

Review Article

Microbial Fuel Cell: Recent Developments in Organic Substrate Use and Bacterial Electrode Interaction

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A new bioelectrochemical approach based on metabolic activities inoculated bacteria, and the microbial fuel cell (MFC) acts as biocatalysts for the natural conversion to energy of organic substrates. Among several factors, the organic substrate is the most critical challenge in MFC, which requires long-term stability. The utilization of unstable organic substrate directly affects the MFC performance, such as low energy generation. Similarly, the interaction and effect of the electrode with organic substrate are well discussed. The electrode-bacterial interaction is also another aspect after organic substrate in order to ensure the MFC performance. The conclusion is based on this literature view; the electrode content is also a significant challenge for MFCs with organic substrates in realistic applications. The current review discusses several commercial aspects of MFCs and their potential prospects. A durable organic substrate with an efficient electron transfer medium (anode electrode) is the modern necessity for this approach.

1. Introduction

Over the past few years, there has been an increase in energy demand. Nonrenewable energy sources, such as fossil fuels and nuclear power, are widely used in the world [1]. When it comes to fossil fuels, this source of energy does more damage to the environment and continuous use of fossil fuels emits carbon dioxide, which becomes toxic when there is too much of it in the air. The rapid depletion of fossil fuels had a significant impact on human life through air pollution and global warming [2–4]. However, many nations worldwide have made outstanding attempts to find a viable alternative to address the energy issue by focusing on renewable sources of energy, such as solar energy, water energy, and wind energy [5–7]. These attempts provided a new way to produce electricity that utilizes a fuel cell through the use of metal catalysts of high value (in the conventional version). As a matter of fact, many benefits can be obtained by using fuel

cell compared to other energy producers, such as emission of zero environmentally polluting gases, for example, CO₂, CO, NO_x, and SO_x, greater efficiency, and the absence of mobile components, resulting in less sonic 'pollution' [8]. The only shortcomings of these new energy sources, on the other hand, are their high cost and high mass generation [8]. Microbial fuel cell (MFC) is a type of bioelectrochemical fuel cell that requires the presence of active bacteria that function as biocatalyst for bioenergy generation in anodic chambers [9, 10]. In the year 1911, Potter [11] reported that bacteria were capable of generating current, but only after a period of 50 years, good results can be observed even they were very low in quantity [12]. Nevertheless, the fuel cell became the center of attention at the beginning of 1990s and as a result, the MFC technology also received attention [13]. In addition, more research was done in the year 1999, as it was known that the presence of a mediator is not a must for an MFC [14]. In general, MFC is composed of the chambers of

cathode and anode isolated by the presence of proton exchange membrane (PEM), as shown in Figure 1. Organic substrates will undergo oxidation via the active biocatalyst located within an anode to form protons and electrons [16]. The PEM facilitates the migration of protons towards the cathode, whereas electrons are transferred via an external circuit. Both electrons and protons form a reaction in the cathodic chamber with simultaneous reduction of oxygen to form water. It is the biocatalyst existing in the anode chamber that facilitates the oxidation sources of substrate for the production of protons and electrons. At an anode, electricity generation is prevented by the presence of oxygen; thus, a design of a realistic system that can avoid oxygen from reacting with bacteria should be created, thus the anaerobic condition for the anode chamber [17]. In the past 10 years, the system of MFC has been drastically enhanced but with certain limitations in regard to practicability and scale-up issues, for instance, the resistance of membrane during transportation of protons and problems in both chambers [18, 19]. In addition to the previous issues, MFC is also facing constriction in producing energy because energy generation through the MFC system is dependent on the concentration of the substrate. If the concentration of the substrates present is larger than a certain value, production of power will be obstructed [20, 21]. The organic substrate is the most critical challenge. The lower stability of the organic substrate may have had an impact on remediation efficiency and energy generation because the organic substrate did not provide enough power to the bacterial population, resulting in poor MFC output. The long-term stability of organic substrates in MFC for industrial use should be the subject of future research. According to the literature survey, there is no similar information available on the interaction of electrode-bacteria and electrode effect in the presence of different organic substrates in MFC. This review provides a direction to researchers to improve the organic substrate factor with electrode development to ensure the strong interaction of bacteria with the electrode material. In the present review article, different organic substrates are extensively studied with bacterial interaction. The effects of different previous electrodes and electrode-bacteria interactions are also summarized in the present study.

2. List of Organic Substrates Used in MFC

One of the most crucial aspects of MFC is the substrates used due to their effect on the generation of electricity [22, 23]. Various substrates can be utilized in the MFC technology to facilitate energy generation, either pure substances or complex mixture from organic materials present in wastewater. The aim of all treatment processes is mainly to reduce the number of pollutants in water in order for it to be safe for utilization and the environment. Activated sludge process or ASP is often used for the treatment of wastewater; however, the downside of this substance is that it consumed a huge amount of energy and based on estimation, the total energy required to provide oxygen for ASPs in the United States of America is equal to near 2% of the total electricity used in the country [22]. In addition, in ASP treatment, a second

treatment step is needed for the process, which ultimately classified the streams produced using this treatment for agroindustry as “raw material” instead of “waste” that can be further used to synthesize specific energy or chemical [24]. Furthermore, these days, waste management is more focused on how energy can be reused and recovered, which results in a new view on ways that these streams can be managed. A record of substrates that had been utilized for various MFC research is presented in Table 1. Due to the differences in parameters used for each MFC study, such as surface area, operational conditions, and electrode used, it is hard to compare the performances of MFC from the literature. Moreover, various researchers applied different units to indicate their MFC performances. A unit most often used is called current density that can signify the produced current per unit area of the surface area of anode (mA/cm^2) or the current produced per volume of the cell (mA/cm^3). From Table 1, the current density is written in the unit of mA/cm^2 for differentiation purposes. For articles that expressed the current density in terms other than mA/cm^2 , the current density is obtained according to the dimensions of the electrode used and the reported current value at maximum power. The most frequently used substrates and the effects they bring towards the performance of MFC are discussed in detail.

2.1. Acetate. Acetate seems to be the common substrate for energy production in the majority of MFC research so far. As opposed to acetate, the recalcitrance of certain forms of wastewater makes them somewhat challenging to use [27]. Acetate behaves as a straightforward substrate that is widely utilized as a source of carbon to stimulate electroactive bacteria [22]. Due to its inert behavior to alternate conversions of microbial such as methanogenesis and fermentation at ambient temperature, acetate is widely utilized as substrate as an ideal model for new MFC materials, operating conditions, or reactor prototypes [47]. Furthermore, acetate is the final result of many metabolic routes for sources of carbon with higher order (along with the glucose metabolism Entner–Doudoroff pathway) [48].

Liu et al. [49] stated that using a single-chambered MFC fed with the provided power by acetate ($506 \text{ mW}/\text{m}^2$, $800 \text{ mg}/\text{L}$) was almost 66 percent larger than the one emitted by butyrate ($305 \text{ mW}/\text{m}^2$, $1000 \text{ mg}/\text{L}$). Chae et al. [50] recently differentiated the power generation and CE efficiency of four types of substrates. MFC fed with acetate resulted in the largest value of CE (72.3%), led by butyrate (43.0%), propionate (36.0%), and glucose (15%). In contrast to wastewater enriched with protein as a substrate in MFC, the acetate-fed MFC obtained over two times the maximum power of electric and one-half of optimum resistance of external load as opposed to the MFC with protein-rich wastewater as the substrate [6]. Nonetheless, since wastewater with high amounts of protein is a dynamic substrate, there is a potential to enrich various microbial populations as compared to acetate. A variety of microbial populations aids in the utilization of different substrates or the conversion of organics to simple substances like acetate that can

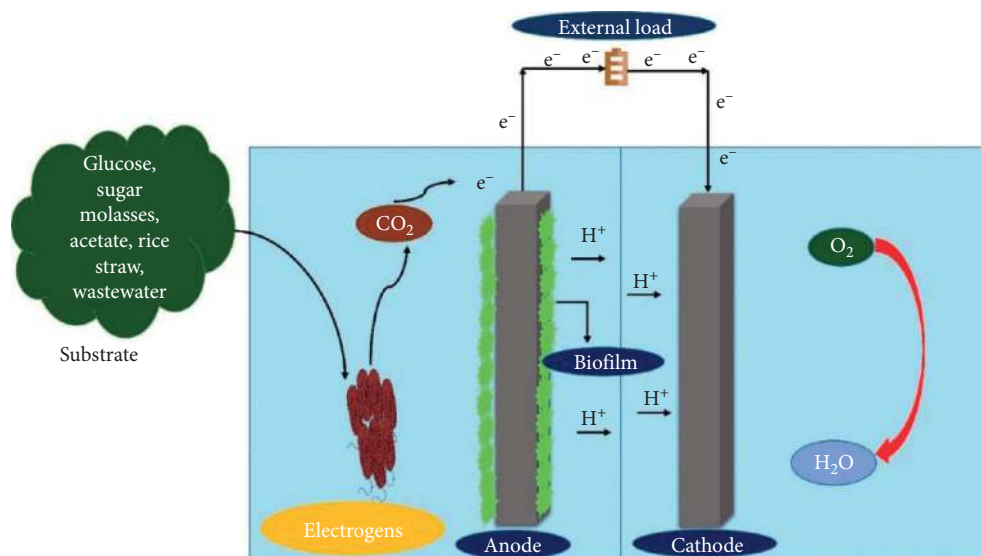


FIGURE 1: Systematic presentation of MFC (adapted from reference [15] with MDPI permission).

TABLE 1: Summary of the used substrates in MFC with performance.

List of organic substrates	Used concentration	Inoculum source	Type of MFC (with surface area of electrode and/or volume of cell)	Current density (mA/cm ²)	Reference
Acetate	1 g/L	Preacclimated bacteria	One-chamber MFC in the form of a cube with a graphite fiber brush anode (brush volume: 7170 m ² /m ³)	0.8	[25]
Arabitol	1220 mg/L	Preacclimated bacteria	MFC (12 mL) with nonwet proofed carbon cloth as an anode (2 cm ²) and wet proofed carbon cloth as a cathode (7 cm ²) in one chamber	0.68	[26]
Azo dye with glucose	300 mg/L	Mixture of anaerobic and aerobic sludge	MFC with a single-chamber air-cathode and a carbon paper anode (36 cm ²)	0.09	[27]
Carboxymethyl cellulose (CMC)	1 g/L	<i>Clostridium celluloticum</i> and <i>G. sulfurreducens</i>	MFC with two chambers and graphite plates as electrodes (16 cm ²), as well as ferricyanide catholyte	0.05	[28]
Cellulose particles	4 g/L	Pure culture of <i>Enterobacter cloacae</i>	Carbon cloth anode (1.13 cm ²) and carbon fibers as the cathode in a U-tube MFC	0.02	[28]
Corn stover biomass	1 g/L	Domestic wastewater	Carbon paper anode (7.1 cm ²) and carbon cloth cathode in a one-chamber membrane-less air-cathode MFC	0.15	[29]
Cysteine	385 mg/L	Sediment sample from depth of 30 cm	MFC with two chambers and carbon paper electrodes (11.25 cm ²)	0.0186	[30]
1,2-Dichloroethane	99 mg/L	Microbial consortia from MFC enriched with acetate	Two-chambered MFC with graphite plate anode (20 cm ²) and graphite granules cathode	0.008	[31]
Furfural	6.8 mM	Preacclimated bacteria from anode of a ferricyanide-cathode MFC	Carbon paper anode and cathode in a one-chamber air-cathode MFC (7 cm ²)	0.17	[32]
Galactitol	1220 mg/L	Preacclimated bacteria from MFC	MFC (12 mL) with nonwet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²) in one chamber	0.78	[26]

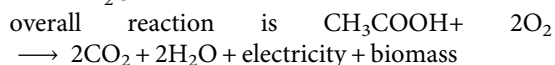
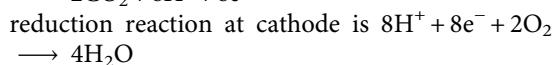
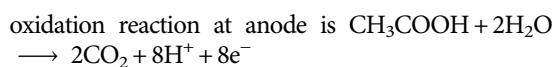
TABLE 1: Continued.

List of organic substrates	Used concentration	Inoculum source	Type of MFC (with surface area of electrode and/or volume of cell)	Current density (mA/cm ²)	Reference
Glucose	6.7 mM	Mixed bacterial culture maintained on sodium acetate for 1 year (<i>Rhodococcus</i> and <i>Paracoccus</i>)	MFC (12 mL) with a one-chamber air-cathode and nonwet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.70	[33]
Lactate	18 mM	Pure culture of <i>S. oneidensis</i> MR-1	MFC with two chambers and graphite felt electrode (20 cm ²)	0.005	[34]
Landfill leachate	6000 mg/L	Leachate and sludge	MFC with two chambers and a carbon veil electrode (30 cm ²)	0.0004	[35]
Macroalgae, <i>Ulva lactuca</i>	2500 mg/L	Primary clarifier overflow of a wastewater plant	Graphite brush anodes and a platinized cathode in a one-chamber air-cathode MFC (25 mL)	0.25	[36]
Microalgae, <i>Chlorella vulgaris</i>	2500 mg/L	Primary clarifier overflow of a wastewater plant	Graphite brush anodes and a platinized cathode in a one-chamber air-cathode MFC (25 mL)	0.20	[37]
Ribitol	1220 mg/L	Preacclimated bacteria from MFC	MFC (12 mL) with nonwet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²) in one chamber	0.73	[33]
Sodium formate	20 mM	Anaerobic digested fluid from a sewage treatment plant	MFC with two chambers and graphite felt electrodes (4.5 cm ²)	0.22	[37]
Sodium fumarate	25 mM	Pure culture of <i>G. sulfurreducens</i>	Half-cells of stainless steel cathode (2.5 cm ²) poised at -600 mV against Ag/AgCl	2.05	[38]
Sucrose	2674 mg/L	Anaerobic sludge from septic tank	MFC in two chambers of woven graphite anode (7 cm ²) and ferricyanide catholyte	0.19	[39]
Xylose and humic acid	10 mM	Domestic wastewater	MFC with two chambers and plain carbon paper as electrode (76.5 cm ²)	0.06	[40]
Brewery wastewater	600 mg/L	Anaerobic mixed consortia	One-chamber air-cathode MFC with carbon fibers as anode	0.18	[41]
Chocolate industry wastewater	1459 mg/L	Activated sludge	MFC with two chambers and ferricyanide as catholyte, with graphite rods as electrodes (16.485 cm ²) and ferricyanide as catholyte	0.302	[42]
Domestic wastewater	600 mg/L	Anaerobic sludge	MFC with a two-chambered mediator-free chamber and a simple graphite electrode (50 cm ²)	0.06	[43]
Protein-rich wastewater	1.75 g/L	Mesophilic anaerobic sludge	MFC with two chambers and graphite rods as electrodes (65 cm ²)	0.008	[44]
Real urban wastewater	330 mg/L	Domestic wastewater	A salt bridge connects the anolyte (1000 cm ³) and catholyte (100 cm ³) chambers; graphite cylinder anode (20 cm ²)	0.018	[45]
Starch processing wastewater	4852 mg/L	Starch processing wastewater	MFC with a single-chamber air-cathode and a carbon paper anode (25 cm ²)	0.09	[44]
Synthetic wastewater	16 g	Granular sludge from upflow anaerobic sludge blanket (UASB) reactor	MFC with glassy carbon electrodes (160 cm ²) that is membrane-less and mediator-free	0.17	[46]
Synthetic wastewater	510 mg/L	Anaerobic culture from a preexisting MFC	Stainless steel as anode (170 cm ²) and graphite rods as cathode (150 cm ²) in a dual-chamber MFC	0.008	[39]

function as donors of electrons in current processing. Bacteria in MFCs oxidize organic substrates, such as acetate, and other sugars to produce electrons. The oxidation

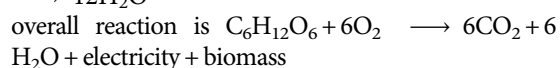
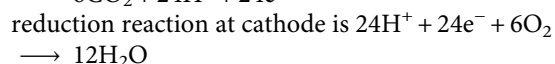
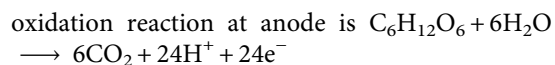
reaction is carried out by the anode, whereas the reduction process is carried out by the cathode. The biological reaction of acetate can be written as follows:

(a) If acetate is used as organic substrate,



2.2. Glucose. Another type of popular substrate for MFC is glucose. According to Kim et al. [51], the output of an MFC comprising *Proteus vulgaris* relies on the sources of carbon in the microorganism's initial medium, whereas glucose prompted cells within MFC ran for a shorter span of time than galactose-initiated cells. Fed-batch MFC enriched with glucose employing 100 mM ferric cyanide as the oxidation agent for cathode yielded a peak power density value of 216 W/m³, as reported by Rabaey et al. [52]. Hu et al. [53] contrasted the viability of using sediment of anaerobic conditions as a MFC fuel for energy production to glucose. Anaerobic sludge was utilized as a very small substrate in a baffle-chamber membrane-less MFC, and only 0.3 mW/m² of power was provided. In the same method, however, glucose produced the highest output value of 161 mW/m². One more research compared the energy conversion efficiency (ECE) of glucose and acetate substrates for MFC [54]. With acetate, the ECE value was 42 percent, but using glucose, it was just 3 percent, resulting in reduced power density and current. According to Chae et al. [50], MFC fed with glucose had the poorest ECE due to electron depletion by bacterial competition, but their comparatively distinct bacterial composition allowed for much broader substrate use and the highest PD. The poor ECE value was attributed to the assumption that glucose can be a fermentable medium, meaning that it is used by a variety of competitive metabolisms that do not generate energy, like methanogenesis and fermentation. Chae et al. [50] suggested that the existence of an even more dynamic mixed consortium of different electricigens or their syntrophic bacteria as an outcome of the development of diverse fermentation byproducts through degradation of glucose to justify the glucose-enriched MFC much broader substrate specificity than the others. Organic substrates such as glucose, acetate, sucrose, and other sugars are oxidized by bacteria in MFCs to generate electrons. The anode performs the oxidation reaction, whereas the cathode performs the reduction reaction. The glucose biochemical reaction can be written as follows:

(b) If glucose is used as organic substrate,



2.3. Lignocellulosic Biomass. Due to the availability and renewability, lignocellulosic compounds derived from residues of agriculture are a favorable feedstock for low-cost electricity

generation [55]. Nonetheless, microorganisms in MFC are unable to explicitly use lignocellulosic biomass for energy production. It must be degraded into monosaccharides or other reduced matters [56]. Catal et al. [33] showed that every monosaccharide, which can be produced directly from lignocellulosic biomass hydrolysis, was a decent energy source in MFC. Utilizing cellulose as base, energy production necessitates the presence of a microbial population that is capable of both exoelectrogenic and cellulolytic activities [57]. Zuo et al. [29] investigated energy production in MFC from biomass of corn stover waste through samples synthesized by acidic or neutral steam-exploded hydrolysis operations that form soluble sugars from hemicellulose. The maximal PDs for neutral hydrolysates and acid hydrolysates (1000 mg COD/L, 250x) were 371 mW/m² and 367 mW/m², respectively, by making use of an air-cathode with a layer of diffusion and improved conductivity of solution (20 mS/cm). Raw corn stover was recently demonstrated as a single-chambered MFC substrate for the production of electricity, but the power output was significantly lower than when glucose was utilized as the substrate [58]. There are yet to be discovered any appropriate microorganisms to convert pentose (an important part in lignocellulose hydrolysates) into bioethanol, leaving a significant portion of residual plant matter unfit for bioethanol development. At a 10 mM concentration of xylose (typical pentose), the PD was 69 mW/m², a lower value as compared to that of PD for glucose (97 mW/m² with similar concentration), meaning that xylose is harder to be used for the production of power unlike glucose [40].

2.4. Synthetic Wastewater. Several researchers use artificial or chemical wastewater of clear composition because it is simple to monitor in regard to loading pressure, conductivity, and pH. Venkata Mohan et al. [36] achieved variable efficiency by using synthetic wastewater at varying rates of loading in MFC with similar configurations. A few media used during bacterial growth contain large quantities of redox mediators, including high-intensity wastewater composed of reduced species of sulphur and cysteine that can serve as an abiotic donor of electrons and improved power output for a brief period of time [59]. However, this does not adequately reflect the system's performance. One solution for this is by utilizing a minimum salt solution containing only one electron donor, like glucose or acetate. Rodrigo et al. [45] fed MFC with different types of synthetic wastewaters but with the same organic contaminants, such as peptone and glucose, and organic loading of 315 mg/dm³ but with a distinct ratio of readily biodegradable substrate to observe how wastewater composition affected MFC efficiency. The waste-fed MFC that is slowly biodegradable generates higher energy, most likely because of the formation of intermediates that favor the production of electricity.

2.5. Brewery Wastewater. 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 [60]. A variety of

wastewater from breweries differs in their concentration, approximately more concentrated as compared to domestic wastewater by 10-fold ranging from 3000 to 5000 mg COD/L [61]. As it contains a high amount of carbohydrate and less concentration of ammonium nitrogen, brewery wastewater comes off as a decent MFC substrate. Feng et al. [62] had studied the treatment of beer brewery wastewater, making use of an air-cathode MFC. According to the report, a peak PD value of 528 mW/m² was obtained after the addition of a 50 mM phosphate buffer into the wastewater. This is a lower value of maximum power in contrast to the one obtained from domestic wastewater at comparable strength. This may be the result due to the different conductivities of both wastewaters. When using deionized water to dilute the concentration of the wastewater from a brewery, it results in a decreased value of conductivity of solution, which was 0.12 mS/cm. Wen et al. [41] had recently utilized a model based on the MFC polarization curve and from the findings, the loss of mass transport and reaction kinetic (0.248 V for both at 1.79 A/m² current density) was reported as the main factors that affected MFC performance for wastewater from the brewery. These losses can be evaded by raising the wastewater concentration and the temperature of the reaction and utilizing rough electrodes to give more sites for the reaction to occur.

2.6. Dye Wastewater. The biggest chemical class of synthetic dyes is made up of azo dyes, which are abundant in the effluent of textile and dye manufacturing industries. It is very crucial to get rid of dyes from these effluents before release as the presence of high dye concentrations will result in very serious environmental issues like blocking the transfer of oxygen and light into water, which will gravely affect aquatic life [63]. Other than that, in nature, dyes can be toxic; thus, in recent years, a lot of efforts are put into using dyes as an MFC substrate for removal of color from wastewater containing dye with simultaneous electricity production. A report by Sun et al. [27], when confectionary and glucose wastewater were utilized as cosubstrates in MFC, a model azo dye, ABRX3, or active brilliant red X-3B was quickly removed. However, dye with a high concentration of up to 1500 mg/L did not show decolorization, but the generation of energy from glucose is achieved with an increased concentration of more than 300 mg/L ABRX3. The competition between anode for electrons from sources of carbon and azo dye contributed to this result. Consequently, treatment of wastewater containing azo dye and wastewater containing organic matter that is easily biodegraded simultaneously can be performed by combining the two different types of wastewater in MFC that can both improve cost and energy [64]. The downside is that this process still needs significant development to find a dense bacterial community capable of applying dyes mixed with simple sources of carbon to provide a solution that is realistic using MFC to treat this wastewater.

2.7. Inorganic and Other Substrates. A research group investigated the production of electricity from anodic sulphide oxidation, which yielded a PD value of 39 mW/L [65].

Simultaneous treatment of wastewater of paper recycling plants and generation of electricity by MFC gives a maximum value of 672 mW/m² PD after using phosphate buffer [66]. Wastewater that is not treated with a phosphate buffer only has a power output = 144 mW/m² because the conductivity of the solution is low. Both production of current and phenol degradation were reported by Luo et al. [67], where the produced power is low as compared to glucose. There is also a substantial loss, which was less than 10% of ECE. According to Kaparaju et al. [68], a possible energy source can be found from the high amount of wastewater from integrated biorefineries. Borole et al. [69] had reported a simultaneous use of MFC for fermentation inhibitors such as vanillic acid, 5-hydroxymethylfurfural, furfural, 4-hydroxyacetophenone, and 4-hydroxybenzaldehyde removal from cellulosic biorefineries along with energy generation. A continuous anaerobic process by combining MFC with a fermenter of carbon monoxide had also been studied [70]. Acetate was synthesized by enrichment of fermented CO to feed MFC for electricity generation. The transformation of electricity from syn-gas (mainly CO) can be shown even if the yield of conversion was small through microbial processes. Degradation of 1,2-dichloroethane using acidophilic bacteria was also reported [31]. Moreover, a single-chambered MFC with *Desulfovibrio desulfuricans* as source inoculum was investigated for the elimination of thiosulfate and sulfate and 0.115 mA/cm² of peak current was produced [71].

3. Effect of Anode in MFC

In order to produce electrons, microorganisms have a significant role in an anode chamber. After the produced electrons pass through the external circuit of a cell, they will facilitate the acceptors of electron reduction in the cathode. Similarly, the generated protons must also thrust through the proton exchange membrane (PEM) towards the cathode from the anode so that the circuit can be completed. These steps will simultaneously work to generate electricity and remove organic waste [21]. As the anaerobic anode chamber is part of the most important component for an MFC, all vital factors are present in this chamber for biomass degradation. An anode chamber consists of a microorganism, mediator, which is optional, substrate, and acceptor of electron electrode [21]. The energy of activation needed for reactions at anode should be lowered in proportion with the catalysts. The catalyst is normally the bacteria present in the chamber of the anode [21, 72–74].

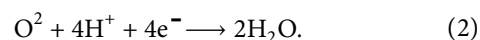
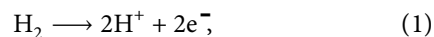
In general, there are a lot of factors that can affect MFC performances, such as the configuration of equipment and the material used for electrodes [75]. It is crucial to stabilize these factors for the development of MFC. Anodic microbial transfer of electron is deemed as a part of the most important agents that can effectively improve MFC performances to raise the transfer rate of the microbial electron by various types of ways that can be applied, for instance, optimizing the design of electrode and cell and mediators of electron addition. Based on these factors, it can be clearly seen that

electrodes are crucial parts for an MFC that can make or break its performance. Thus, a lot of research to study many types of materials for an electrode had been performed [59,76]. Moreover, for an electrode to be ideal, the materials should possess certain features: (1) satisfactory conduction of electricity and little resistance; (2) corrosion resistance and chemical stability; (3) biocompatibility; (4) suitable toughness and mechanical strength; (5) high surface area [9]. Materials that are made of carbon are the most common for an electrode, including those that contain carbon cloth and paper, graphite rod and fiber brush, felt of carbon, and reticulated vitreous carbon or RVC due to the good electrical conduction, stable microbial cultures, and high surface area [9]. Other than that, granular activated carbon (GAC), also known as graphite granules (GGs), also showed a high degree of activities of catalysts and microporosity, good conductivity, and low cost [77, 78]. Modified anode electrodes can improve MFC performance and because of this factor, more studies recently have focused on modifying anode using various techniques of nanoengineering to simplify the transfer of electrons. Furthermore, improvement of power density and increasing the potential of accepting electrons through modifying nanomaterials and techniques of heterogeneous fabrication had been researched [79]. Based on a report by Qiao et al. [80], utilizing carbon nanotubes or CNTs with polyaniline materials for anode can increase the feasibility of electron transfer and surface area of the electrode. A practical method to increase the output power of MFC is through using an anode made up of metal and modified carbon along with conductive polymers [81]. Attention should be given to the stability of electrodes when dealing with microorganisms present in substrates and organic polymers. Among various types of conductive polymers, the most commonly used for anode electrode modification is polyaniline (PANI) [82]. It has been reported that densities of current could be improved by using PANI polymers that had been modified, such as fluorinated PANI [23] and mixture of PANI/titanium dioxide [83]. Another suitable material for anode electrode is the composite of CNTs with polyaniline [84]. In addition, the performance of MFC could also be developed by the determination of anode composition [85]. Poly-tetrafluoroethylene (PTFE) that possesses hydrophobic behavior and chemical stability was used as an electrode for MFC. Zhang et al. also studied that composite of graphite/PTFE with 30% w/w of PTFE is a great anode to generate electricity with power density production of 760 mW/m² and *Escherichia coli* biocatalyst [86]. Some respective anode performances of previous studies are presented in Table 2.

4. Effect of Cathode in MFC

In the chamber of the anode, protons will be produced, and these protons will transfer to the cathode by proton exchange membrane; as a result, the circuit is completed. The generated electron (equations 1 and 2) will transfer onto oxygen after moving towards the cathode chamber. The resulting oxygen is radical oxygen and its positive ions will be synthesized at anode producing water that will spread

along the permeable membrane of ion at the cathode with the help of catalyst as follows [99]:



In this process, a steady current is produced when the cathode and anode are connected with wire [100]. The acceptor of electron species and concentration, the performance of catalyst, presence of protons, and structure of electrode have a tremendous impact on the cathode reaction yield. For cathodic and anodic reactions, catalysis is required, and a suitable catalyst can definitely help in improving the rate of reaction and decreasing the energy of activation [101]. Oxygen, which is abundant in the cathode chamber, is commonly utilized as the last acceptor electron because of the high potential of oxidation and formation of water, which is nonpoisonous at the end of reaction and is beneficial for the environment [102].

However, the issue of slow oxygen reduction kinetic even if a high potential is generated when plain graphite is utilized in MFC needs to be addressed by using an equivalent catalyst [91]. One catalyst that has been suggested to solve this problem is Potassium Ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) [92]. On the other hand, $\text{K}_3[\text{Fe}(\text{CN})_6]$ needs to be refilled manually with passing time because of regeneration issues as $\text{K}_3[\text{Fe}(\text{CN})_6]$ is not sufficiently oxidized by oxygen [93]. At the same time, $\text{K}_3[\text{Fe}(\text{CN})_6]$ modified the anaerobic environment of the anode chamber via PEM [9]. Ferricyanide is beneficial as it produces small overpotential on simple electrodes made from carbon. The abiotic platinum catalyst is also often used for reactions occurring at cathode, but platinum itself is not an ideal catalyst for MFC because of its toxic behavior towards certain substrates [103]. Researchers usually added another oxidant, for example, artificial mediators for electron redox into the cathodic chamber, i.e., potassium permanganate, in order to raise the performance of MFC [104]. Najafpour et al. [105] reported that power, current, and voltage values can be increased with a low amount of potassium permanganate acting as an agent for oxidation. In MFC, the cathode is put into the compartment at one side and external surrounding on another. On the air side, the existence of Co catalyst facilitates the performance of MFC, but waterproofing may cause a limit of protons towards the catalyst [106]. Biocathodes have been a probable solution for the issues of catalysis specifications for oxidation of oxygen at cathode [107]. Biocathodes required utilization of microorganisms catalyst to facilitate reactions at the cathode for the improvement of energy generation using MFC, which will be an ideal cathode substitute rather than synthetic mediators or nitrate, tetrachloroethene, perchlorate, fumarate, sulfate catalysts, and electron acceptors such as Co, trichloroethene, Fe(III), U(VI), H₂, and Cr(VI) without utilizing exogenous [108]. Lower cost and configuration of biocathodes give them an advantage for them to be utilized instead of abiotic cathode, and as a result, high-cost mediators and catalysts like Pt are unnecessary [21]. Biocathodes also produce practical end products and the

TABLE 2: Different MFC performances with their respective anode.

Substrate	Anode	Bacteria	Configuration of the system	Maximum PD (mW/m ²)	References
Glucose	Carbon paper	<i>Geobacter SPP</i> (firmicutes)	Two-chamber	40.3 ± 3.9	[87]
Glucose	Graphite	<i>Saccharomyces cerevisiae</i>	Two-chamber	16	[88]
Acetate	Carbon paper	<i>G. sulfurreducens</i>	Two-chamber	48.4 ± 0.3	[87]
Lactate	Carbon paper	<i>Geobacter SPP</i>	Two-chamber	52 ± 4.7	[87]
Ethanol	—	Betaproteobacteria	Two-chamber	40 ± 2	[89]
Cysteine	Carbon paper	Gammaproteobacteria and <i>Shewanella affinis</i> (KMM3586)	Two-chamber	36	[30]
Marine sediment reached in acetate	Graphite	Deltaproteobacteria	Two-chamber	14	[90]
Marine sediment	Noncorroding graphite	<i>Desulfuromonas</i> spp.	Two-chamber	25.4–26.6	[91]
Sewage sludge	Graphite with Mn ⁴⁺	<i>Escherichia coli</i>	Single chamber	91	[92]
Sewage sludge	Graphite with neutral red (NR)	<i>Escherichia coli</i>	Single chamber	152	[92]
Sewage sludge	Platinum and polyaniline-co-modified	<i>Escherichia coli</i>	Single chamber	6000	[93]
Glucose	Composite electrode (graphite/PTFE)	<i>Escherichia coli</i>	Single chamber	760	[86]
Glucose	Teflon-treated carbon fiber paper	Electrochemically active bacteria	Two-chamber (H-type MFC)	15.2	[94]
Lactose	“	“	“	17.2	[95]
Cellulose	Nonwet proof carbon paper	Cellulose-degrading bacteria	“	188	[96]
Glucose	Graphite plates	Mixed culture	Two-chamber air-cathode MFC	283	[97]
Glucose	Carbon paper with PPY-CNTs	<i>Escherichia coli</i>	DCMFC	228	[98]

metabolism of microbe allows the removal of side products. Other than that, by dismissing the possibility of needing to use mediators of electrons at cathode and toxic Pt chamber, this will directly improve the sustainability of MFC [109]. There are two types of biocathodes, which are anaerobic and aerobic. For aerobic biocathodes, reduction of oxygen with simultaneous transition metal oxidation catalyzed with biofilm present on the cathode surface like Mn (II) and Fe (II) is possible. Furthermore, a larger power density can be obtained as compared to using anaerobic biocathodes [110]. Biocathode-based MFC is also capable of treating surplus streams of wastewater in the cathode. Besides, microbial metabolites will accumulate in the cathode compartment, which will obstruct the microbial activities; based on the report by Zhou et al. [111], removing various overpotentials, the resulting oxidation voltage of organic carbon and combination reaction of redox may be within minute value of the MFC.

The major limitation is the cathode performance. For an MFC to be of conventional use, cathode design is very crucial. Nonetheless, the power output for an MFC is independent of the cathode surface area, whereas the capability of the cathode can be increased with materials that have a large surface area or granular materials [112]. However, it is important and a challenge to recognize material that can increase and improve the electricity generation of an MFC to its fullest and reduce the cost at the same time. Carbon paper, brush, felt, and fiber, as well as many graphites, Pt which is also utilized as catalyst, Cu-Au, Cu, granular graphite (great material), reticulated vitreous

carbon (RVC), and tungsten carbide, have been used as cathode materials [113, 114]. The characteristics of catalysts differ with cathode materials. Table 3 lists down the different values obtained with various heterogeneous cathode materials.

5. Electrical Interaction between Electrodes-Bacterial Species

In order to study the electrical interactions between pure and mixed cultures with anode of the system, cyclic voltammetry is frequently utilized as the process of indirect and direct transfer of electrons can be easily distinguished from that within biofilms connected to anode [119]. The use of cyclic voltammograms enables data interpretation of the process of electron transfer that differs according to the growth of the biofilm, the community of biofilm, and the energy produced by the biofilm [120, 121]. However, interpretation through cyclic voltammograms can be quite difficult; for instance, for analysis of *G. sulfurreducens* using this technique, a lower rate of scans was used to increase the complexity and to raise the discernible systems redox from 2 to either 4 or more [49]. The cyclic voltammetry is capable of examining the nearest biofilm to the electrode; thus, the adequacy of this method to examine the large biofilm is questionable because of the notable gradient of potential that exists in biofilms [122]. This method is not suitable to work independently for physiochemical nature determination of mediators present in the transfer of electrons [123]. A lot of microorganisms such as *Pseudomonas* species, *Geothrix fermentans*, and

TABLE 3: List of electrodes used in MFC with maximum power, voltage, and current produced.

Cathode	Maximum PD	Maximum CD	Maximum voltage (mV)	References
Activated carbon fiber felt (ACFF)	315 mW/m ² (0.7 W/m ³)	1.67 * 10 ⁻³ mA/m ²	679	[115]
Air-cathode with graphite	283 mW/m ²	1210 mA/m ²	440	[97]
Carbon felt	77 mW/m ² (0.2 W/m ³)	6 * 10 ⁻³ mA/m ²	575	[115]
Plain carbon	67 mW/m ² (0.1 W/m ³)	1.5 mA/m ²	598	[115]
Pt-coated carbon paper	0.3 W/m ³	4.69 mA/m ²	644	[115]
Tubular ACFF	784 mW/m ²	3.17 A/m ²	716	[115]
ACFF granules (1 cm)	667 W/m ³	3.34 A/m ²	658	[115]
Biocathode	19.53 W/m ³	41.78 A/m ³	432	[20]
Graphite felt	539 mW/m ²	3145 mA/m ²	742.3	[116]
Parallel sheets of carbon paper secured by carbon fiber coated with Pt	7.29 W/m ³	13.16 A/m ³	553	[117]
Air-cathode with carbon cloth	50 W/m ³	363 A/m ³	710	[118]

Shewanella species have been recognized for mediators of electron formation, but applications in MFC that can generate high current have been debated in contrast to bacterial species that are capable of transferring electrons directly to electrode [123].

The processes of indirect and direct transfer of electrons and their possible mechanisms have been recognized through research of dissimilatory metal that can reduce bacteria using nonsoluble iron oxides as the terminal acceptor of electrons [124]. Identified routes for the movement of electrons, including shuttles of electron formation, direct contact of the external surface of c-type cytochromes, and long-distance contact through nanowires or electrically conductive pili. Various reviews have discussed the benefits of direct transfer of electron and pili application in the synthesis of conductive biofilm for shuttles formation [125]. *G. sulfurreducens* is the most frequently researched bacteria in MFC that can generate high-range densities of current. It represents the *Geobacter* communities to explain the processes involving bacterial species in the MFC system with environmental inoculum [126]. Other than that, pure cultures of this organism are capable of generating power that is close to or larger than the maximum for biofilm of mixed-species biofilms. The sequence of the full genome can be obtained and modification of gene is possible, the analysis of the full microarray genome can also be obtained, and lastly, the availability of metabolic model of *in silico* based from the genome [127]. Moreover, *G. sulfurreducens* is classified as bacteria called electricigens capable of saving energy for development through complete oxidation of organic material to CO₂ along with transfer of electrons directly to MFC anode [106]. Other names that refer to bacteria that can facilitate electron transfer have been suggested, such as exoelectrogens, anode-respiring bacteria, anodophilic bacteria, electrochemically active bacteria (EAB), and electrogenic microorganisms [128]. Electron transfer to electrode allows organic material to be completely oxidized and, as a result, produces a large value of Coulombic efficiency; bacteria species facilitate direct transfer of electron towards anode removing the requirement of exogenous and electrons mediator's formation; lasting stability related to the ability of energy conservation for development due to electron transfer capability is one of the strong points of the

utilization of electricigens in MFC [105]. The bacteria-anode interaction is systematically shown in Figure 2.

Indication of direct contact between anode and cells through interaction via c-type cytochromes on the external layers is recognized by analysis of electrochemical and expression of genome-scale gene [130]. The thickness of biofilms formed from *G. sulfurreducens* is more than 50 μm and the cells are active metabolically and facilitate the generation of energy [131]. The investigation of the expression of genes proposed that for the long-distance transfer of electrons via the biofilms of *G. sulfurreducens*, it is crucial to form microbial nanowires [132]. Formation of the high value of current is also anticipated by representative studies via biofilm of thick anode if the conductive biofilm is present [133]. The generation of biofilm that is conductive is unlikely because the majority function as insulators [134]. In the field of MFC, it will be a great discovery to explore the conductivity determination, measurement, and the materials that affect the biofilm conductivity.

The focus that energy is gained by bacterial species for the electrode or shuttle of electrons directly is a common misunderstanding. In actuality, energy is received from the activities of protons pumping over the internal membrane that produces a gradient of protons that enhances the production of ATP via ATPase from ADP. In this regard, the external transfer of electron function is to migrate the electron towards the surface of anode, but no energy is obtained during the process for the growth of bacteria; instead, the proton gradient enables the formation of ATP, which in return supplies energy for the bacterial species [135].

The effort has been made to enhance the generation of current by genetic engineering but is unsuccessful [136]. The power generated does not increase, albeit there is an excess of microbial nanowires or cytochromes; similarly, ATP drain formation as forecasted by metabolism representative to raise the metabolic activity also did not have any effect on the current. Based on these investigations, it is clear that the production of current in an MFC is a difficult process that needs more than a few gene modifications or rates of bacterial respiration to have an impact on the current rate of generation. Adaptive selection has shown more positive results in strain production to increase the density of current

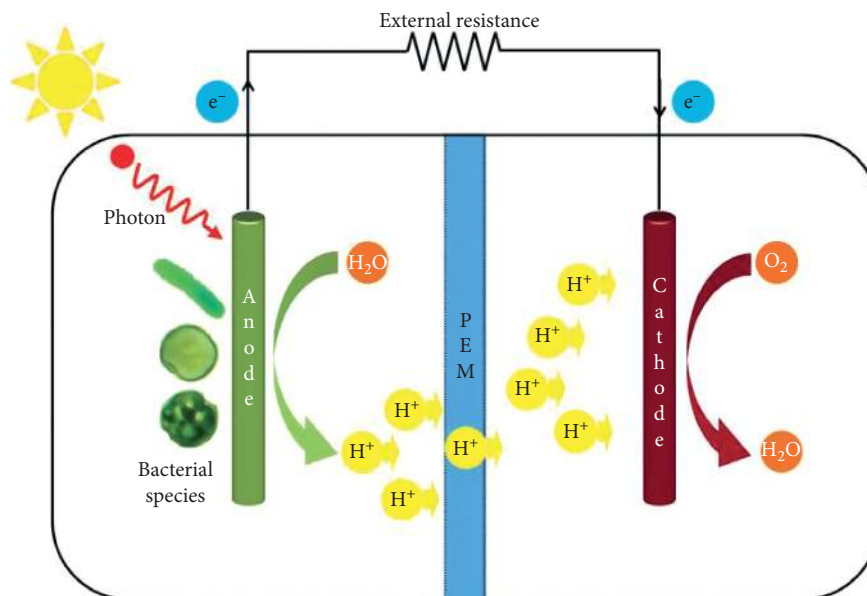


FIGURE 2: A dual-chamber PMFC with photosynthetic microorganisms serving as electricigens in the anodic chamber is shown schematically (adapted from [129] with Springer Nature Open Access Permission).

in MFC. One example is MFC with a low operating potential that was deployed for a period of more than 5 months and resulted in the separation of a *G. sulfurreducens* variant called KN400 that allows the rise in power density up to 8 times. The strain formed generates biofilm that is thinner along with cytochromes of small external surface area but a large number of nanowires that may enable the examination of the transfer of electrons occurring at increased current densities.

6. MFC Commercialization and Future Perspectives

The commercialization of technology can be deemed successful if more people become aware of the product and it is marketed in large amounts to a wide range of consumers. The commercialization of MFC will bring more benefits due to its function in energy production through the utilization of wastes. For instance, electricity can easily be generated at homes; energy generation with low expense can be accomplished all year long as waste materials. The xenobiotics are abundant in low-income countries like Africa; the MFC will be beneficial because of its low operating cost as compared to large foundations needed to set up energy generation plants that are unavailable [137]. Lastly, MFC could be an alternative for the remediation process to remove hazardous substances from xenobiotics and wastes [138]. The performance of an MFC technology is dependent on the number of variables used to observe the system, for example, the utilized substrates, the setup, microbes present, catalyst, concentration, ideal membrane, and electrode materials. A large number of accessible reports have shown that MFC could be configured from sizes of a few milliliters to a few thousand liters. Based on these reports, the outcome is that the power generated is affected by the MFC scaling, which is a major weakness in marketing MFC. The space

between anode and cathode is one of the components that can affect the power production for MFC [139]. With bigger cell size, the size of electrodes used will also increase; however, the separation itself does not change to the same extent to avoid bulky cells and this causes the low generation of power. Another reason that limits MFC scaling is the price of electrodes, which rationally should be inexpensive, but as the electrodes are bought instead of being produced in the industry, the price becomes higher, as well as because of the material itself. The membrane present in MFC is also created using high-cost material such as nylon. Substrates also play an important role for MFC and depending on the type of substrate utilized, the power output will differ. As an example, the pure substrate will result in high power production, but by using wastes as substrates, the value of power generated decreased significantly. The reason for this is that microorganisms are incapable of metabolizing waste and pure sources of carbon [140]. These are some of the limiting factors towards MFC commercialization. MFC is capable of utilizing various organic materials in producing energy. Nevertheless, MFC still has few weaknesses that need to be addressed in order for the technology to be applied in a practical situation. The biggest challenge of an MFC is the poor density of power, which can be sorted out either by separating strong microbes that are capable of transferring electrons towards anode or by synthesizing engineered strains via DNA recombination that provides a higher transfer rate of electrons. Various consortia of bacteria are proven to be more efficient in transferring electrons than pure cultures and more strains of bacteria can generate mediators for a more successful electron transfer. New types of mediators are recognized to enhance MFC performance. The small surface area of an MFC is also a big challenge as a limited number of microorganisms can adhere to it. Further

studies reported new techniques that can improve the performance of MFC, which provide a more efficient configuration of small-scale MFC. Some of the techniques are stacked reactors, assemblies of cloth electrodes, and utilization of air cathodes. Utilization of air-cathode MFC is the most efficient method out of all techniques because it utilizes oxygen sources from air efficiently, in return removing the requirement of water aeration and employing chemical catholytes like ferricyanide that need to be regenerated. Distinct cell designs are used in evaluating the consequences of utilizing various shapes and positions for MFC improvement and optimization of air-cathode is also done for MFC utilization. Great results have been achieved through these attempts in which a power output of more than 1000 W m^{-3} was generated from an efficient small-scale MFC (~20 ml volume of anode) [141]. Nevertheless, producing a large-size MFC that can provide increased energy generation and performance stability is still a difficult task. According to a study done by Liu et al. [142], the highest value of power density 20 W m^{-3} can be achieved by using MFC of 500 ml in volume. The final downside of the MFC system is in regard to wastewater treatment and MFC scaling up. These are crucial issues needing solutions as scaling up can result in MFC application for a large-scale setup that can help in thoroughly improving MFC performance, especially in the case of wastewater treatment that is abundant.

7. Conclusion

Electricity generation through the MFC system by utilizing organic substrates that are oxidized by bacterial species can provide a promising technique for the future. In this review, major waste materials such as acetate, brewery wastewater, synthetic wastewater, inorganic compounds, and azo dyes, which are harmful to the environment and living beings, have been discussed, which can explore new potential through electricity generation via MFC as substrates. In addition, some of the toxic substances can also be treated and converted into less harmful substances that are beneficial for the management of waste and can decrease the amount of environmental pollution. Until recently, a wide range of substrates had been used in MFC for energy production. Even so, major challenges for practical use of MFC need to be addressed and solved, such as the poor output of power and reduction of energy production as a result of scaling up. These factors contribute to difficulties in MFC commercialization. Thus, more efforts are needed to provide a feasible, applicable, and efficient technology that can be approved and acknowledged by the industry.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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