on microbial fuel cells (MFCs):

Table 5. Highlighting the effects of reactor design on microbial fuel cells (MFCs)

Reactor Design	Advantages:	Disadvantages:
Single-Chamber MFC	Simpler design and operation Lower cost and easier maintenance Higher power density due to reduced internal losses	Limited control over anode and cathode environments Greater susceptibility to oxygen crossover Lower efficiency in terms of substrate utilization
Dual-Chamber MFC	Better control over anode and cathode environments Reduced possibility of oxygen crossover Higher efficiency in terms of substrate utilization	More complex design and additional components Higher cost and more challenging maintenance

12.3 *Effect of separator*

The use of different membranes for separation in MFCs has a direct impact on both the internal resistance and construction cost. While membranes help reduce coulombic losses, the choice of membrane type plays a crucial role in MFC performance.

Table 6. Illustrating the effects of the separator in MFCs

Separator Effect	Advantages:	Disadvantages:
Proton Exchange Membrane (PEM)	Efficient proton transport, preventing anode-cathode short circuits Enables selective ion transport for enhanced performance and efficiency Offers flexibility in system design and operation	Expensive compared to other separator materials Susceptible to fouling and degradation over time
Ion Exchange Membrane (IEM)	Good ionic conductivity and selective ion transport Can be more cost-effective compared to PEMs Suitable for various MFC configurations and applications	Limited availability of suitable IEMs for MFCs Vulnerable to fouling and membrane degradation
Non-Ionic Materials	Low-cost alternative to traditional ion exchange membranes Can be resistant to fouling and degradation Provides freedom in material choice and customization	Lower ionic conductivity compared to PEMs and IEMs May require additional optimization and modifications
No Separator ("Open Circuit")	Simpler design with reduced costs and maintenance Facilitates direct electron transfer between anode and cathode Suitable for certain MFC configurations and specific applications	Prone to anode-cathode short circuits and performance limitations Limited control over ion transport and potential cross-contamination

For instance, a Cation Exchange Membrane (CEM) may exhibit a slow transfer rate of protons, leading to a rapid accumulation of acidity in the anode. This accumulation of acidity can adversely affect the activity of Exoelectrogens, the microorganisms responsible for electricity generation in MFCs.

As a potential solution, some researchers have explored the possibility of omitting the membrane from MFCs altogether. By doing so, it could help in balancing the pH levels between the anode and cathode, thus improving the overall efficiency of the system. However, this approach warrants careful consideration and experimentation to optimize MFC performance.[69] Table 6 illustrates the effects of separators in MFCs.

12.4 *Effect of electrode properties*

Table 7. Highlighting the effects of electrode properties in MFCs

Electrode Property	Effect
Surface Area	Larger surface area provides more sites for microbial attachment and electron transfer
	Increased power generation potential and improved overall performance
	Enhances substrate utilization efficiency and microbial activity
Material Composition	Different materials can affect the electron transfer kinetics and conductivity
	Choice of material influences the catalytic activity and electrochemical performance
	Material selection can impact the cost, stability, and long-term performance
Porosity	Porous electrodes promote better mass transfer and nutrient availability
	Facilitates efficient microbial colonization and substrate utilization
	Improves access to dissolved oxygen, enhancing oxygen reduction at the cathode
Surface Modification	Surface modifications can enhance microbial adhesion and biofilm formation
	Promotes direct electron transfer and reduces electron transfer resistance
	Modifying electrode surfaces can optimize charge transfer and overall efficiency
Conductivity	Higher electrode conductivity minimizes electrical losses and resistance
	Improves electron transfer efficiency and overall power generation
	Lowers internal resistance, leading to higher current and power density
Geometry	Different electrode geometries can affect mass transfer and fluid dynamics
	Optimized geometry promotes efficient substrate distribution and utilization
	Proper electrode spacing and design can enhance overall MFC performance

The aggregation of Exoelectrogenic biofilm and transfer of electrons in MFCs can be affected by the properties of electrodes. Biofilm formation, structure and microbial communities be able to affect by electrode materials. For internal resistance, different materials contribute differently. The current production can be increased by 40% due to the use of electrode materials with highly friendly microbial accessible surface. The design of the anode chamber and electrodes can affect the transportation of the proton and substrate. The use of nanotechnology can modify the surface of electrodes or the application of effective electrodes.[70] Table 7 highlights the effects of electrode properties in MFCs.

12.5 *Operating factors*

If the reactor design is put apart the performance and microbiology of MFCs are influenced by the operational factors applied such as substrate type, external resistance, concentration, feeding rate, pH, temperature, conductivity, and mixing velocity.[71]

12.6 *External resistance effect*

External resistance is one of the important electrical factors to produce power and it plays a vital role to control the ratio between the electric current and the working voltage. Low external resistance leads to a low working voltage and high current which increase the high substrate conversion rate these can be the opposite in term of high external resistance.[72] If the external resistance is equal to the internal resistance of the fuel cell, then the maximum power is gained, in this case by altering the external resistance and recording the voltage and current generated, the internal resistance could be predictable.[73] The power generation and biofilm growth of MFCs can be promoted when external resistance is equal to or lower than internal resistance.

12.7 *Effect of Temperature, pH, conductivity and shear rate*

These parameters are important in group and continuous operation. Temperature and pH are the most important environmental factors which impact bacterial cell growth and physiology. The pH gradients formed in MFCs by using CEM can alter the function of MFCs, the acidic pH in the cathode and neutral pH in the anode chamber is favored for electricity generation.[74] The bacterial growth and power production in MFCs are also affected by temperature. The environmental temperature increases the power production of MFCs the temperature of about 30 to 40 °C increases the current by 80%. Table 8 outlines the operating factors in MFCs and their effects.

Table 8. Outlining the operating factors in MFCs and their effects

Operating Factor	Effect
Substrate	Type and concentration of substrate affect microbial activity and electron transfer
	Different substrates can result in varying power generation and efficiency
	Optimal substrate selection can enhance overall MFC performance
pH	pH levels influence microbial community composition and metabolic activity
	Optimal pH conditions promote higher microbial activity and electron transfer
	pH variations can affect electrochemical reactions and overall MFC performance
Temperature	Temperature affects microbial growth, metabolism, and enzymatic activity
	Optimal temperature promotes higher reaction rates and electron transfer
	Temperature fluctuations can impact microbial community dynamics and MFC efficiency

Table 8. (cont.)

Operating Factor	Effect
Hydraulic Retention Time (HRT)	HRT determines the duration of substrate exposure to the microbial community
	Longer HRT allows for increased substrate utilization and enhanced power output
	Shorter HRT may result in incomplete substrate degradation and reduced efficiency
Electrode Potential	Electrode potential influences electron transfer kinetics and microbial activity
	Appropriate electrode potential ensures efficient electron transfer and power generation
	Improper potential may lead to microbial inhibition or suboptimal performance
External Resistance	External resistance determines the load on the MFC and current generation
	Optimal resistance balances power output, substrate utilization, and efficiency
	High resistance can result in reduced power, while low resistance can overload the system
Electron Acceptor	Selection of electron acceptor influences microbial metabolism and electron transfer
	Different acceptors affect overall MFC performance and power generation efficiency
	Suitable electron acceptors enhance microbial activity and electron transfer

13. Losses in microbial fuel cell

To understand the performance of MFC, it is necessary to consider sources of energy loss occurring in an MFC. These losses comprise the resistance to electron transport in terms of charge-transfer resistance (activation loss), ohmic resistance, and diffusion resistance (mass transfer). Among these losses, ohmic resistance loss plays a major role as far as the performance of an MFC is concerned.[75] When designing an MFC, each type of polarization loss should be minimized to decrease overall potential losses and thus increase power generation.

13.1 *Activation losses*

Activation losses occur due to the activation energy needed to overcome the initial energy barriers for an oxidation/reduction reaction during the electron transfer or to form a compound reacting at the electrode. This compound can be a soluble mediator, a mediator present at the surface of bacteria, or an electron acceptor at the cathode. It is essential to break the energy barrier to initiate electron transfer from Exoelectrogens to the electrode or to transfer electrons to an oxidizing agent that works as the final electron acceptor.[76] This results in voltage loss or activation over potential shown by a steep fall of MFC voltage especially in the early phase of electricity production. It has been suggested that attachment of firm thick biofilm to the electrode, an increase in the electrode surface area, catalyst modification for electrodes, and a slight increase in temperature may result in reducing activation losses, impedance associated with the anode is the dominant loss in an MFC, even with an established biofilm. They also reported that the charge transfer resistance dropped from 2.6 to 1.5 kΩ cm^2 after five days of closed-circuit operation and decreased further to 0.48 kΩ cm^2 after three weeks of operation. This indicates that as the biofilm is established, biocatalytic oxidation of the substrate is enhanced and thus activation losses are reduced.[77]

13.2 *Ohmic losses*

One of the significant and problematic parameters that limit the electricity generation in an MFC is the high internal resistance of the system. The electrons and ions must overcome minimum ohmic resistance to flow through the electrochemical system. This internal resistance is rooted in the MFC internal structure. Indeed, higher ohmic resistance and current density lead to higher ohmic loss in the MFC system.[78] Electrolyte ohmic loss and electrode ohmic loss are two major ohmic losses relevant for MFCs. The electrolyte ohmic loss is caused by the movement of electrons through electrodes, while the electrolyte voltage loss is caused by the movement of ions through the electrolyte.[79] Much real wastewater has very low conductivity causing considerable electrolyte ohmic losses. However, MFC performance improvement by reducing internal losses can be achieved either by engineering advancements or by conducting an in-depth microbiological study to better understand bacterial morphology and classification. Furthermore, it is convenient to achieve high conductivity by keeping a high salt concentration or with the help of employing a buffer solution during the laboratory study, but in the case of actual sewage and industrial effluent electrical conductivities are too low typically of the order of 1 ms/cm.[80]

To reduce the internal resistance of an MFC, one of the options is to reduce the distance between the anode and cathode. Another way is to increase ionic strength, which increases the solution conductivity of the MFC system. High ohmic resistance and energy losses are related to the restricted proton movement to and from the electrodes in an MFC. Although ionic species control the charge balance, protons generated at the anode and consumed at the cathode must be in balance.[81] Another approach to reduce ohmic losses is an up-flow anode design in which the organic substrate can flow through biofilm continuously, which results in higher power production and less internal resistance. Several researchers have focused on methods to reduce the MFC internal resistance as this is the major impediment to harnessing more power.[82]

13.3 *Mass transfer losses*

A limited mass transfer of substrate or electron acceptors toward the electrodes leads to concentration or mass transfer losses, which results in a sharp drop in cell voltage. This phenomenon is more predominant at maximum current densities during polarization. Concentration polarization also occurs when there are different anodic and cathodic mass transfer rates. This means anodic oxidation occurs at a faster rate than the transport of species toward the cathode.[83] As discussed before, this phenomenon observed near maximum current density (due to non-conductive thick biofilm formed at the anode) leads to a very slow rate of diffusion. In addition, the competition between organisms that can consume the substrate before an endophilic organism is encountered also plays a role in concentration polarization to occur.[84] Mass transfer limitations in the bulk electrolyte solution also hinder substrate transport, which is another kind of concentration loss. With the help of polarization curves, the starting point of concentration losses can be determined.

13.4 *Bacterial metabolic losses and electron reducing reactions*

Bacterial metabolic losses are caused by the energy used by bacteria, for example, growth and maintenance, instead of the energy captured by an MFC. The higher the difference in redox potential between the substrate and anode, the higher the energy gained by bacteria, but the lower the energy captured by the MFC or the lower the MFC voltage, the anode potential should be low enough to minimize metabolic losses but high enough to prevent bacteria switching to fermentative metabolism instead of using the anode as the electron acceptor.[64] Besides this, other side reactions such as fermentation, methanogenesis, and respiration (in the presence of oxygen) lead to electron loss and partial substrate conversion into endophilic mass. Another possibility is substrate leakage into the cathode compartment. This can contribute to electron loss in terms of load, and this is a kind of short-circuiting since the actual path (circuit) is bypassed.[85]

14. Conclusion

Microbial fuel cells have shown great potential as a sustainable and renewable energy technology. They offer several advantages, such as the ability to directly convert organic matter into electricity through microbial activity, compatibility with various waste streams and renewable resources, and their potential for decentralized energy production. MFCs have also gained attention for their applications in wastewater treatment, biosensors, and remote power generation.

Despite the progress made in microbial fuel cell (MFC) technology, several hurdles still hinder its widespread adoption and commercialization. A primary area of focus is improving power output and efficiency. This entails optimizing the selection of microbial species, electrode materials, and system configurations to maximize electricity generation.

To further enhance MFC performance, a deeper understanding of microbial communities, biofilm formation, and electron transfer mechanisms is essential. This knowledge can pave the way for more effective strategies to boost electricity production.

In addition to technical aspects, scalability, cost-effectiveness, and durability are critical considerations for the future development of MFCs. Addressing these factors requires advancements in electrode materials, separator designs, and reactor configurations. These improvements will play a significant role in overcoming challenges and making MFCs more practical and economically viable for various applications.

Integration of MFCs with other renewable energy technologies, such as solar or wind, could create hybrid systems that offer enhanced reliability and energy generation capabilities. Furthermore, research efforts should continue to explore novel applications and potential synergies with other industries, such as agriculture, bioremediation, and wastewater treatment

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported here.

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