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Overview of Microbial Fuel Cell (MFC) Recent Advancement from Fundamentals to Applications: MFC Designs, Major Elements, and Scalability

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Abstract: Fossil fuels and carbon origin resources are affecting our environment. Therefore, alternative energy sources have to be established to co-produce energy along with fossil fuels and carbon origin resources until it is the right time to replace them. Microbial Fuel Cell (MFC) is a promising technology in the field of energy production. Compared to the conventional power sources it is more efficient and not controlled by Carnot cycle. Its high efficiencies, low noise, and less pollutant output could make it revolutionize in the power generation industry with a shift from centrally located generating stations and long-distance transmission lines to dispersed power generation at load sites.

In this review, several characteristics of the MFC technology will be highlighted. **First**, a brief history of abiotic to biological fuel cells and subsequently, microbial fuel cells is presented. **Second**, the focus is then shifted to elements responsible for the making MFC working with effeciency. Setup of the MFC system for every element and their assymbly is then introduced, followed by an explanation of the working machinary principle. **Finally**, microbial fuel cell designs and types of main configurations used are presented along with scalability of the technology for the proper application.

Keywords: Microbial fuel cell (MFC); fuel cell elements; design; energy generation; Scaling up; configuration.

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1. Introduction

Conventional waste and wastewater treatment systems need high-energy requirement depending on the alternative treatment technology, which will consume less energy for efficient operation. Moreover, with the insufficient use of fossil fuels and their products, global energy environmental concerns and crises urge the world to find out renewable/sustainable and clean energy sources [1, 2]. Employing the microorganisms to convert biodegradable waste materials (substrates), and whatever considered once as expensive organic wastes to dispose of, into electricity [3-5], grab the attention to MFC and made it the technology of choice [6]. With this technology, bacteria are attached to the surface of anode electrode forming a biofilm, which is then used as an electrochemical reaction catalyst to produce electricity. When used for waste and wastewater treatment, treatment using MFC is effective as it is clean energy, safe, quiet in performance, low in emissions, highly efficient, and has direct electricity recovery.

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Herein, the issue of designing and scaling-up MFCs will be addressed, especially with regard to their prospective configuration. In addition, the history of MFC evolution and mechanism of how does it work were mentioned briefly. Lastly, some recommendations and suggestions were listed and discussed with some detals.

2. Microbial fuel cell revolution

On the year 1790, Italian physician and physicist called Luigi Galvani who investigated the nature and effects of electricity in animal tissue was the first to observe the bioelectric phenomenon when he noticed twitching of isolated frog leg upon passing_a brief electrical discharge through it, and the term bioelectricity was coined after that observation. On the year 1910, Michael Cresse Potter, a professor of botany at the University of Durham, UK, demonstrated that organisms could generate a voltage and deliver current when he was researching how microorganisms degrade organic compounds. Later in 1911, Potter discovered electrical energy from cell cultures of Escherichia coli and Saccharomyces using platinum electrodes. This discovery led him to construct a basic microbial fuel cell [7]. In 1931, Cohen at Cambridge, UK, revived Potter's idea when he described how a batch of biological fuel cells produced more than 35V. On the 1960s, the idea of fuel cell became popular when the National Aeronautics and Space Administration exhibited interest in turning organic waste into electricity on its long-haul space flights. Algae and bacteria were among the first organisms used in biological fuel cells. During this period, Rohrback et al. designed the first biological fuel cell in which *Clostridium butyricum* was used as a biological material to generate hydrogen by glucose fermentation [8]. Right after that, biological fuel cells were already commercially available for use as a power source, but they were unsuccessful. Therefore, they disappeared from the market.

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On 1966, Williams showed that rice husk is a potential source of lignocellulose as it can produce, up on fermentation, many useful enzymes and biofuels resulting in 40 mA at 6 V using biological fuel cells [9]. Also, few more attempts have been done to follow William's demonstration [10, 11]. However, the fuel cell revolution started when M. J. Allen and H. Peter Bennetto from Kings College in London, UK, have developed and demonstrated improved biological fuel cells using various microorganisms to enhance both the efficiency of electron-transfer and the reaction rate using mediator systems [12]. Hence, they combined an understanding of the electron transport chain and significant advancements in technology. Moreover, to answer the question of how do the electrons get from the electron transport chain to the anode? Certain electrochemically active bacterial species using no mediator molecules were discovered by Byung Hong Kim group, (Korean Institute of Science and Technology, South Korea) to transport electrons to the electrodes [13]. Later, Chaudhuri and Lovely have confirmed the finding by Byung Hong Kim group by demonstrating that *R. ferrireducens* microorganism can recover up to 83% of electrons from glucose oxidation in the presence of Fe^{3+} without a mediator.

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Table 1: MFC History.

Scientist	Country	Year	Contribution
Luigi Galvani (Physician and physicist)	Italy	1790	Observe the bioelectric phenomenon when he noticed twitching of isolated frog leg upon passing a brief electrical discharge through it, and the term bioelectricity was coined after that observation.
Potter, M.C., (Professor of botany at the University of Durham)	Durham, UK	1910 - 1912	Demonstrated that organisms could generate a voltage and deliver current. Discovered electrical energy from cell cultures of Escherichia coli and Saccharomyces using platinum electrodes
Cohen, B.,	Cambridge, UK	1931	Described how a batch of biological fuel cells produced more than 35V.
Rohrback, G. H., Scott, W. R. and canfield, J. H.		1962	Designed the first biological fuel cell in which Clostridium butyricum was used as a biological material to generate hydrogen by glucose fermentation
Williams, K. R.		1966	Showed that rice husk is a potential source of lignocellulose as it can produce, up on fermentation, many useful enzymes and biofuels resulting in 40mA at 6 V using biological fuel cells
Allen, R. M. & Bennetto, H. P. from kings college in London, UK	London, UK	1993	Developed and demonstrated improved biological fuel cells using various microorganisms to enhance both the efficiency of electron-transfer and the reaction rate using mediator systems
Chang, I. S., Moon, H., Bretschger, O., Jang, J. K., Park, H. I., Nealson, K. H. & Kim, B. H. from Korean institute of science and technology (KIST)	South Korea	2006	Discovered that certain electrochemically active bacterial species using no mediator molecules transport electrons to the electrodes.

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3. MFC Elements, Setup, and Working machinery Principle

Typically, MFC apparatus (Figure 1) consists of two chambers (anodic and cathodic) made up of glass, polycarbonate or Plexiglas, with a respective electrode of graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black or reticulated vitreous carbon (RVC). These two chambers are separated by a proton exchange membrane (PEM) (Nafion or Ultrex) [14]. The anodic chamber contains organic substrates that are metabolized by microbes for growth and energy production while generating electron and proton. Cathode chamber contains a high potential electron acceptor to complete the circuit. To increase power density, an ideal electron acceptor is needed and it must be sustainable with no interference or toxic effect to microbial community or any other elements of the system in any way. Oxygen worked as an ideal electron acceptor due to its non-toxic effect and preferred as an oxidizing agent as it simplifies MFC operation. Instead, a standard oxidizing agent with a suitable electron acceptor such as ferricyanide can be used [15, 16]. Since microorganisms can produce electrochemically active substances that may be either metabolic intermediaries or final products of anaerobic respiration [17], MFC can be defined as a device using microorganisms to convert chemicals into electrical energy in a catalytic reaction [18]. MFCs are exceptional in using microorganisms to oxidize the organic matters biologically and to produce and transfer electrons to the anode electrode surface to form a biofilm. This biofilm is used as a biocatalyst instead of precious metal catalysts such as platinum (Pt) to perform the required electrochemical redox reaction (catalytic oxidation at the anode and chemical reduction at the cathode).

At the anode chamber, organic matter is the fuel of MFCs and microorganisms carry out the degradation of organic compounds by oxidizing (reduces other substances and loses

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electrons; electron donor) biodegradable substrates to produce CO_2, protons, and electrons. The generated electrons produced from the metabolic activity of microorganisms are transferred to and collected on the anode electrode surface by redox-active proteins or cytochromes and then passed to the cathode, thus reacting with the electronic acceptor (usually oxygen) through electrical circuit (copper wire) [19, 20]. Simultaneously, protons are transferred internally through the membrane to form water molecule at the cathode. Due to the difference in solution concentrations, a potential difference is produced between the anode and cathode. The electron flow through the external electric circuit is responsible for the electric power generation.

At the cathode chamber, usually, an electron acceptor is provided, e.g., oxygen or ferricyanide, for the reduction of electrons to take place, and among other possible reactions electrons combine with protons and oxygen forming water molecules, i.e., oxygen is reduced to water [15, 21].

Usually, the oxygen reduction is catalyzed by a precious metal, such as platinum, and non-precious metal, such as palladium catalyst [22, 23]. Furthermore, the microorganisms contain true catalysts because the energy they gained is from the fuel oxidation at the anode. Thus, this oxidation is not an actual catalysis step, where overall energy loss is created. Depending on the energy (the bacterial energy gain at the anode and the loss at the cathode), 0.3–0.5 volts is usually obtained for fuels such as acetic acid or glucose. Almost any substrate of any biodegradable organic matter and complex organic matter mix, present in human, animal, and food processing wastewaters, could be used to generate electricity in MFC. Technically, microorganisms are ideal catalysts and self-regenerating systems that can to produce electrical energy from a variety of renewable chemical sources. These chemicals are the carbon source for

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bacteria in MFCs and they could be either simple carbohydrate sources, such as sucrose, glucose [24], acetate [25], alcohols [26, 27], grape juice [28]; or complex carbon sources such as wastewaters from different origins [29], starch [30, 31], chocolate industry [32], food processing [33], beer brewery [34], and sewage sludge [35]; and food industry wastes [36]. As a result of microorganism's flexibility in consuming a broad range of fuels harnessed from waste, the MFC considered globally to be an ideal technology for bioelectricity generation from renewable biomass.

According to how electrons are transferred from the bacteria to the anode, MFCs can be classified into two types: mediator and mediatorless MFCs. In mediator type of MFCs, microorganisms have no electrochemically-active-surface-proteins for transferring electrons to the anode electrode. As a result, they need agents to help them increase the availability of the transferred electrons and, to transfer these electrons between the microorganisms and the electrode at relatively higher concentrations. These agents are called electroactive metabolites (or mediators). Nevertheless, depending on the microorganism species involved, mediators may be synthetic, like neutral red; or natural, like sulfate/sulfide [37, 38]. However, the cells are set to enter the cell electron transport chain and to accept the electrons that are produced using inorganic mediators under anaerobic conditions. At this point, the mediators cross the outer cell lipid membranes and plasma wall, and start liberating electrons from the electron transport chain, which is usually taken up by oxygen and other intermediates. Afterward, the reduced mediator exits the electron-loaded cell and deposits the carried electrons on the surface of the anode electrode. Hence, anode electrode becomes negatively charged. Upon releasing the electrons, the mediator returns to its original oxidized state prepared to repeat the process. By contrast, under aerobic condition, oxygen (has a higher electronegativity than the mediator) will collect all the

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electrons. Some mediators have been suggested to be used in microbial fuel cells including methylene blue, natural red, resorufin or thionine [39]. The reduced mediator is oxidized when it deposits the carried electrons from the cell to the anode electrode. Through the external circuit, the electrons are then flow to the cathode electrode (acts as an electron sink) and passes to an oxidizing material to complete the process. It should be noted that these electrons are consumed as electric current to power electronic devices before they ultimately get to oxygen at the cathode, which is what is required for bacterial optimal growth. Due to high costs and toxicity of these mediators, it is necessary to develop alternatives to improve the power production and decrease the capital cost making it suitable for wastewater treatment process; mediator-less MFCs. Furthermore, there are many drawbacks when using exogenous mediators such as short lifetime, high cost, and toxicity to both the microorganisms and the natural environmental-eco systems. Nevertheless, when the bacteria produce their own mediators or directly transfer electrons to the electrode, the system can operate at a high-sustained activity level.

On the other hand, electricity can be generated without mediators using some microorganisms. The mediator-less type of MFCs has an advantage over the former type, as it is less costly and non-toxic. They function without exogenous electron carriers, and they mostly rely on metal-reducing bacteria such as *Shewanella* [4, 5, 40] including *Shewanella putrefaciens* [41]; *Rhodoferax* [42]; *Geobacteraceae* [43] [44] including *Geobacter sulfuurreducens* [4], *Geobacter metalli reducens* [45]; *Aeromonas hydrophila* [46]; and *Klebsiella pneumoniae* [47]. Nonetheless, when mediatorless MFC is operated, some factors such as presence of electrochemically active redox enzymes for efficient electron transfer to the anode, fuel oxidation at the anode, the circuit external resistance, oxygen reduction at the cathode, and proton transfer through the membrane to the cathode have to be considered as they are limiting

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the generation of electricity. However, when the membrane proton permeability is poor, the cathode chamber proton transfer can be a limiting factor. Under these limitations, microbial activity and electron transfer to the electrode in anode chamber can be reduced due to change in pH, besides the slow cathode reaction due to limited proton supply [30].

Geobacteraceae sp. transfer electrons directly to the anode via redox enzymes in the outer membrane. Nevertheless, this mediator-less MFCs, capture monosaccharides and methanol from food leftovers converting them into hydrogen and food for the bacteria.

For using the bacterial electron flow for electricity generation, this process has to be apprehended in a fuel cell, and to create a useful current; it is necessary to create a fast, complete, and non-stoppable circuit. Furthermore, for the microorganisms to feed on a suitable substrate such as glucose or wastewater, the mediator and the microorganism have to be mixed in one solution. This solution mix has to be contained in a sealed chamber (the Anode) under an anaerobic environment in the absence of oxygen (normally slows down bacterial growth) enforcing the microorganism to use anaerobic respiration. Usually, when substrates are metabolized in an aerobic environment, electrons in the substrate-containing monosaccharides, fats, proteins, or other bio-available molecules are biodegraded by the bacteria and electrons are transferred using bacterial metabolic pathways for cell energy. During this process, oxygen is the driving force and the ultimate electron acceptor owing to its electrons-high-affinity. Since MFCs operate under anaerobic environment, none of this electron transfer called aerobic respiration will occur. Moreover, in an anaerobic environment, cells act differently in using the waste as substrate molecules, and the amount of energy they receive is greatly reduced with limited growth. To solve this limitation problem, microorganisms should transfer their electrons to an

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electrode in the anode solution and, hence, facilitate fuel electrochemical oxidation. Any electrically conductive path such as copper wire in the presence of a resistor (acts as a break in a car to slowdown the speed of electron flow not to be quickly depleted) connects this negatively charged anode creating an external circuit toward another electrode in the cathode chamber. In the presence of oxygen, the cathode electrode sank in the solution-containing oxidizing agent, such as oxygen. This agent, in turn, picks up the electrons on the electrode surface to promote the electrochemical reduction of the oxidant. However, the cathode is positively charged representing the oxygen sink equivalent at the end of the electron transport chain, the process of which involves a large volume of circulating gas, which is not practical. Henceforth, a solution of a solid oxidizing agent, potassium ferricyanide ($K_3Fe(CN)_6$), is used. To connect the two chambers and complete the circuit, there has to be a salt bridge (or electrolytes) or an ion or proton exchange membrane (PEM) that allows the passage of the produced protons only in one direction; from the anode toward the cathode chamber.

In principle, the electrons released in MFCs after microbial oxidation of a substrate are transferred to the anode. Then leave the anode and flow through an external electrical circuit before reaching the cathode to produce electricity. Finally, these electrons react with protons and oxygen at the cathode (in the case of an oxygen reduction reaction), producing water as the final and clean product. Moreover, as long as the current flows over a potential difference, power will directly be generated from microbial fuel via bacterial catalytic activity [44].

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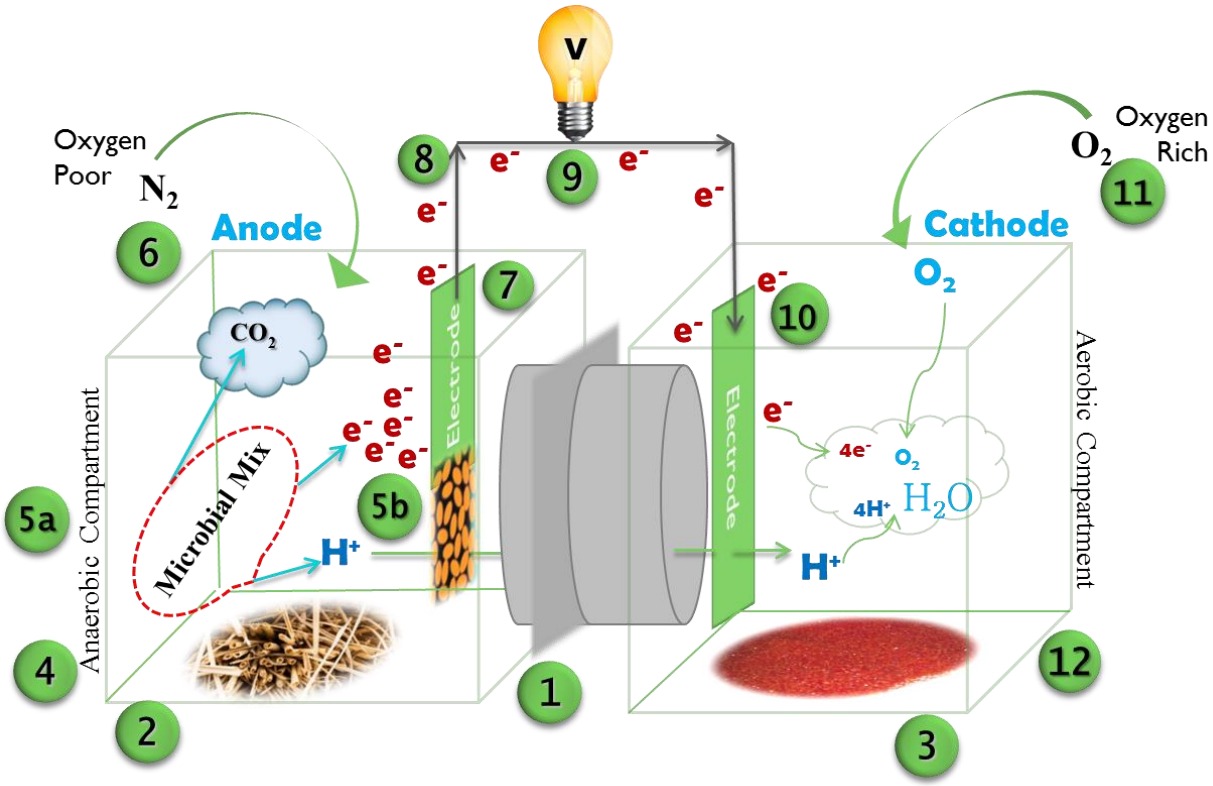

Figure 1: Major elements of MFC reactor: 1. Proton Exchange Membrane (PEM) selective to H^+ cation only separating the two chambers; 2. Anode chamber under anaerobic conditions; 3. Cathode chamber under aerobic (open air) conditions; 4. Substrate or Biomass for bacteria to feed on; 5. Pure or mixed bacterial culture (a) and biofilm (b); 6. Nitrogen gas to remove oxygen and maintain the anaerobic condition; 7. Anode electrode, on which bacterial attachment occurs; 8. Copper wire for transferring electrons to the cathode; 9. Ω resistor; 10. Cathode electrode to receive the electrons from the anode; 11. Air oxygen; 12. Electrons reducing agent (Taken from [48]).

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4. Design of MFCs

Reactor size, shape, and configuration vary widely, it is entirely up to the designer, and there is no standard design to recommend. Reactor configurations with different volumes, oxygen supply, membrane area, and electrode spacing can control the MFC overall performance.

Among variously available configurations, double chamber “H” type MFCs have been typically used due to the existence of ion exchange membrane, which helps in protons diffusion and in limiting the substrate and oxygen crossover [49]. However, the designs depend merely on research planning and the aim of the project findings. To date, reactors have been cube-shaped, cylindrical, horseshoe-shaped, two chamber and single chamber, and H-type configured and made of glass and various types of plastic, even buckets. Sizes also vary widely with some reactors having volumes of a few square centimeters and others of up to a square meter with volumes ranging from microlitres to thousands of liters. Fuel cell design is a significant key element in the success of a microbial fuel cell (MFC)/microbial electrolysis cell (MEC).

Single-chamber cells have evolved from the original two-chamber design in an attempt to eliminate the need for a membrane [50]. Moreover, single chamber reactors may show the most promising results, but this has not discouraged people from using two chamber types. Regarding construction difficulties, a single chamber reactor can be the harder of the two options. Based upon assembly of anode and cathode chambers, a simple MFC prototype can either be a double chambered or single chambered. Besides these two common designs, several adaptations have been made in a prototype of MFC design and structure [51].

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Table 2: MFC performance based on configuration.

Configuration	Inoculum	Substrates	Electrode		Electron acceptor	Power Density	Ref.
			Anode	Cathode			
Single chamber	Primary clarifier overflow (mixed culture)	Glucose	Graphite carbon fiber brush treated with ammonia Gas	30% wet-proofing pt coated carbon cloth (type b-1b)	----------	2400 mWm^{-2}	[52]
Single-chamber	Domestic wastewater (14 ml, ~300 mg-cod/l)	Glucose	Plain toray carbon paper (without wet proofing	30% wet-proofing pt coated carbon paper	----------	1330 mWm^{-2}	[53]
Two-chamber	Anaerobic sludge	Glucose	Carbone paper	Carbone cloth	permanganate	115.60 mWm^{-2}	[54]
Two-chamber	Anaerobic, Granular, methanogenic sludge	Glucose	Graphite plate	Graphite plate	hexacynoferrate	4310 mWm^{-2}	[55]
Stacked	Mixture of anaerobic and aerobic sludge	Sterile synthetic influent containing sodium acetate	Graphite granules	Graphite rod	hexacynoferrate	258 Wm^{-3}	[56]
Stacked	50% Of fresh activated sewage Sludge and 50% Of fresh urine	Neat Undiluted Urine .	Untreated Carbon fibre Veil	Coating activated Carbon (AC) paste on polytetrafluooe thylene (PTFE)	----------	0.8 Wm^{-3}	[57]

4.1 Design and Development of Microbial Fuel Cells

Design and construction are one if not the most important elements in MFC. There are fundamental components of MFCs, which are important in constructions. Electrodes, wire connection, glass cell and salt bridge have an important role. The salt bridge is replaced with proton exchange membrane in PEM fuel cell. However, the cost, handling, and the power generation get enhanced, thus increasing the portability and efficiency of the system. However, MFC usually consists of an anodic chamber and a cathodic chamber separated by a PEM as

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shown in Figure 1 using a one-chamber MFC by exposing the cathode directly to the air eliminates the need for the cathodic part. Generally, the two-chamber MFC is operated in water-cathode mode and single-chamber MFC is operated in air-cathode mode. The major advantage of two-chamber MFC over the single-chamber MFC is that the performance of cathode can be improved by controlling pH, purging pure oxygen, increasing flow rate, and adding electron-mediators in cathode, leading to total enhancement of the MFCs performance.

4.2 Double-chambered fuel cells

In general, this configuration has an anodic and a cathodic chamber separated by a PEM that facilitates proton transfer from the anode to cathode with simultaneous prevention of oxygen diffusion into the anode. Therefore, this configuration is generally used for waste treatment with simultaneous power generation. Both the anode and cathode are different compartments separated but also connected to each other through a proton exchange membrane (PEM) [58], which mainly acts as a proton transfer medium to complete the circuit between the two chambers (Figure 2b). This completes the reaction process and prevents anode from coming into direct contact with oxygen or any other oxidizers. They typically run in batch mode often with a chemically defined medium such as glucose or acetate solution to produce higher energy power output and can be utilized to give power in many inaccessible conditions.

Moreover, in the double chamber, MFC, the relatively longer distance of electrodes can decrease MFC performance due to an elevation of internal resistance. Choi et al. demonstrated that for to decrease internal resistance from 672 Ω (103 mW m^{-2}) [59] to 93 Ω (57 mW m^{-2}), electrodes have to be clamped along with membranes with closer electrodes spacing [60]. As a consequence of this closer of the electrode to the membrane, higher oxygen diffusions from the

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cathode to anode will eventually occur, which will directly affect the power production and power density.

Furthermore, Figure 2a illustrates a cubed mini square-shaped compact flat plate MFC (FPMFC) resembles that of a conventional chemical fuel cell with only a single electrode/PEM assembly, where the cathode is hot pressed to a Nafion PEM and in contact with an anode to form an electrode/PEM assembly. The FPMFC with two non-conductive polycarbonate plates is bolted together. The PEM links the anodic and the cathodic chambers as shown in Figure 2a. The anodic chamber can be fed with wastewater or other organic biomass, and dry air can be pumped through the cathodic chamber without any liquid catholyte, both in a continuous flow mode [61]. Nonetheless, Kim et al. have tested the cube type MFCs against the bottle type MFC and they found that the former produced about 14 times higher power generation (214 mWm^{-2} power density with 84 Ω internal resistance) than that of the latter (38 mWm^{-2} power density with 1272 Ω internal resistance) [62]

The A miniature microbial fuel cell (mini-MFC) of about 2 cm in diameter shown in Figure 2c, provide a high volume power density of 24 and 10 mWm^{-2} using reticulated vitreous carbon (RVC) and graphite felt (GF) electrodes respectively without the addition of exogenous mediators in the anolyte as reported by [40]. Therefore, they can be useful in powering self-directed sensors for long-term operations in less accessible regions. The compartments can take various practical shapes.

Upflow MFC (UMFC) configuration (Figure 2d and 2e) can be suitably designed to scale up to treat a large volume of wastewater and another carbon source. He et. al. employed two different UMFC configuration in their experiments and they manage to get a maximum power

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density of 170 mW m^{-2} with 84 Ω internal resistance (Figure 2d) [63] and the other one generated a power density of 29.2 W m^{-3} with 17.13 Ω internal resistance (Figure 2e) [64]. Although fluid pumping and recirculation are used in both configurations, it costs much greater energy than their power outputs. Therefore, the primary function of Upflow configuration is wastewater treatment, not power generation. However, this configuration falls between the single chambered and double chambered MFCs. They are mediators and sometimes membranes and can be used for large-scale electricity production from the wastes. Nonetheless, the configuration in Figure 2e offers a low internal resistance of 4 Ω because the anode and cathode are nearby over a large PEM surface area. Furthermore, one significant disadvantage of the two-chamber system is that the cathode part needs a regular solution replacement with aeration to provide oxygen to the cathode [65].

Eom, H. et al. proposed a new MFC system, which they named as M2FC as shown in Figure 3, which consists of two compartments; a ferric-based MFC and a ferrous-based fuel cell (FC). In this reactor, the FC system efficient regeneration of ferric ion (the catholyte in the MFC compartment) with the generation of additional electricity has been successfully accomplished. In this system, ferric ion, a cathodic TEA, is converted to ferrous ion in the MFC cathode chamber, and ferrous ion, an FC fuel, is oxidized to ferric ion in the FC anode chamber. When both compartments were operated separately, depletion of ferric ion in the catholyte was observed in about four days resulting in a decrease of power production. However, when combing the two units together and with continuous restocking from ferrous ion in the FC unit, the ferric ion from ferric-based MFC unit can be continuously operated with the production of additional electricity. Moreover, M2FC system yielded a power density of up to 2 W m^{-2} (up to 20 times higher than air-cathode based MFC system). Furthermore, they found that the types of

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catholytes and chelating agents as anolyte were found to play essential roles in the reduction of ferric ions and oxidation of ferrous ion [66].

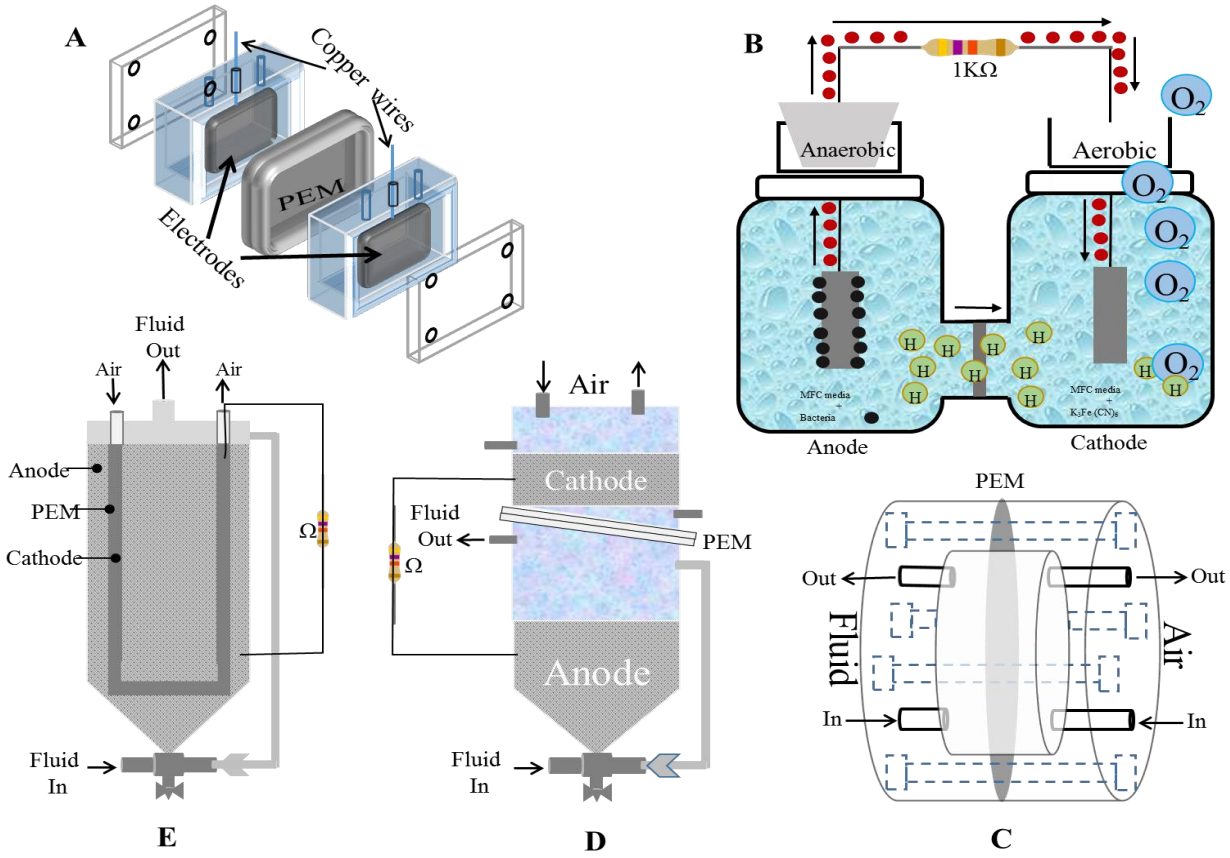

Figure 2: Schematic design of microbial fuel cell configuration.

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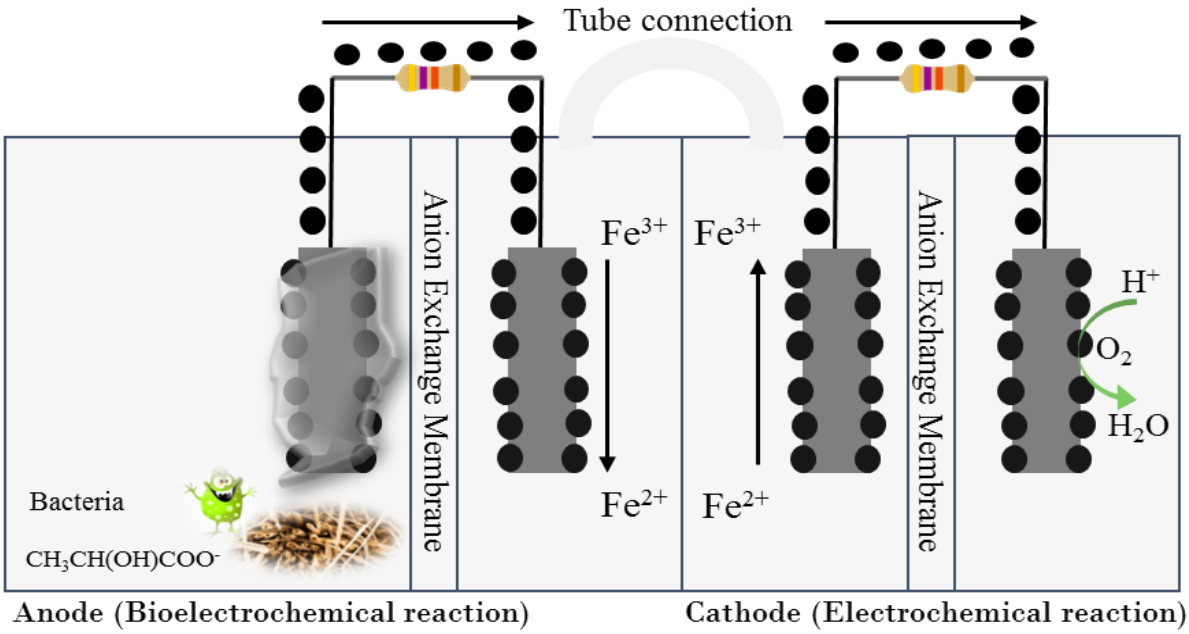

Figure 3: Schematic design of M2FC configuration.

4.3 Single Chambered Fuel cells

Hydrogen fuel cells consist of a cathode directly attached to a PEM allowing air oxygen to react directly at the electrode [65, 67]. This principle used for designing a single chamber MFC where the anodic chamber is linked to a porous air exposed cathode separated from each other by a gas diffusion layer (GDL) or a PEM leading to a passive oxygen transfer to the cathode. Electrons are then transferred to the porous cathode through the electrically conductive wire to complete the circuit. Un-necessity to aerate the cathode when using oxygen as final electron acceptor led to the development of single chamber MFCs with air cathode assembly. This type of MFC configuration attracted researchers' attention due to several advantages such as simple nature of the operation, decrease in internal resistance, enhanced oxygen reduction rate on

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the cathode, increased proton diffusion and reduced electrode spacing. The limited requirement of regular changing of oxidative media and aeration makes this configuration more adaptable. Furthermore, Logan et al. compared between single chamber cube MFC and bottle type MFC and they found that the former produced higher power generation (2400 mW m^{-2} power density with 8 Ω internal resistance) than with latter (1200 mW m^{-2} power density with 20 Ω internal resistance) [52].

These fuel cells are simple anode compartment without any definitive cathode compartment and may not contain proton exchange membranes as shown in Figure 4. Porous cathodes form one side of the cathode chamber wall utilizing oxygen from the atmosphere and allow protons to diffuse through them. This configuration is quite simple to scale up than the double-chambered fuel cells and thus have extensively utilized in research recently. While anodes are normal carbon electrodes, the cathodes are either porous carbon electrodes, or PEM bonded with flexible carbon cloth electrodes. However, cathodes are frequently covered with graphite in which electrolytes are poured into a steady state that behaves as catholyte and prevents the membrane and cathode from drying. Thus, water management or better fluid management is an important issue in such single chambered fuel cells.

Moreover, one of the major cons of using a single chamber MFC is liquid leakage, evaporation, and high oxygen diffusion. Cheng et al. solved this problem using polytetra fluroethylene (PTFE) diffusion layers on the cathode to improve oxygen diffusion and water loss and this, in turn, will increase the Columbic efficiency and maximum power density [68].

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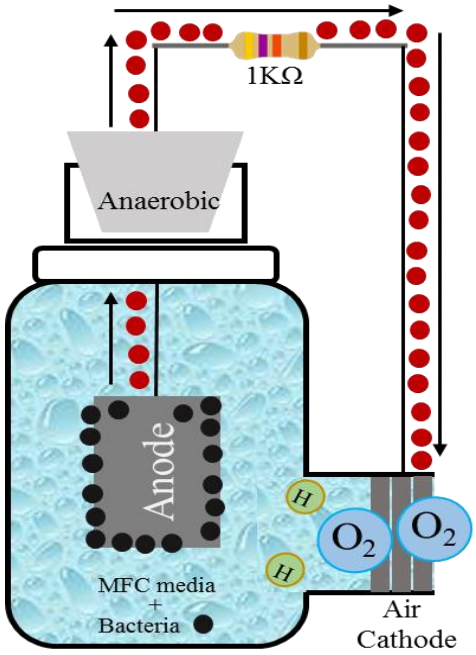

Figure 4: Single chamber microbial fuel cell.

4.4 Stacked Microbial fuel cell

It is a type of configuration where fuel cells are stacked to form a battery of fuel cell. This kind of construction does not affect each cell's distinct Coulombic efficiency, but together it increases the output of the overall battery to be comparable to conventional power sources as shown in Figure 5. These can be either stacked in series or stacked in parallel. Both have their importance and are high in power efficiency and can be practically utilized as a power source.

Stack yields higher voltage and current by the series connection and parallel connection, respectively; accordingly, the required voltage, current, and power in electronic devices can be satisfied. MFC can be connected in series, and parallel circuits hence called stacked MFC. Designing stacked MFC efficiently is a critical issue. The type of electrode, stack direction

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(horizontal or vertical type), shape of the reactor, determination of connection methods, and modulation should be considered.

4.4.1 Bipolar electrode stack

Initial MFC reactors for stacking consist of anode chamber, a bipolar electrode, membrane, a cathode chamber, and end plates that were externally similar with proton exchange membrane (PEM) fuel cell stack (Figure 6A). The bipolar electrode for fuel cell stack has several advantages such as 1) minimizing resistive losses (good electrical contacts), 2) minimizing iR losses in current collectors, and 3) ease of fabrication. Shin et al. [69] used five bipolar plates stacked MFCs in series, resulted in total voltage equal to the sum of individual MFCs and no electrical degradation in performance. The bipolar stacked MFC is composed of an H-beam shape of bipolar graphite that has two compartments at each side (anodic and cathodic compartment), a Pt/C catalyst coated Nafion membrane at one side (positioned between each bipolar plate), and end plates. The titanium (Ti) plate functioned as both anode and cathode can also be used for MFC stack [70]. However, the bipolar stacked MFC in series has often observed in voltage reversal [70-72], wherein the polarity of voltages of some MFCs among the stacked MFC are suddenly reversed (from a positive value to a negative value), while the voltages of the other MFCs still remain positive values. When the reversed MFCs operate for long-term, the carbon corrosion makes the biofilm on the anode damage; therefore, the whole MFC system fails [73]. This compact design of bipolar electrode would be difficult to separate and maintain individually [71]. In addition, even if the bipolar electrode is separated from whole stacked MFC, it cannot be guaranteed that the voltage reversal phenomenon will not appear in the other bipolar stacked MFCs. An et al. [72] suggested membrane-less single-chambered MFC using a bipolar

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plate-electrode assembly (BEA) that can easily be separated and stackable in series (Figure 6B). An individual MFC unit was made of a cylindrical acrylic body, an anode (graphite felt) at the bottom, a cathode (graphite felt) at the top, and a rigid graphite plate under the anode to protect water leakage and air exposure. When two individual MFCs (MFC 1 unit and MFC 2 unit) are stacked in series, for example, the anode in MFC 1 unit with the graphite plate directly connect the cathode in MFC 2 unit without external wires. Whenever some MFCs show reversed polarity, the MFCs using BEA can easily be detached, and then other MFCs can be connected. Though the bipolar electrode distributes to reduce the internal resistance during series stack, shortcut assembly and disassembly should be considered due to voltage reversal phenomenon. The stack using external metal wires such as copper (Cu) and Ti wire would be a better choice for easy connection of MFC units in series and in parallel rather than bipolar electrode stack.

4.4.2 Horizontal stack & vertical stack

The MFC can be stacked horizontally and vertically. Many researchers have used a rectangular parallelepiped two-chamber MFC for the horizontal stack. Initial MFC stacks are containing bipolar electrode MFC stack were also arranged in the horizontal direction [70, 71, 74]. Individual MFC unit for horizontal stack without bipolar electrode consists of anode and cathode in the rectangular frames that have two ports (one for inlet and other for an outlet), and a CEM or PEM between two frames (anode chamber and cathode chamber) [71, 75-79]. The MFC units are horizontally stacked with each other by connecting electrically metal wires to current collectors for making series or parallel circuits. The MFC units are also divided by rubber sheets or acryl sheets for not sharing anolyte and/or catholyte from other MFC units. All the MFC units with end plates and the sheets for separation of each MFC units are strongly tightened via several

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long screws, such called horizontal MFC stack. Aelterman et al. [71] reported a maximum power density of 308 W m^{-3} for series connection and 263 W m^{-3} for parallel connection based on the polarization curves using horizontal stacked MFC system consisting six individual MFC units. Recently, similar shape for pilot-scale horizontal MFC stack has been constructed and then operated. Wu et al. showed that the maximum power density of 51 W m^{-3} and a COD removal efficiency of 97% were achieved using the scaled-up stacked MFC in parallel connection (total volume of 72 L) [75]. Vilajeliu-Pons et al. [76] presented the power density of between 2-4 W m^{-3} using a couple of six stacked MFC in a combination of series and parallel connection from swine manure as a substrate for more than six months. The total volume of the six stacked MFC was 115 L.

The tubular air-cathode MFC design has also been used for the horizontal stack. Zhuang and Zhou reported the tubular air-cathode MFC stack horizontally [80]. The tubular MFC unit (void volume of 0.75 L) is constructed with polyvinyl chloride (PCV) plastic tube serving as the frame of anode chamber and tubular membrane cathode assembly by hot-pressing carbon fiber cloth to CEM. The substrate hydraulically flows between anode chambers. They used Ti wires to serially connect each cell. This design could be adaptable for a drain pipe in the wastewater treatment processes, have continuous plug flow. Zhuang et al. [81] constructed 10-liter serpentine-type MFC stack using forty tubular air-cathode MFC units and then operated it for 180 days (Figure 6C). The OCV of 23.0 V and maximum power density of 4.1 W m^{-3} were obtained from brewery wastewater by the series stack. They concluded that the serpentine-type air-cathode MFC stack has the potential for application in wastewater treatment process due to reduced capital costs, easy modulation, high scalability, and a simple wastewater distribution system. However, each MFC unit would have different electrical performances due to different

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concentration of organic matter and ion conductivity faced with biofilm on the anode in continuous operation [82]. The low COD concentration near end MFC unit can reduce electrical performances or even produce negligible power compared to the initial MFC near inlet. In long-term operation, organic matter depletion near end MFC would result in extreme power loss and then may fail to operation due to inactivate microorganisms in the biofilm. Also, when multiple MFC units are connected in series, the voltage reversal could occur due to imbalance of substrate loading, causing a kinetic imbalance between MFC units or between anode and cathode [83-85].

The vertical cascade stacked MFC system (Figure 6D) can also be confronted with the voltage reversal phenomenon because of the substrate imbalances. However, the cascade stacked MFC can be used as inexpensive way because the fluid flow of electrolytes was mostly gravity driven without pumps. The horizontally stacked MFC system usually require extra pumps for supplying substrate, *e.g.*, wastewater, for microbes metabolism to anode chamber and oxidized catholyte, *i.e.*, air-saturated solution, for reduction reaction to the cathode chamber. It has been reported that the first self-sustainable MFC stack capable of self-maintenance using vertical cascade single-chamber MFC stacks consisting of 40 identical 20mL units (total volume of 0.8 L) [86]. Feeding, hydration, sensing, and reporting were performed by using the power from MFC stack, and even the MFC stack produced extra energy, demonstrating net energy excess. Also, the researchers achieved a COD removal of over 95% from artificial wastewater (containing 5 mM acetate) during 5.7 h using six continuous-flow cascades MFCs by connection in parallel [87]. Recently, the smartphone was successfully charged by scaled-up cascade MFC stack (10.5 L of whole stack volume) employing urine as substrate [88]though the MFC stack produced a low power of near 110 mW (maximum power density of ~10 W m^{-3}). The phone call was allowed for 1 h 45 min after 3 h of charge using the MFC stack. However, still, the methods

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for maintaining flow rate, reduced organic concentration near end MFC, the volume of the reactor, and height of the whole system would be considered for vertical cascade MFC stack to maintain electrical and biological performances (*i.e.*, power generation and wastewater treatment) of MFC stack.

4.4.3 Modularized multiple electrodes MFC stack

The electrolyte has been not shared from other electrolytes by rubber sheets [71], bipolar plate [70, 72, 74], thin inter-connecting tubes [81], and overflow [89] to prevent ionic cross-conduction, causing voltage reversal of stacked MFC in series connection. The ionic cross-conduction occurs when the same electrolyte (anolyte or catholyte) is shared by different MFC in series connection [80]. These designs for separation of individual MFC reactors would require additional materials, leading to the complex structure of reactor and increase of capital costs for construction and maintenance. Modulation of MFC stack would be an efficient way to construct a stacked MFC system simply and reduce construction costs. He et al. [90] developed the stackable MFC with the anode and dual-cathode modules for simple construction, easy access, and maintenance of the electrodes. The highest power density (11.0 W m^{-3}) was achieved using raw domestic wastewater with the MFC module. After that, a larger MFC module (6.1 L) using four anode modules and three cathode modules were constructed and then operated, resulting in the maximum power density of 6.0 W m^{-3} and COD removal of between 40-60% [91], but the researchers were not conducted to stack operation by electrical series and parallel connection. A promising development direction for the modularized MFC stack is to combine electrical arrays with series connection and parallel connection of MFC units and/or MFC module. The MFC units in a module are connected in parallel first to be capable to prevent voltage reversal and

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increase current. Feng et al. [92] have shown that the increased high current (0.435 A) was obtained using modularized MFC stack in parallel connection of thirty-two carbon brush anodes. After series connection of modules in parallel-connected MFC units, the increased voltage and power can be satisfied with the required standard input voltage and power of an electrical device depends on how many MFCs are parallelly and serially connected. Ge and He [93] developed a 200 L modularized MFC stack, which generated power of ~200 mW. The MFC system is comprised of eight MFC modules, containing twelve tubular MFC units (2L/each). The twelve MFC units are connected in parallel using external wires, and then eight MFC modules are connected in series (Figure 6E). They tried to avoid short circuit connection between MFC modules because two modules (one set) are hydraulically connected for wastewater feeding. This system achieved removals of over 75% of COD, 90% of the suspended solids, and 68% of ammonia nitrogen.

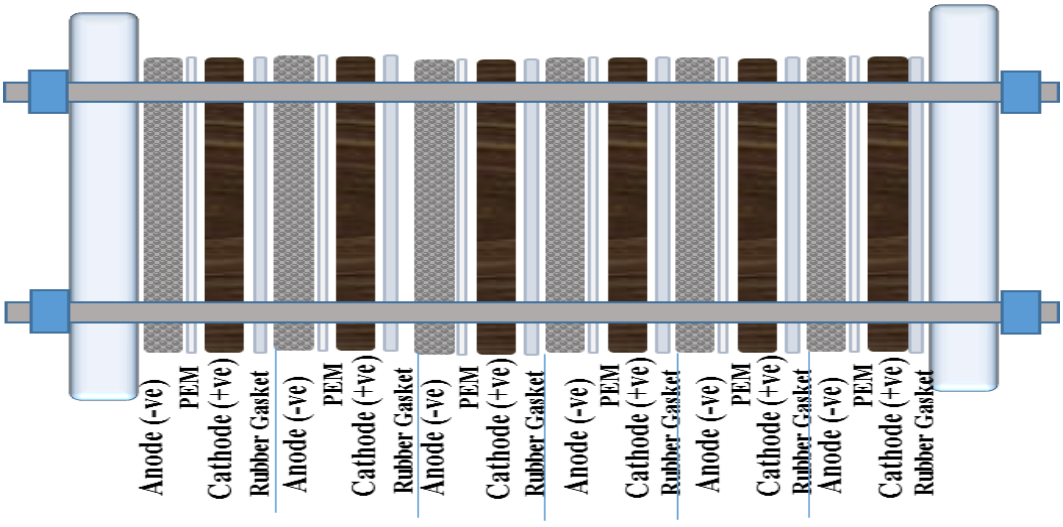

Figure 5. Stacked microbial fuel cell.

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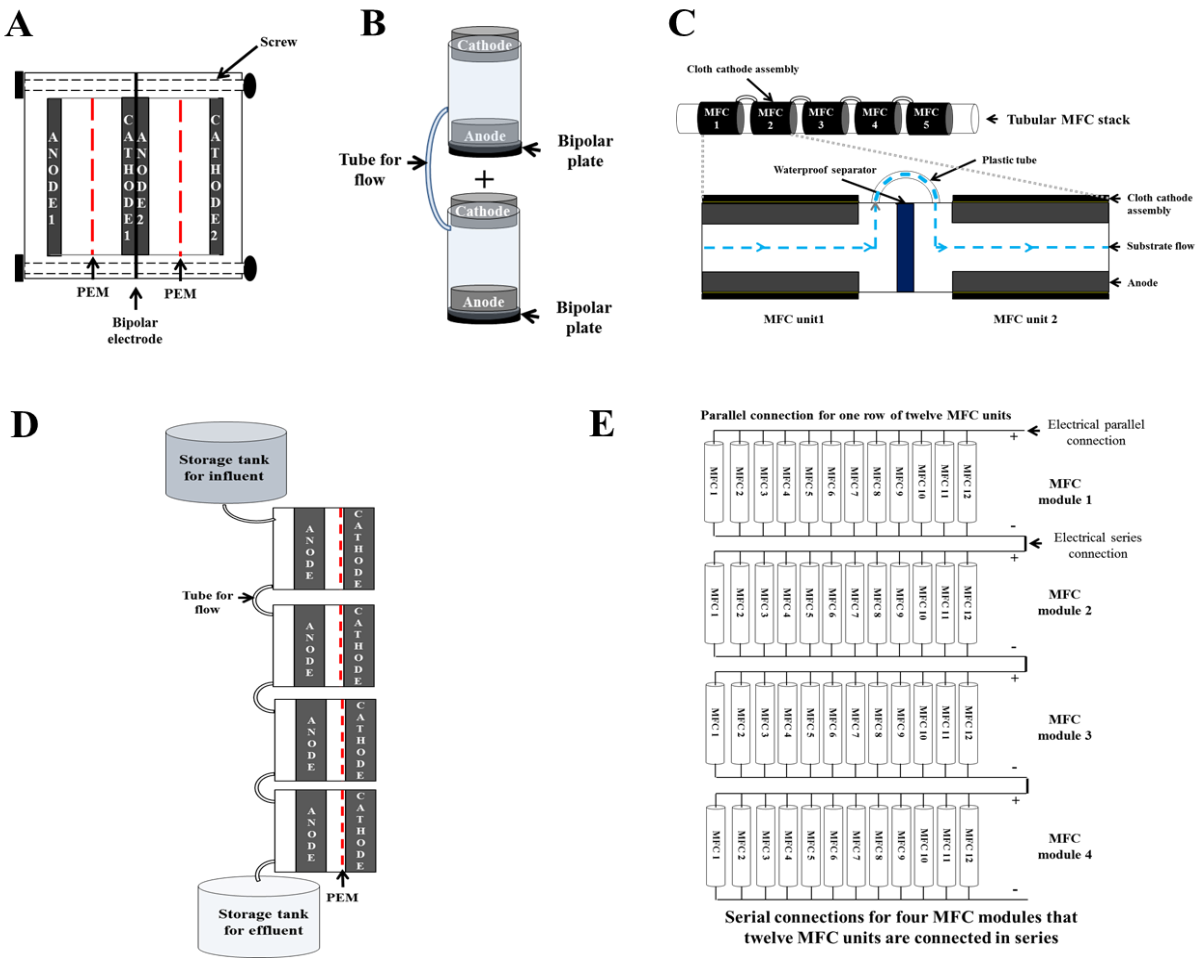

Figure 6. The schematic diagrams of stacked MFCs configuration. A. a bipolar plate two-chamber MFC horizontal stack; B. a tubular bipolar plate membrane-less two-chamber MFC vertical stack; C. a tubular air-cathode MFC horizontal stack; D. a cascade air-cathode MFC vertical stack; E. the module MFC stack in combination of series and parallel connection.

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Table 3. Summary of configuration, operational condition, and performance of MFC stack reported in literatures.

Configuration	Reactor No.	Total volume (L)	Electrode	Connection	Internal resistance (Ω)	OCV (V)	Maximum power density ($W m^{-3}$)	Maximum current density ($A m^{-3}$)	Organic conc. or OLR	Ref.
Two-chamber MFC stack	6	0.936	Graphite granules	Cu wire	6.5 (s)[1)]	4.16 (s) 0.67 (p)	308 (s) 263 (p)[2)]	0.085 A (s) 0.425 A (p)	1.62 g COD L^{-1} d^{-1}	[56]
Bipolar two-chamber MFC stack	4	20	Ti plates	Ti plates	1.2 mΩ m^{-3} (s)	4.06 (s)	144 (s)	2.8 A m^{-2}	-	[94]
Two-chamber MFC stack	3	1.8	Graphite	Cu wire	11.5 Ω m^{-2} (s) 1 Ω m^{-2} (p)	1.042 (s) 0.687 (p)	0.11 W m^{-2} (s) 0.13 W m^{-2} (p)	0.098 A m^{-2} (s) 0.381 A m^{-2} (p)	30 g L^{-1} of G-F-S[4)]	[95]
Two chamber MFC stack	4	-	Carbon cloth	-	-	3.27 (s) 0.82 (p)	2.22 W m^{-2} (s) 1.98 W m^{-2} (p)	16.9 A m^{-2} (s) 4.45 A m^{-2} (p)	0.5 g COD L^{-1}	[96]
Single-chamber MFC stack	10	0.063	Carbon fibre veil	-	-	3.6 (s)	0.97 (p)	~7.1 (p)	5 mM of acetate	[97]
Tubular type of single-chamber MFC stack	5	1.475	A: Graphite felt C: Carbon fiber cloth	Ti wire	10-15 (p)	2.1 (s)	67.5 W m^{-2} (s) 175.7 W m^{-2} (p)	0.128 A m^{-2} (s) 0.675 A m^{-2} (p)	4.9 g COD L^{-1} d^{-1}	[98]
Tubular type of single-chamber MFC stack	40	10	A; Graphite felt C: Metal catalyst	Ti wire	800 (s) 15 (s-p)[3)]	23 (s) 3.25 (s-p)	4.1 (s) 6.0 (s-p)	2.1 (s) 13.8 (s-p)	1.06 g COD L^{-1} d^{-1}	[99]
Cascade type of single-chamber 3D-printed MFC stack	40	0.8	Carbon veil	-	-	13 (20 units used)	-	-	25 mM of acetate	[100]
Horizontally stackable type of single-chamber MFC	1 (32)[5)]	250	A: Carbon brush C: Carbon mesh	Ti wire	2.3×10^{8} Ω m^{-2}	0.8 (p)	0.116 W	0.435 A	~0.32 g COD L^{-1}	[101]
Bipolar plate single-chamber MFC stack	3	0.35	Graphite felt	Graphite plate	634	1.58 (s)	0.023 W m^{-2} (s)	0.037 A m^{-2}	10 mM of acetate	[102]

[1)] (s): series connection, [2)] (p): parallel connection, [3)] (s-p): series and parallel connection, [4)] G-F-S: glucose-fructose-sucrose, [5)] : Carbon brushes of the number of 32 were parallel connected in a MFC.

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5. Scalability of MFC

Compared to other renewable energy sources, MFC is still considered as a low energy producing system because of its thermodynamic limitations and different voltage losses associated with redox reactions. The theoretical maximum voltage that could be achieved from the best MFC configurations mentioned so far is about 1V using acetate as a carbon source in anodic chamber and oxygen as the cathodic electron acceptor [103]. Still, the actual voltage obtained from an MFC is always lower than the theoretical maximum voltage due to several voltage losses, often called as overpotential losses [14, 103]. The major problems restricts the application of this technique from commercialization is the technology application (restricted by design aspects, and technological, electrochemical and microbiological limitations), cathodic electron transfer limitation, slower Coulombic efficiency (CE), lower power production and high capital cost compared to other conventional wastewater treatment processes [104]. Although improvement in power and the current density was reported from the scalable design of MFC, this increase in electrical output is disproportional with the corresponding increase in anodic chamber volume [105]. Microbes are relatively slow electron transformers even at its fastest growth rate and have to compete with other non-electrogens for food as in case of mixed inoculum. Another limitation is represented by substrate diffusion towards anode in anodic chamber of larger volume MFC, which is not at sufficient rate to reach acceptable levels of current and cell potential (to minimize the diffusion losses) and it needs proper mixing condition for better proton transfer and to maintain homogenous condition throughout the electrolyte solution [106].

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Before thinking of commercial manufacturing, bioreactor and the processes within are required to scale-up. This involves many steps of volume increase in bioreactors. Proportional volumetric power generation decreased with increase in the volume of MFC. The MFCs real applications scalability is decisive, concerning not only increased fuel utilization efficiency and capacity, but also concerning increasing electricity power generation. Hence, this concept has always been a critical issue that affects their practical applications. Scale-up of MFCs from the laboratory to pilot scale is a challenging step in the development of the process. The challenges for bringing MFC technologies for practical applications underlying the fact the factors that might influence the MFC performance have to be considered. The primary challenge is scaling the energy production along with reactor liquid volume. Total power generation can be improved either by increasing the reactor volume or by electrically connecting some MFCs in series or parallel. Nonetheless, any scale-up work made from the bench-scale bioreactor of one to thousand liters of volume is not an easy task [107-109]. Therefore, it is challenging when one considers all the added difficulty in operational complexity, logistic supports, utility supplies, regulatory and safety compliance besides already broad technical issues have to be addressed. With the limited information and knowledge available, the researcher must integrate many different approaches and tailor them to the specific or general situation.

For MFC system better optimization and to improve its efficiency, different aspects have to be considered. These include inoculums, the substrate (fuel), proton exchange membrane material, fuel cell internal and external resistance, the ionic strength of the solution, electrode materials, and electrode spacing [110]. In addition, interesting factors that could influence the overall performance of the system such as mode of operation [111], effects of gravity and

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geometric flow [112], effect of culture time [113, 114], and chaotic application for stimulation of microbial activity [115] have to be considered.

Finally yet importantly, based on internet news posts, conference presentations, and researchers discussion forums, there are very few large-scale tests of microbial fuel cells. The largest of all was conducted at Foster's brewery in Yatala, Queensland (Australia), by the Advanced Water Management Center at the University of Queensland. The reactor consisted of 12 modules, each 3 m high, with a total volume of approximately 1 m^3 (Figure 7). The reactor contained carbon fiber brush anodes inside tubular reactors covered with graphite fiber brush cathodes, with flow up through the tubes and out over the outside of the reactor. This design was similar to one tested in the laboratory with a ferricyanide catholyte [116]. Little is known about MFC performance at the site, other than solution conductivity was low, limiting current generation, and that excess biochemical oxygen demand in the wastewater leaving the anode chamber resulted in the build-up of excessive biofilm on the cathodes as the wastewater was exposed to air.

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Figure 7: Brewery in Yatala, Queensland (Australia) at the University of Queensland, conducted a large scale MFC under the direction of Jurg Keller and Korneel Rabaey (www.microbialfuelcell.org).

6. Picturing the difficulties when designing and scaling up

When we think of the designing and its importance to solve the problem of MFC, we have to picture the process and what calculations and planning steps involved to constructing a building and try to do the same when designing the MFC reactors. Either it is of one floor or hundred-floor building, it does not matter because both will have the same process and same steps with different volumes and proportions but what matters is the perfect design to serve the cause with whatever budget available.

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The first step in the plan to construct a ten-floor building is to plan everything on paper from the foundation to the tenth floor and have your blueprint ready for that. Drawing a blueprint and make it ready is not an easy task because it involves planning for the general and specific details of the building depending on the cause. Whoever preparing the blue print should ask themselves many questions, and the answers should be clear before they start sketching how the building will look like. The first big question should be what is the cause of this building? Is this building for personal use or public use? Is it for individuals or the government? Is it like a shopping mall and hospitals with different entrances and exits leading to different parking lots or it is like a private or company building with one or two entrance and one or two exits leading to one reasonably big parking lot? and so on. Answers to these questions will lead to the next step in the designing and drawing of the blue print, which how much funding and space are available to proceed and make this project to happen. Accordingly, the designer will start thinking about what the design should look like and what are the materials should be used with whatever cost included within the budget. At this point, the designer should have a broad and unstoppable imagination about the interior and exterior design and what it should look like to serve the cause? However, part of the process of designing is to make a prototype (small scale) of the design to view what is going to be real and try to think of any pros and cons that this design might have after consulting with other expertise. The prototype design would help the designer to think more about some details and experimental testing what should go wrong with the design if this prototype brought to scale up to face the real daily life problems.

Moreover, to perfictionize the process, one crucial issue should carefully be considered; engineering mathematical calculations. Every single detail such the volume and ratio of cement versus water, size of doors and hinges, electrical and plumbing layouts, health measures against

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rodents and insects, and safety measures in case of fire should be calculated and considered. Nevertheless, any issue that we might think it is insignificant should not be ignored or underestimated as it might help to serve the case, but sometimes we need to dig in little more to be able to picture the real problem and find a solution for it.

7. Conclusions

For the microbial fuel cells to function appropriately with the highest production and efficiency possible, all the elements involved in the reaction have to be considered. A better understanding of these elements, how they function, what factors that could affect them, and the impact of these factors is the key part of the puzzle or what and this impact is it due to multiple or due to one individual effect. Therefore, more and better understanding of the system and its components will be useful in controlling the power efficiency and the type of energy produced.

The development of MFC technology needs a selection of suitable and cost-effective electrode materials and separator, and engineering design of scalable architecture to find its feasibility for wastewater treatment under optimum operating conditions.

Unfortunately, with the current publications, no one ever has the accurate study that integrated various science and engineering scale-up approaches and applied them correctly to the industrial situations with a case study as a demonstration.

Furthermore, compared to what researchers have started with, the amount of progress made to date is impressive with the small amount of funding available in this field of research. Many companies are now looking into commercialization of these systems with promising future.

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It is hoped that with more funding and continuous progress in research using cost-effective materials and designs, such systems could be soon commercially available.

Moreover, if researchers start to think using the strategy recommended in this review, there should not be any problem in scaling up or even scaling down. As per group opinion, the key point why most researchers somehow failed to scale up their successful experiments using whatever reactor design, because they do not fully understand not only the engineering mathematics behind scaling up but also other fields. Therefore, it is recommended to collaborate as a team with a variety of expertise in different fields like architecture engineering, civil engineering, environmental, artists and designers, electrochemical engineering, materials science, electrical and electronic engineering, fluid and thermodynamics, biology and microbiology, environmental science, and so on.

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