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# Đánh giá tổng quan tế bào nhiên liệu vi sinh: Những tiến bộ gần đây về cơ chất

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**TÓM TẮT**

Trong hai thập kỷ qua, pin nhiên liệu vi sinh vật (Microbial fuel cells - MFCs) đã được chú ý vì chúng có thể chuyển đổi trực tiếp năng lượng hóa học từ các hợp chất hữu cơ để tạo ra điện sinh học. Với việc sử dụng MFC, năng lượng sinh khối có thể được thu trực tiếp dưới dạng điện năng, đây là năng lượng sạch, phổ biến và tiện lợi nhất. Do đó, MFC được xem là một phương pháp đầy hứa hẹn khác để khai thác năng lượng bền vững trong sinh khối. Có nhiều cơ chất đã được nghiên cứu để sử dụng làm nguồn cấp nguyên liệu sử dụng trong MFC. Chúng bao gồm nhiều loại nước thải nhân tạo, nước thải thực và sinh khối lignocellulosic. Trong số các yếu tố ảnh hưởng đến hiệu suất của MFCs: thiết kế, vật liệu điện cực, cơ chất, màng trao đổi, vi sinh vật hoạt động điện, pH, nhiệt độ vận hành,… Cơ chất là thách thức quan trọng nhất trong công nghệ MFCs, đòi hỏi sự ổn định lâu dài. Việc sử dụng cơ chất không ổn định ảnh hưởng trực tiếp đến hiệu suất của MFC. Tương tự, ảnh hưởng của các chất lên cộng đồng vi sinh vật cũng được thảo luận. Bài tổng quan này cập nhật những tiến bộ gần đây trong việc cải tiến công nghệ MFC để tăng cường hiệu suất phát điện, đặc biệt là các cơ chất khác nhau được dùng trong MFC cho đến nay cũng như các cơ chất tiềm năng trong tương lai.

**Từ khóa:** *Cơ chất, mật độ năng lượng, pin nhiên liệu vi sinh, xử lý nước thải.*

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# A review of the general microbial fuel cell: Recent advances in substrates

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**ABSTRACT**

Over the past two decades, microbial fuel cells (MFCs) have gained attention because they can directly convert chemical energy from organic compounds to bioelectricity. Using MFCs, biomass energy can be directly harvested in the form of electricity, which is the most convenient, widely available, and clean energy. Therefore, MFCs are considered to be another promising way to harness sustainable energy in biomass and add a new dimension to the biomass energy industry. Many substrates have been studied for microbial nourishment. These include a variety of artificial and natural wastewater and lignocellulosic biomass. Among factors essential for long-term stability in MFCs, the substrate is the most challenging. Studies have shown that an unstable substrate directly harms the performance of MFCs. This review discusses the effect of substrates on the microbial community. Furthermore, it provides updates on recent advances in improving MFC technology, particularly the different substrates discovered in MFCs to date, power generation efficiency, and potential substrates in the future.

**Keywords:** *Microbial fuel cell, substrate, wastewater treatment, power density.*

## 1. INTRODUCTION

Due to the unsustainable nature and harmful environmental impacts of fossil fuels, attention to renewable energy has increased.[1] Renewable energy has become an important alternative to fossil fuels since it produces useful energy without being depleted. In addition, water shortage is one of the critical global issues. According to climate change forecasts, this problem will be even more severe in the future.[2] The increase in water demand has led to an increase in the amount of wastewater generated. At the same time, there is an urgent need for renewable energy due to the rapid depletion of fossil fuels and growing concern about climate change.[3] Many countries worldwide are looking for alternative resources such as biomass as a more reliable, sustainable, and environmentally beneficial resource to reduce the need for fossil fuels. To date, biomass can be converted into different types of energy products such as heat, gas, fuel, and electricity.[3] MFCs are a type of power generation device that uses bacteria as biological catalysts to generate electricity by oxidizing organic matter from the wastewater through respiration.[4] It has considerable potential for applications in wastewater treatment,[5] electrical equipment,[6] and biosensors.[7] Recently, reactors with a scale of several hundred liters have been designed.[8] The move to bring this technology from the laboratory scale to the current pilot scale brought it closer to practical application.

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MFCs function through bacterial activities, resulting in electron production from these substrates. To produce power, these electrons are transferred from the negative anode to the positive cathode through a conductive material and a load or resistor (Figure 1).

For a system to be categorized as an MFC, its substrate must be replenished continuously or intermittently; otherwise, it is considered a bio-battery. Electron mediators or shuttles transfer electrons to the anode[9] through electron transfers directly associated with the membrane or through nanowires formed by bacteria,[10] or possibly through some other unexplored ways. Chemical mediators, such as neutral red or anthraquinone-2,6-disulfonate can be used in MFCs to facilitate electricity generation by bacteria that cannot use the electrode without aid.[11] Suppose the system has no added exogenous mediators. In that case, the MFC is considered "mediator-less" even if the electron transfer mechanism may be unknown.[12]

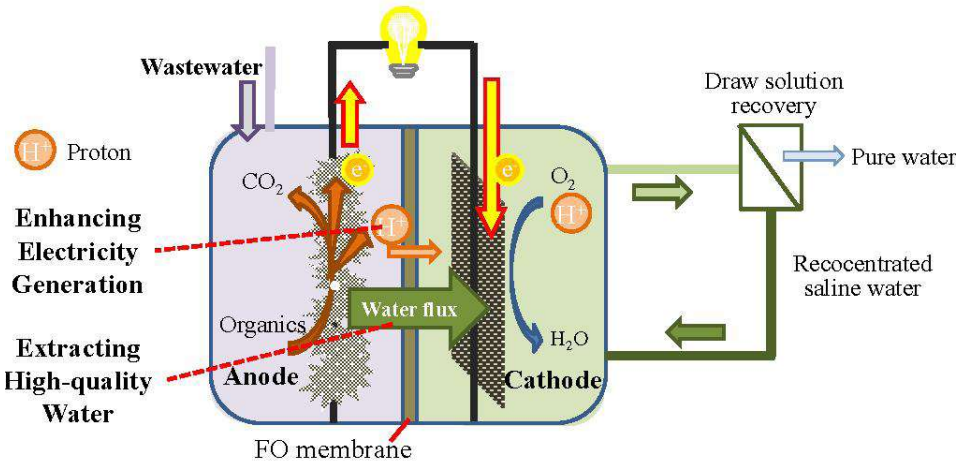


**Figure 1.** Structure diagram of a microbial fuel cell.[13]

Research is increasing on MFCs constructed with various materials and configurations. The operating conditions also differ in temperature, pH, electron acceptor, operating time, surface areas of electrodes, and reactor size. Studies report potentials using different reference states and occasionally only using a single resistor (load). These and sometimes the lack of essential data, such as resistance internal to the system, or power densities obtained from polarization curves using differing methods, have created a challenge in interpreting and comparing results among studies.[14]

MFC has been an interesting topic for more than 20 years now not only because of its electricity generation but also because it is an environmentally friendly wastewater treatment technology.[15] Many types of wastewater today contain various toxic wastes, making them expensive to treat before being discharged into the environment. Previous studies have demonstrated the ability of MFCs to treat contaminated wastewater containing metals, food, and urine, even producing drinking water post-treatment.[16-17] MFCs have coexisted with biological filter tanks in wastewater treatment to enhance pollution control and improve treatment capacity.[18] Most treatment methods aim to remove organic compounds that reduce chemical oxygen demand (COD), azo dyes,[19] and heavy metal waste.[20] The aeration system in wastewater treatment is reported to consume more than 54% of the electricity required in the treatment process, while the MFCs use anaerobic bacteria for the wastewater treatment process, which indicates a potential for energy saving of

MFCs.[19] In addition, MFCs used as biosensors are expected to be one of the promising applications of MFC-derived technology. Such biosensors have been studied to measure various parameters, including COD, volatile fatty acids, dissolved oxygen (DO), biochemical oxygen demand (BOD), toxic substances and microbial activity.[21] This helps to reduce the time and cost required to measure toxicity in water.

Recent advancement in the application of power generation capabilities includes using MFCs to power a small computer (158 mW) directly and continuously without any management equipment or power source.[22] The performance enhancement of MFC is accomplished through many aspects, such as electrode material and surface, electrochemically active bacteria (EAB), substrate, and load resistance.

## 2. DESIGN OF MFCs REACTOR

Many types of MFC designs have been researched and developed. Each design has its advantages and disadvantages and is suitable for specific uses. Various factors are considered in designing MFCs. The size, shape, and configuration of reactors widely differ and are wholly decided upon by the designer. There is no existing recommended standard design yet. The MFC's overall performance is significantly affected by reactor configurations, including the volume, oxygen supply, area of the membrane, and spacing between electrodes. Among the studied structures, double chamber H-type MFC is typically used because of its ion exchange membrane, facilitating proton diffusion and limiting the crossover of substrate and oxygen.[23] It is up to the designer to decide the project's aims and plan the design accordingly. Presently, the available reactor designs are horseshoe-shaped, cylindrical, cubed, dual- and single-chamber, and H-type. Some are made of glass, while others are made up of a variety of plastic materials.

Also, sizes range widely, with reactor volumes of a few square millimeters and others of up to a square meter, ranging from microliters to thousands of liters. The fuel cell design is an important element in the MFC/microbial electrolysis cell (MEC). Single-chamber cells have been created from a two-chamber design to eliminate the membrane.[24] Furthermore, single-chamber MFCs have shown promising results; however, dual-chamber is still widely studied. Dual-chamber cells are easier to construct than single-chamber reactors. A simple MFC device can either be dual- or single-chambered, based on the anode and cathode chamber assembly. Several MFC design and structure adaptations have been made from these two typical designs.[16]

H-shaped fuel cells commonly consist of two bottles connected by a tube containing a separator, usually a cation exchange membrane (CEM) such as Nafion,[11] or Ultrex,[25] or a plain salt bridge (Figure 2).[26] The vital consideration for this design is selecting a membrane that allows proton transfer between the chambers but hinders the cathode chamber's substrate or electron acceptor (usually oxygen) from crossing. Using a glass tube heated and bent to a U-shape is a cost-effective method to connect the bottles. Agar and salt are used as a CEM in the U-shaped glass tube and are inserted through the bottles' lids (Figure 2). However, it was observed that MFCs using salt bridge generates low power because of high internal resistance.

H-shaped MFC devices are generally accepted for basic parameter research. An example is testing the power produced using new materials or new microbes developing from some compound decomposition. However, this MFC type typically generates low power densities. The relative surface area of the cathode to that of anode[27] and the membrane surface[28] affect the power generation.

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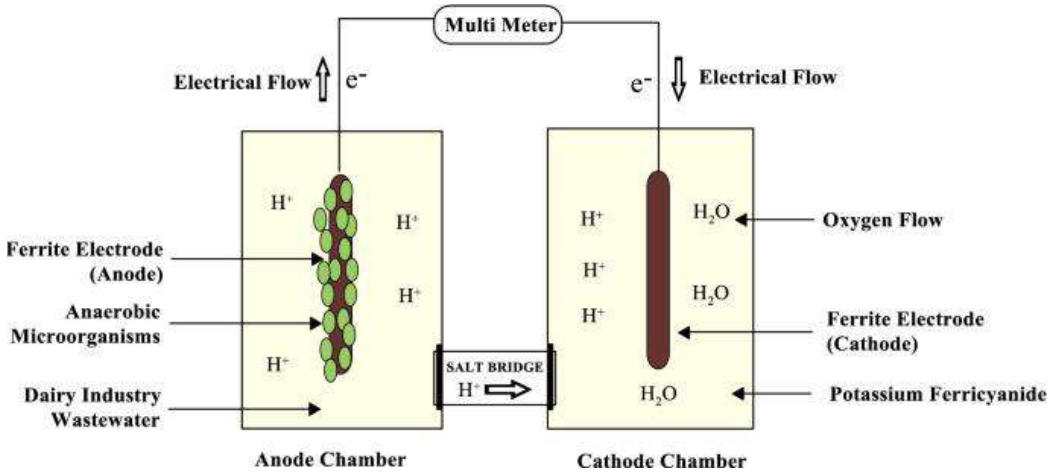


**Figure 2.** MFC types device with a salt bridge (pointed by arrow) which is easily assembled.[29]

Limitations to the power densities generated in these systems are typically due to considerable internal resistance and losses from the electrodes. Therefore, to compare systems' power production, it is logical to evaluate using equally sized anodes, cathodes, and membranes.[28] Ferricyanide, the cathodic electron acceptor, improves power generation because of the high concentrations of electron acceptors. In an H-shaped reactor using Nafion as CEM, compared to a Pt-catalyst and dissolved oxygen in the cathode, ferricyanide increased the power produced by 1.5 to 1.8 times.[28] MFCs with the highest power densities and low internal resistances that have been published so far reported the use of ferricyanide in the cathode chamber.[9] Even though this chemical is excellent as a catholyte for system performance, it is not sustainable to use since it is not chemically regenerated. Therefore, ferricyanide use is restricted to basic laboratory research only. Several studies have also explored using cathode directly in contact with air (Figure 3A, B), either with or without a membrane.[30] In one study, a separator based on kaolin clay and a cathode made of graphite were connected to combine the separator and cathode structure.[31] MFCs using air cathodes improved power densities significantly compared to MFCs with aqueous cathodes. The most straightforward configuration involves placing the anode and cathode on either side of a tube, sealing the anode against a flat plate, and exposing the cathode to air on one side and water on the other (Figure 3A).

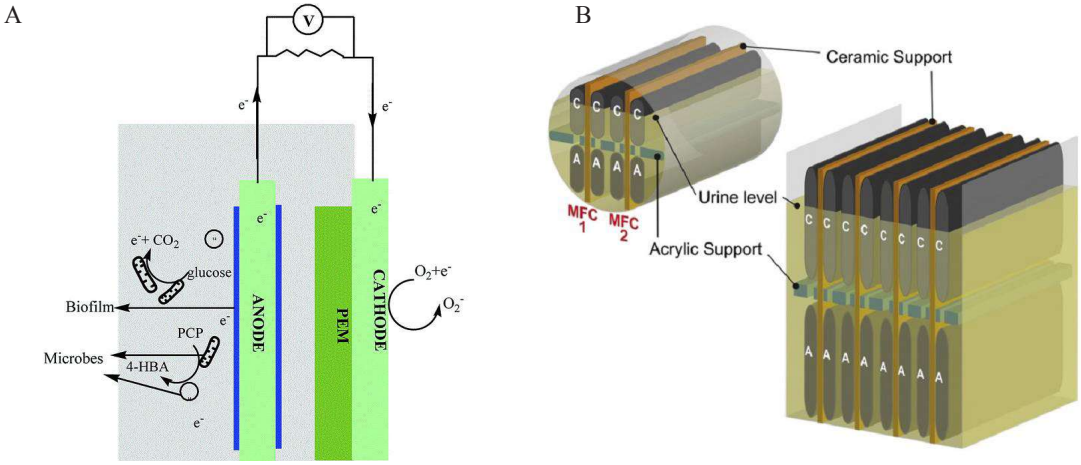


**Figure 3.** MFC types in studies: (A) single-chamber, simple "tube" arrangement of air-cathode[32]; (B) stacked MFC, with one out of two ceramic supports removed.[33]

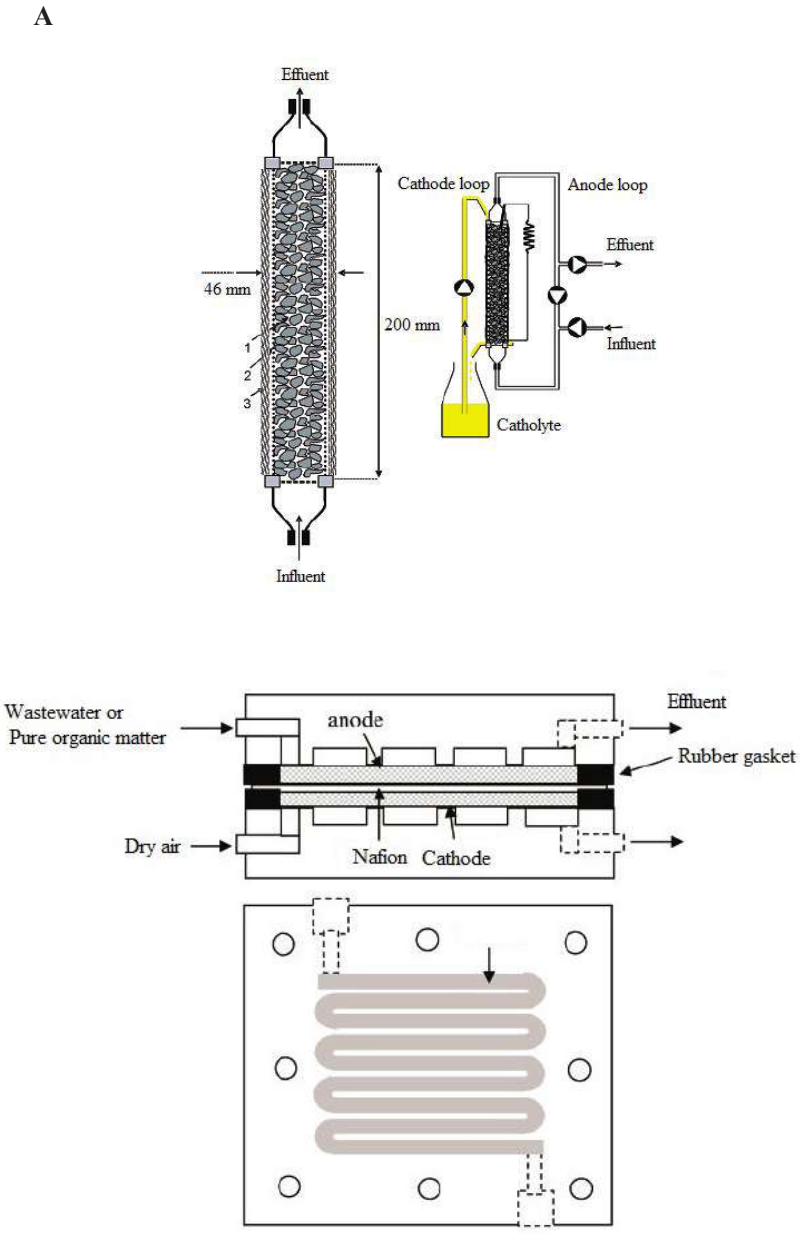


**Figure 4.** MFC operated continuously: (A) upward flowing, tubular type MFC with inner graphite bed anode and outer cathode;[34] (B) flat plate design where a serpentine pattern for fluid flow.[35]

A membrane's purpose in an air-cathode device is to prevent water leakage through the cathode. However, it also decreases oxygen diffusion into the anode chamber. The bacterial oxygen demand in the anode chamber can lower Coulombic efficiency, which is the fraction of electrons recovered over the maximum number of electrons that can possibly be recovered.[30] Although hydrostatic pressure on the cathode will cause water leaks, this can be minimized using coatings, such as polytetrafluoroethylene (PTFE), outside the cathode. These coatings allow the diffusion of oxygen but limit the bulk loss of water.[36]

The systems mentioned so far are batch-operated devices. Several other basic designs also exist that provide continuous flow through the anode chamber. Some designs include an outer cylindrical reactor and a concentric inner cathode tube.[37] Some are the other way around

having an internal cylindrical anode filled with granular media and an outer cathode (Figure 4A).[34] Another design variation is an upward-flowing fixed-bed biofilm reactor, with fluid flowing continuously through permeable anodes to a membrane that separates the anode from the cathode chamber.[38] System designs resembling hydrogen fuel cells have been employed, where a CEM is placed between the cathode and anode (Figure 4B). Stacking systems as a series of flat plates or linking together in series can increase overall system voltage (Figure 3B).[33]

Sediment MFCs have been developed. These are created by putting an electrode inside marine sediment abundant in sulfides and organic substances, and the other electrode is placed in the overlying oxic water. With these, electricity is produced sufficiently to provide power to some marine devices.[39] Graphite disks[11] and platinum mesh electrodes[39] have been used as electrodes. "Bottlebrush" cathodes have a high surface area and are corrosive-resistant. Therefore, these find applications for seawater batteries and are promising for long-term operation.[40] H-tube dual-chamber systems have also been applied to sediment MFCs to study bacterial communities.[11] Modifications have also been done to produce hydrogen. Using a slight external potential in the MFC, the potential produced at the anode by the bacteria was assisted, making cathodic hydrogen generation possible.[41] These are called bioelectrochemical assisted microbial reactors (BEAMRs) or bio-catalyzed electrolysis systems and are not considered as real fuel cells since they are operated to generate not electricity, but hydrogen. Having a second chamber for hydrogen gas capture would make it possible to develop various designs for hydrogen generation.

## 3. ELECTROACTIVE BIOFILMS

Electroactive biofilms (EAB) have been identified in many natural ecosystems such as soils, sediments, seawater, or freshwater and in samples collected from a wide range of different microbially-rich environments (sewage sludge, activated sludge, or industrial and domestic effluents). The microbes transfer the electrons to the electrode through various electron transfer mechanisms. However, the electron transfer mechanism plays a vital role in maximizing the microbe to electrode interaction and helps provide an understanding of how such systems operate in the MFC.[42] Researchers have proposed three extracellular electron transfer (EET) mechanisms.[43] Depending on the mechanisms involved, the distances of EET may vary greatly, from the nanometer-scale in the case of electron transfer across the cell envelope to lengths exceeding one centimeter for cable bacteria.[44]

### 3.1. Direct electron transfer

The first transfer mechanism uses direct electron transfer (DET) between electron carriers in the bacteria and the solid electron acceptors.[45] The mechanism is carried out by the presence of outer membrane cytochromes that can interact directly with the solid surface to carry out respiration.[46] DET can occur through direct physical contact between the cell and an electrode without the involvement of any diffusible redox compounds. This is achieved if the microbe contains redox-active proteins on the outer surface of the cell membrane or cell envelope, e.g., cytochromes,[47] flavoproteins,[48] or multi-copper proteins,[49] which allow transport of electrons between the inside of the cell and an external environment. According to studies reported so far, three different mechanisms accomplishing this type of electrical connection have been proposed[50]: (i) ET through electrically conductive pili,[51] (ii) ET between redox proteins bound to the outer cell surfaces,[52] or (iii) ET through abiotic conductive materials.[53]

### 3.2. Mediated electron transfer

The second transfer mechanism employs an electron shuttle between bacteria and electrodes. The mediated electron transfer (MET) has redox mediators involved in shuttling electrons between bacteria and electrodes.[46] MET takes place by the presence of redox-active mediating

compounds, which shuttle electrons between an external donor/acceptor and a microorganism. Some compounds shown to be effective electron shuttles including both inorganic and organic compounds have been identified potassium ferricyanide,[54] flavin mononucleotide,[55] neutral red,[56] phenazines, phenoxazines, phenothiazines, and quinones,[57] 9,10-anthraquinone-2,6-disulfonic acid disodium salt, safranine O, resazurin, methylene blue, and humic acids.[58] As multiple prior studies have proven that bacteria excrete various primary and secondary metabolites, which may be involved in EET as diffusible mediators.[59] Thus, these disadvantages lead to the general relinquishment of this approach. Because MET uses natural electron shuttles and DET mechanisms, it is generally established that artificial mediators are no longer significant.[60]

### 3.3. Indirect electron transfer

The third type of mechanism is indirect electron transfer (IET), which is based on the electrochemical synthesis of a wide range of microbial electron donors and acceptors. Here, the compounds used as electron donors or acceptors undergo irreversible redox processes, creating new compounds such as hydrogen or formic acid. Additionally, electroactive (metabolic) substances can be secreted by microorganisms and transfer electrons between the microbes and electrodes.[44]

## 4. FACTORS AFFECTING THE ACTIVITY OF MFCs

### 4.1. Electrode materials

An important goal of the anode chamber in the MFC is to serve as an electron receiver for the electric current generation. An effective anode material is electrically conductive, biocompatible, free from corrosion and fouling over time, inexpensive, and with a high surface area.[61] The electrode materials greatly influence the performance of the MFC.[62] Carbon materials are used as electrodes in MFC because they are non-corrosive, highly biocompatible, and exhibit some distinctive surface characteristics of electrode materials. Modification of the electrode material has been shown to be an effective way to improve the performance of the MFC.[63]

Many studies have shown that electrodes with nanoparticle modification are more efficient than simple electrodes. This change in the physical and chemical properties of the electrode helps the microorganism better bind and transfer electrons. The efficiency of MFC can be increased through the improvement of bacterial adhesion and electron transfer with the modification of the electrode surface.[64] The biofilm attached to the electrode is an essential element of electrochemical bioreaction.[65] The growth and development of biofilms on the MFC electrodes, especially on the anode electrode, will help organic matter oxidation and transfer electrons to the cathode.[66] The anode electrode of the MFC must contain a stable and homogeneous bioreactor for enhanced energy generation.[67]

In summary, the electrode properties and the correlation between electrode, substrate, and bacteria are the main factors affecting the performance of MFC. It can be seen that the development of MFC technology is a diverse combination of specialties such as biochemistry, electrochemistry, mechanical engineering, and materials science.

### 4.2. Proton exchange system

In a dual-chamber design, the anode and the cathode compartments are separated by an ion-selective membrane, allowing proton transfer from the anode to the cathode and preventing oxygen diffusion in the anode chamber from the cathode compartment. The membrane in the MFCs plays an important role in MFC performance. The most commonly used materials for PEM affect the internal resistance and concentration of the polarisation loss of the system and influences the power output of the MFCs. There are several significant types of ion exchange membranes used in MFC systems: anion exchange membranes (AEM), cation

exchange membranes (CEM), and polarized membranes (PBM).[68]

The bioreactor architecture, material type, and reactor geometry determine the device's performance and cost. Studies aim to find the optimal combination of materials and design that will result in high performance, low cost, and multiple functions to establish a standard for convenient and economically feasible scaling up. One such effort is using CEM such as Nafion derived from the existing technology on hydrogen PEMFC[69] or treatment systems on water using membranes.[38] Since Nafion is expensive, most research is concentrated on possible alternatives, including investigations on materials such as nylon and glass fibers, j-cloth, biodegradable plastic bags, and ceramics.[69] Several waste materials, such as laboratory gloves and natural rubber, have also been tested.[70] Results show that these materials offer benefits in terms of membrane fouling.

MFC operation was studied using similar metals in different solutions or different metals in similar solutions when liquid electrolytes are employed in the anode and cathode, as long as the cathode is not exposed to open air. Ions are contained in liquid solutions, which creates the need for an ion-exchange membrane. Therefore, in theory, a membrane is not a necessity for MFCs,[30] given that the anode and cathode are either dissimilar (electrochemically separated) or identical but placed at a distance apart to avoid short-circuit.

Membrane-less MFC was therefore developed.[71] While this eliminates the need for high-cost membranes and fouling problems, the downside involves oxygen diffusion, which creates adverse competition with the anode on the available electrons. Most MFCs have been designed to have rigid, inert structural materials for housing anode and cathode half-cells, regardless of the presence of membranes. Currently, studies have emerged using 3-D printing in the fabrication of MFCs.[72] 3-D printing of MFC bioreactors also has the advantage of creating complete reactors. As a result, these products can be employed in various applications and environments.[73]

The challenge of electrode spacing can be addressed by material type. Porosity, strength, chemical inertness, and longevity are the factors that may address this challenge while hindering oxygen penetration. The materials that have been studied so far are canvas,[71] photocopy paper,[74] microporous filtration membranes,[75] and nylon infused membrane.[76]

### 4.3. pH

MFCs are very susceptible to the influence of external pH on their ability to generate renewable energy and remove contaminants. When the external pH changes, many physiological changes occur, including changes in ion concentration, proton shutdown, microbial cell pH, and biofilm formation at the anode electrode. pH value plays a vital role in the growth of microorganisms, and it is necessary to consider the right pH conditions for the microorganisms to achieve maximum performance. The bacteria responsible for generating electricity in the MFC were more active at pH 6 to 8 in the anode chamber and at neutral or slightly higher pH in the cathode chamber. The activities of the bacteria decrease due to the low pH in the anolyte, which has a tremendous effect on the biofilm formation and power output of the MFC. The main effect of pH on the electrolyte influences bacterial metabolism and the cathodic oxygen reduction reaction rate.[77]

### 4.4. Temperature

Temperature affects microbial metabolism, mass displacement, and thermodynamics, leading to an effect on MFC performance. Orellana et al.[78] reports that MFCs operate stability at the temperature range of 25°C to 30°C. MFCs operating at higher temperatures have an advantageous performance and better substrate

removal. This is because the temperature contributes to the initial biofilm formation, augmentation of the bacterial metabolism, and membrane permeability.[79] The optimum temperature for mesophilic microbes ranges from 35°C to 40°C. Warmer temperatures generally positively affect power generation, CE, and COD removal.[80] MFCs operating at higher temperatures have a performance advantage and substrate removal. This is because of the temperature contributes to the initial biofilm formation, augmentation of the bacterial metabolism, and membrane permeability.[81] These results indicate that temperature plays an important role in shaping microbial communities of the anode biofilms and the cell's internal resistance in MFCs through changes in species evenness.[82]

### 4.5. Substrate

The substrate is considered one of the essential biological factors related to the power efficiency in MFCs.[83] Organic substrates range from simple to complex, and their mixtures can be used as a nutrient source by electrically active bacteria for power generation in MFCs. Studies reported that substrates for MFCs range from simple (i.e., glucose, acetate, sucrose, etc.) to complex (i.e., amino proteins, acids, etc.).[84-85] Aside from those, some wastewater such as seafood industrial wastewater,[86] petroleum recycling wastewater,[87] bamboo fermentation effluent,[88] were used as a complex substrate in MFC. In most cases, the ultimate purpose of wastewater use is to remove the pollutants present in the wastewater before releasing them into the environment. Different researchers used varying units to represent MFC performance. A unit most often used is called power density, which can signify an amount of power (time rate of energy transfer) per unit area of the anode electrode surface ($mW/m^2$) or the power density per volume of the cell ($mW/m^3$). This review discusses the most commonly used substrates with the corresponding MFC performance.

## 5. RESEARCH ON SUBSTRATES AND THEIR EFFECT ON MFCs PERFORMANCES

### 5.1. Acetate

In most of the research on MFC, acetate has been the most widely used substrate type for electricity generation. As a simple substrate, acetate is rich in carbon which electroactive microorganisms can easily metabolize. Ions are present in acetic acid that tends to prompt electroactive microbes. Notably, acetate is the final product of various metabolic pathways for higher-order carbon sources.[89] As a simple compound, acetate is easier to degrade in MFCs.[90] Liu et al.[91] observed that the acetate-fed single-chamber MFC obtained a power generation of 506 $mW/m^2$, 800 mg/L, which was approximately 66% higher than the power produced by an MFC with butyrate (305 $mW/m^2$, 1000 mg/L). Liu et al.[83] observed that the MFC fed by acetate substrate and acetate-acclimatized microbial consortia obtained more than twice higher power generation with half optimal external load resistance compared to MFC with protein-rich wastewater as substrate. However, a wide range of microbial community compositions was observed in the anode biofilm for the protein-enriched wastewater compared to the acetate substrate. Chae et al.[92] evaluated the power generation of four diverse substrates where the acetate-fed MFC obtained the maximum power generation with CE of 72.3% while the other substrate-operated MFC achieved the lower power generation with butyrate (43.0%), propionate (36.0%) and glucose (15.0%). Dinh et al.[90] reported that a power density of 593.4 $mW/m^2$ was produced from acetate at the concentration of 10 mM. An MFC using a mixture of acetate and lactate substrates at 30 mM yielded a power density of 956.75 $mW/m^2$.

### 5.2. Glucose

Another common substrate used in MFC is glucose. The presence of glucose in wastewater sludge enhances the conductivity property of the MFC.

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Generally, MFC using glucose as substrate achieves low power generation by lower electron transfer (CE) efficiency between electroactive bacteria and electrode. This is due to methanogenic and fermentative bacteria that are unrelated to electricity production in the MFC system that readily consume glucose.[93] Therefore, a combination of simple and complex substrates is recommended to be used in MFCs.[92]

### 5.3. Synthetic wastewater

Various synthetic or chemical wastewater with known compositions have been used in MFCs as the conductivity, pH, and other parameters are easily controlled. Mohan et al.[94] operated MFCs fed with various loadings of synthetic wastewater to obtain an ideal loading rate.

A few media used during bacterial growth contain large quantities of redox mediators, including high-intensity wastewater composed of reduced species of sulfur and cysteine that can serve as an abiotic donor of electrons and improve power output for a brief period.[95] However, this does not adequately reflect the system's performance. One solution utilizes a minimum salt solution containing only one electron donor, like glucose or acetate. Sun et al.[96] used synthetic wastewater prepared fresh by dissolving glucose in tap water until the COD concentration was about 300 mg/L. The power density was 112.36 mW/m$^2$. The COD removal efficiency was about 60%, and the effluent COD was about 100 mg/L. Rodrigo et al.[97] studied the production of electricity and the oxidation of the pollutants contained in a synthetic wastewater fed with glucose and peptone of soybean as carbon sources. Waste-fed MFC that is slowly biodegradable generates higher energy. It is most likely because of the formation of intermediates that favor the production of electricity.

### 5.4. Brewery effluent

Brewery effluent is generally nontoxic and characterized by high COD and total nitrogen content in the presence of higher organic contents, mainly consisting of protein and starch components.[98] Wang et al.[99] reported the power generation efficiency from brewery wastewater in the non-diaphragm MFC with a maximum power density of 483 mW/m$^2$ at 20°C. The test results show that MFC can generate electricity from high-intensity wastewater with COD concentrations ranging from 400 to 1,400 mg/L. Brewery wastewater is a preferred substrate in MFC because the strength is low and also because the organic matter is derived from food, thus resulting in low inhibitory compound concentrations, such as ammonia contained in animal wastewater.[100]

### 5.5. Dye wastewater

Azo dyes are abundant in the textile and dyeing industries. The presence of high concentrations of dyes will have severe environmental effects, such as blocking the passage of oxygen and light into the water, which will seriously affect aquatic life.[101] Therefore, removing dyes from these effluents before discharge into the environment is necessary. Some processing technology, such as physical, chemical, and electrochemical treatments, are being effectuated before discharging into the land.[102] Recently, MFC technologies have converted chemical energy directly to electricity through a biological pathway.[103] Qiu et al.[104] used a novel combined process of constructed wetland-microbial fuel cell and three dimensional biofilm electrode reactor to treat reactive brilliant red X-3B dye wastewater. The results showed that the decolorization rate of the combination process was over 96%, and the COD removal rates ranging from 78.9% to 90.8% were achieved. Fatima et al.[105] designed and optimized for efficient treatment of recalcitrant textile wastewater. A maximum power density of 120 mW/m$^2$ was obtained under optimized conditions. As a result, the MFC's color removal and COD reduction were up to 81 and 58%. Consequently, treating wastewater containing azo dye and wastewater containing organic matter that is easily bio-degraded simultaneously

can be performed by combining the two different types of wastewater in MFC that can improve both cost and energy.[106] The downside of this is that significant development is still needed in finding a dense bacterial community suitable for dyes mixed with simple carbon sources to provide a realistic solution in the wastewater treatment of MFCs.

### 5.6. Lignocellulosic biomass

Lignocellulosic biomass is abundantly available in nature, mainly generated from agricultural waste, and considered a promising and cost-effective feedstock for energy production.[107]

Due to their new availability and reproducibility, lignocellulosic compounds derived from agricultural by-products are favorable raw materials for low-cost electricity generation.[108] However, microorganisms in the MFC cannot use its lignocellulosic biomass for energy production. It must be broken down into monosaccharides or other reducing agents.[89]

Until now, much biomass, including forest detritus in a forested wetland,[109] kitchen waste,[110] chitin,[111] cow dung,[112] orange peel waste biomass,[113] wheat straw,[114] algae grown,[115] rice straw,[116] potato wastes,[117] hydrothermal liquefied cornstalk biomass,[118] food waste,[119] lemon peel biomass,[79] has been exploited as fuel sources for bioenergy production in MFCs.

Dai et al.[88] used wastewater from biohydrogen fermentation as a potential substrate. The wastewater used is reported to contain end-product metatabs (acetic acid, lactic acid, and butyric acid), which constitute a rich source of substrates for bacteria. This is a type of wastewater with a stable composition, rich in nutrients and low in toxic components. Hou et al.[110] reported that anaerobically digested kitchen waste produced a power density of 6255 mW/m$^3$, biomass concentration which was 325 mg/L, and COD removal efficiency of 43.59% when Golenkinia SDEC-16 was cultivated in the single chamber MFC. Miran et al.[113] demonstrated bioelectricity production from orange peel waste without chemical pretreatment or adding extra mediators. The maximum power density was 358.8 ± 15.6 mW/m$^2$. Liu et al.[118] has shown the continuous production of electricity from cornstalk biomass after hydrothermal liquefaction treatment. A maximum power density of 680 mW/m$^3$. About 80% of COD and TOC were effectively removed. In the study of Li et al.,[119] electricity recovery was achieved with efficient organic biodegradation in the MFC using canteen-based food waste as substrates. A maximum power density of 5.6 W/m$^3$ and an average output voltage of 0.51V was obtained.

Biomass quality is often determined by its inherent properties such as moisture content, bulk density, yield, size and shape, which affect its bioconversion and ability to bioelectricity production in MFC.[120] Most of the raw biomass or biomass derived from organic waste can be used as a substrate for MFC, implying that MFC is widely adaptable for energy harvesting from biomass.

## 6. APPLICATIONS OF MFC

Using MFC technology is very attractive because waste can be reduced and converted into energy, reducing waste disposal costs and increasing economic efficiency. This technology has led to many important applications, such as bioelectricity production, wastewater treatment, metal removal/recovery, biohydrogen production, biosensors, etc.

### 6.1. Wastewater treatment with bioelectricity production

Wastewater treatment and bioelectricity generation have become MFC's most heavily researched areas. Food processing wastewater, domestic wastewater, sewage sludge, and many other types of wastewater are rich in organic matter and can provide a wide range of microorganisms used in MFC. Furthermore, wastewater containing heavy metals and other harmful pollutants has also been used as a

substrate in MFC to reduce pollution.[121] They can remove COD up to 90% and Coulumbic efficiency up to 80%.[122] Mehmood et al. reported that this is an effective and highly cost-effective method to remove nitrogen and organic matter from leachate, biological treatment.[123] The traditional wastewater treatment methods have many limitations, such as energy consumption for the aeration of wastewater and generation of harmful emissions to the environment.[124] MFC technology is used to treat wastewater with a completely different method because of its ability to capture energy.[125] Also, MFC could be an efficient method of electricity generation. Du Z. and et al. reported that MFC has potential applications for waste treatment and energy generation in spaceships.[126] Wang et al. reported a maximum power of 6.0 $W/m^3$, with the current being 1.9 ± 0.4 mA and high biomass retention.[122] Rojas-Flores et al. reported having a peak voltage and current of 1.127 ± 0.096 V and (1.130 ± 0.018 mA). The maximum power density was 3.155 ± 0.24 $W/cm^2$ at 374.4 $mA/cm^2$ current density was achieved using blueberry waste as substrate.[127]

### 6.2. Biosensors

MFCs used as biosensors have attracted increasing attention because of their simplicity and robustness in various applications. One such sensor measures the amount of hydrogen peroxide produced and the lack of oxygen, having the advantages of being easily fabricated and assembling small-sized systems.[128] MFC-based biosensors are reported to be much more stable and durable than traditional BOD biosensors.[129] MFC-based biosensors are alternative dissolved oxygen (DO) measurement strategy. They are based on the fundamental principle that is based on cathode behavior. Wang et al. used a mini autonomous MFC-based for monitoring hexavalent chromium in wastewater.[130] Also, the potential of remediating toxicants, such as phenols, formaldehyde, and petroleum compounds, is another application of MFC.[131-132]

### 6.3. Biohydrogen production

Hydrogen may be produced in MFCs, such as secondary fuels, instead of electricity. To generate hydrogen gas in a typical MFC, the anodic potential must be increased with a supplemental voltage of about 0.23V or more to overcome this thermodynamic barrier. The oxygen in the cathode chamber should also be enhanced.[125] MFCs provide simultaneous wastewater remediation along with hydrogen generation has proven to be a sustainable process for energy production.[133] Chae et al. demonstrated a solar-powered microbial electrolysis cell for hydrogen production. Their work demonstrated that solar energy could be coupled into an MFC device and provide a critical driving force for the bioelectrochemical reaction.[134] The production of hydrogen by MFC is an environmentally friendly method compared to glucose fermentation.[135]

## 7. CONCLUSION

Power generation through an MFC using quality substrates oxidized by bacterial species offers a promising technology for the future. In previous studies, simple substrates such as lactate and acetate were widely used; however, in recent years, many available substrates have been used as anode chamber nutrients to utilize waste biomass and their treatment. The production of electricity from renewable waste biomass using MFC techniques has been considered a major development compared to traditional non-renewable biofuels. It is expected that the MFC technology will be designed to adapt a variety of substrates to make it a sustainable source of bioenergy.

The power generation efficiency of the systems is still very low, which prevents them from being widely used and limits their practical applications. It has been discovered that power density and combined efficiency can be increased through the appropriate selection of microorganisms, modes of operation, suitable materials for construction, and improved design

of MFCs. In addition, the large-scale application of MFCs is limited by its high cost and low wastewater buffering capacity. Therefore, more technological advances are needed, especially in the design of low-cost materials. The use of available substrates, especially wastewater, creates complexity in MFCs due to their high organic loads and inhibitory agents. Establishing a diverse and efficient microbial community is necessary to utilize the wastewater substrates efficiently and improve system performance. Combining MFC with conventional wastewater treatment technologies may be the best possible alternative for this technology.

This review discusses substrates such as acetate, brewery water, bamboo fermentation wastewater, inorganic substances, and azo coolants, which are harmful to the environment and organisms. New functions can be explored through power generation through MFC as substrate. Until now, a variety of substrates have been used in MFC for high efficiency/ performance. Even so, the important factors that limits the practical use of MFCs need to be worked out and addressed, such as power output reduction, in order to expand regulation. These factors contribute to the difficulty of commercializing MFC, so more efforts are needed to provide a viable technology that can be effectively applied to commercialize MFC technology.

Optimal and cost-effective design for scaled-up versions of laboratory-scale reactors is one of the most challenging issues in commercializing MFCs. In the case of COD removal and power generation, laboratory-scale MFCs have shown outstanding results. However, many aspects must be addressed in order for it to be scalable for practical applications, including the separator configuration, mechanical strength, electrode cost, and its low output power. Since most microbial electrochemical processes are based on redox reactions, various elements, such as electrodes, could be improved to enhance their performance. Using well-surfaced electrodes that promote microbial affinity can improve the performance of MFCs.

MFC performance has been illustrated when it comes to removing contaminants. The key to fully implementing MFCs in the field is to improve long-term operational stability. Most of the current MFC research was performed in the laboratory. Therefore, more scaled-up MFC research is needed to better understand how large-scale MFC systems operate and to enhance power production while maintaining economic feasibility. This is essential in the commercial adoption of this technology.

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