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REVIEW ARTICLE

Review of Progress and Prospects in Research on Enzymatic and Non-Enzymatic Biofuel Cells; Specific Emphasis on 2D Nanomaterials

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Abstract: Energy generation from renewable sources and effective management are two critical challenges for sustainable development. Biofuel Cells (BFCs) provide an elegant solution by combining these two tasks. BFCs are defined by the catalyst used in the fuel cell and can directly generate electricity from biological substances. Various nontoxic chemical fuels, such as glucose, lactate, urate, alcohol, amines, starch, and fructose, can be used in BFCs and have specific components to oxide fuels. Widely available fuel sources and moderate operational conditions make them promise in renewable energy generation, remote device power sources, *etc*. Enzymatic biofuel cells (EBFCs) use enzymes as a catalyst to oxidize the fuel rather than precious metals. The shortcoming of the EBFCs system leads to integrated miniaturization issues, lower power density, poor operational stability, lower voltage output, lower energy density, inadequate durability, instability in the long-term application, and incomplete fuel oxidation. This necessitates the development of non-enzymatic biofuel cells (NEBFCs). The review paper extensively studies NEBFCs and its various synthetic strategies and catalytic characteristics. This paper reviews the use of nanocomposites as biocatalysts in biofuel cells and the principle of biofuel cells as well as their construction elements. This review briefly presents recent technologies developed to improve the biocatalytic properties, biocompatibility, biodegradability, implantability, and mechanical flexibility of BFCs.

Keywords: Biofuel, cells, renewable, energy, nanocomposites, biocompatibility.

1. INTRODUCTION

The uneven distribution of fuels throughout the geographical locations insisted many researchers and governments explore energy and fuels from sustainable energy and green sources. In the pursuit of fuels from renewable sources, fuel cells (FCs) are environmentally friendly and generate electrical energy from chemical energy [1]. In conventional fuel cells, noble metals and their alloys are used as catalysts in the oxidation of pure fuels (methanol, hydrogen, *etc*.) at the anode and the reduction of oxidant (Oxygen) at the cathode. They are very efficient and can work in acid/basic electrolytes. The limitation of these metals is they are costly with restricted availability. The need for renewable energy has opened fields like harvesting energy from biological sources [2]. Applications in biomedical, microelectronics, and sensor devices have attracted more attention in utilizing energy from electric impulses, walking, running, muscle contraction, relaxation, body heat, *etc*. [3]. However, significant challenges are posed in biocompatibility and durability for health and safety concerns.

Enzymatic biofuel cells (EBFCs) are subareas of fuel cells that employ redox reactions by oxidoreductase enzymes [4-9]. Yahiro *et al*. in 1964 first described the EBFCs concept. With the demand for potential applications, the EBFCs are designed in numerous configurations, which are quite different from conventional FCs stacks, but their components are the same [10]. Like other FCs, EBFCs comprise a proton-conducting medium separating two-electrode cells that stream through the external electrical circuit to the biocathode, where the oxidants peroxides [11] or oxygen [12] are reduced to water. The advantages of using EBFCs are that the catalysts are renewable, diverse, operationally safe, and mild.

Sugars [13], organic acids [14], alcohols [15], and many other materials in the body are digested by living organisms through their respective enzymes. They can be utilized as

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fuels for EBFCs. The enzymatic reactions occur at room temperature, physiological pH, and ambient pressure. The recent use of enzymatic reactions at high temperatures like 85 °C and acidic pH of 2 have expanded the scope of the EBFCs work under varying conditions [16, 17]. The redox enzymes have an additional extraordinary selectivity towards their respective substrates, allowing circumvention of the purification step. Furthermore, the EBFCs can be considered environmentally friendly because their components are mostly biodegradable. These advantages and properties of EBFCs have opened new fields for green power generation applicable in a wide range of applications.

The review article evaluates enzymatic and non-enzymatic 2D nanomaterial-based biofuel cells in considerable detail and their diverse synthesis methods and catalytic properties. The usage of nanocomposites as biocatalysts in biofuel cells is examined in this review article. It also discusses the basic idea of biofuel cells and its components. It discusses current technological advancements to enhance the mechanical flexibility, biocatalytic characteristics, biocompatibility, and biodegradability of BFCs.

2. BACKGROUND RESEARCH ON BFCS

Potter's pioneering work in BFCs was initiated in 1912, engaging yeast cells for glucose oxidation at the anode. They proposed the concept of biofuel generation employing enzymes for the energy conversation giving rise to the development of enzymatic biofuel cells [18]. Microbial biofuel cells (MBFCs) have been studied for more than a century, but in the 1960s, eBFCs were developed. The idea of EBFCs with a platinum cathode and an anode based on the glucose oxidase (GOx) enzyme was created by Yahiro *et al*. (illustrating the first enzymatic biofuel cells with the platinum as a cathode and glucose oxidized as an anode). Even though the created EBFC had a very low open circuit potential (0.17-0.35 V), it proved the theory that an oxidoreductase enzyme could be used to start a half-reaction fuel cell. By following this route, $C_6H_{12}O_6$ is converted to $C_6H_{10}O_6$, generating electrons that can be transferred to the electrode surface with a redox conducting support. EBFCs, which used substrate-specific enzymes to circumvent the limitations of traditional fuel cells, were built on the groundwork of this groundbreaking research. The use of an electron relay to facilitate mediated electron transfer (MET) from purified oxidoreductase enzymes to the electrode surface was documented in several biofuel cell studies from the 1960s [10]. Their work proved that enzymes could swap a noble metal catalyst for the FCs reaction. However, this primitive biofuel cell has limitations of lower open-circuit potentials and negligible current density due to the lack of a mediator molecule. In 1984, Cass *et al.* also investigated electron transfer using mediators and enzymes to improve the current density. Since bioelectrocatalysis of direct electron transfer (DET) has the capacity to transfer electrons from the enzyme cofactor to the electrode directly, it does not require a mediator of redox activity.

In contrast to MET, which may experience a dip due to the difference in potential between the mediator and enzyme active sites, DET results in smaller potential losses. This research work inspired the primary goal of BFCs in the future. The research in this field focused on enhancing enzymatic bioelectrocatalysis as an application in biosensors. By bypassing the redox mediation, in 1978, Berezin and coworkers introduced the concept of electron transfer to the electrode from proteins directly. As shown in Fig. (**1**), mediators have carried out several innovative research works for electron transfer that produces high current densities [19]. Laane *et al*. in 1984 used organometallic redox mediators in solution and incorporated them into polymers for biofuel cells [20].

After 1984, the concept of utilizing biological catalysts on a BFCs cathode was first proposed. Instead of using the enzyme for direct bio-electrocatalysis at the cathode, a gold-supported cathode was utilized, which produced peroxide from oxygen, which was then eliminated by the chloroperoxidase enzyme [20]. Therefore, this innovative study expands the range of BFCs that use enzymes at either the cathode or the anode. While numerous studies have defended the use of facilitated electron transfer because it typically results in higher current densities. Even yet, there are still significant problems with EBFCs, such as their low mediator stability and high potential losses. Cass *et al*. employed organometallic redox mediators for the first time in the 1980s [21].

Redox mediators were used in two ways: they might be dissolved in a solution or immobilized in conducting polymers. The integration of organo-metallic-based redox-active mediators into redox polymers was the Heller group's groundbreaking work that was later applied in other research domains. Future development in this field focuses on developing various bio-anodes, constructing microbial and enzymatic biofuel cells with improved current densities, and extending their lifetime up to five years [22]. The stability of proteins in the solutions is a major drawback of the enzymatic fuel cells. Most enzymes lose three-dimensional structure and catalytic activity over 8-72 hours. The researchers aim to improve the energy density and lifetime *via* enzyme cascades. The strategic employment of enzyme cascades has resulted in the encashment of the degree of oxidation of EBFCs. There has been a growing interest in developing novel immobilization strategies for alternative electron transfer pathways and enhancing the enzymes' lifetime at the electrode surface [23]. Yu *et al*., 2010, developed biofuel cells by immobilizing the selective enzyme at the cathode and anode to form novel membrane-less BFCs, as shown in Fig. (**2**).

3. CATEGORIES OF BIOFUEL CELLS

The classification of BFCs based on the biological catalyst opens up Enzymatic biofuel cells (EBFCs), Microbial fuel cells (MBFCs), and Abiotic biofuel cells (ABFCs). EBFCs use enzymes as catalysts at both cathode and anode, MBFCs use living cells as catalysts, and ABFCs are nonbiological fuel cells that use nonbiological catalysts.

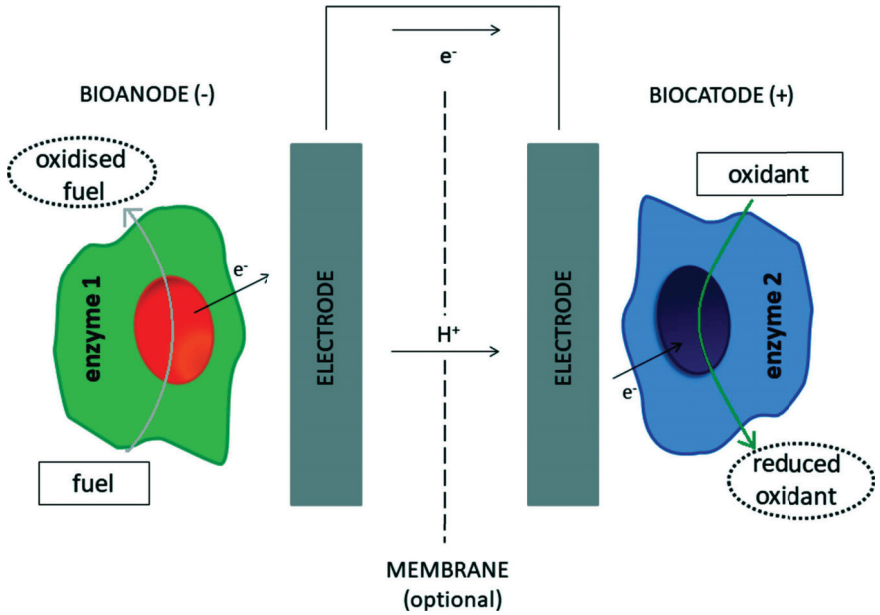

Fig. (1). Schematic diagram of an EBFC utilizing a mediator at bio-anode and a direct electron transfer bio-cathode. Figure adapted from permission with [19] copyright open access. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

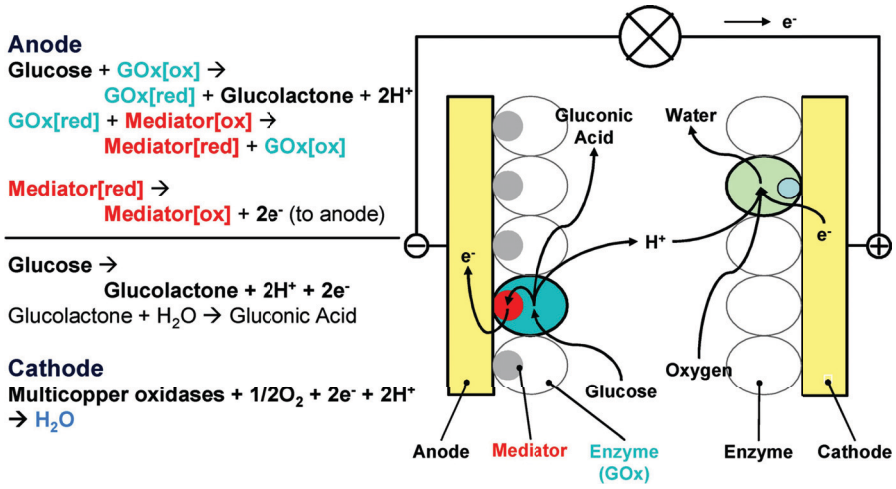

Fig. (2). Schematic diagram of the membranes and compartmentless EBFC. Figure adapted from permission with [24] copyright open access. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.1. Microbial Fuel Cells (MBFCs)

MBFCs are bio-electrochemical fuel cells that produce electrical energy from chemical energy with the help of microorganisms or microbes in the organic substrate. In 1990, Habermann and Pommer first testified about an MBFC, where a specific kind of bacteria donated electrons indirectly through soluble mediators. This study with sulfide storage capacity was employed for five years in utilizing municipal wastewater [25]. The MBFCs can further be classified into mediator MBFCs and mediator-less MBFCs based on the mediator usage. In mediator MBFCs, microbes generate electricity in the presence of mediators. The mediators are usually chemical agents that are involved in the reactions. One of its kinds is anthraquinone-2,6-disulfonate, humic acid, neutral red, *etc*. Logan mentioned that potassium ferricyanide, neutral red, and methyl viologen are the chemical mediators used in MBFCs, also denoted as active electron metabolites [26].

On the other hand, as the name indicates, mediator-less MBFCs are those FCs that do not use mediators to generate electricity. Here, microbes convert chemical energy into electrical energy without the interference of chemical molecules. These mediator-less MBFCs have few advantages over mediator MBFCs. These are less expensive and non-toxic. In mediatorless MBFCs, the microbes use the enzymes produced in them. A few factors limiting electricity generation in mediator-less MBFCs are redox enzymes for electron transfer to the anode, electrical resistance in the circuit, fuel oxidation at the anode, oxidation, and reduction at the cathode, and variation of pH near electrodes may hinder microbial activity. Researchers have conducted many studies discussing electricity generation through MBFCs, applications,

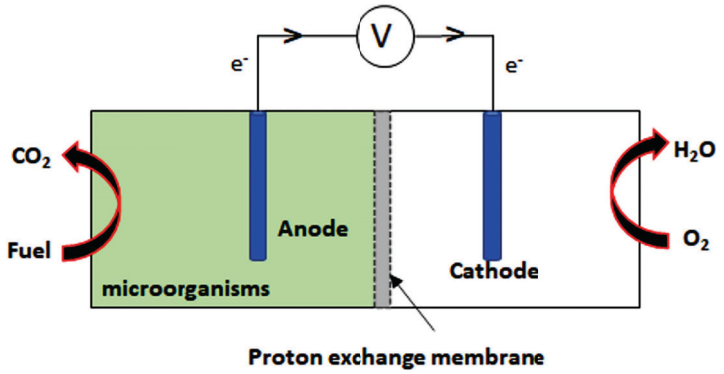

Fig. (3). Schematic diagram of a simple MBFC. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

performance, and design of MBFCs. However, configuration, design, economics, and cost-effective materials are the scope required to construct MBFCs [27, 28].

The construction of an MBFC comprises two compartments identified as electrodes, one cathode and another anode, which is divided by a proton exchange membrane (Fig. **3**). The anodic chamber consists of electrochemically active microbes, and the cathodic chamber is abiotic. The microorganisms behave as biocatalysts, stimulating the degradation of the organic constituents to harvest electrons that travel to the cathodic section through the electric circuit [29]. Oxygen and electrons at the cathode react with protons from the anodic and produce water in the internal circuit by passing through the external circuit [30, 31]. Therefore, MBFCs are potential candidates for green "electricity."

Based on the design, the MBFCs can also be categorized into single-chamber reactor MBFC, double-chamber reactor MBFC, up-flow MBFC, and stacked MBFC. Single-Chamber reactor MBFCs: It contains both anode and cathode in one chamber. Protons are transferred inside the anodic and cathodic common chamber, and released water is produced by the interaction between protons and electrons in the presence of oxygen [32]. It has a few advantages like requiring less space, less internal resistance, simple operating procedures, and low cost. Contrarily, the major drawback of single-chamber MBFC is the presence of oxygen concentration. If excess oxygen is present, the efficiency of the MBFC will reduce (Fig. **4**).

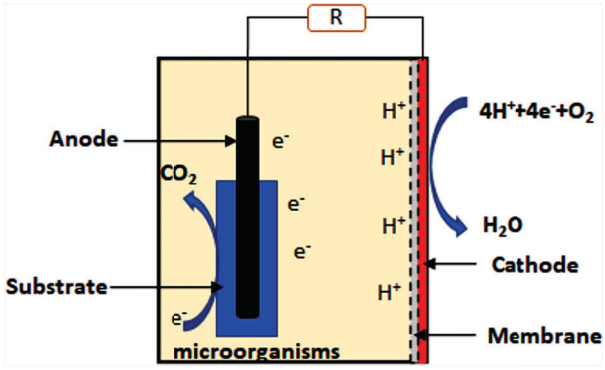

Fig. (4). Single chamber MBFCs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Double-Chamber MBFCs: Double-chamber MBFCs generally have a similar design to the traditional fuel cell. The proton exchange membrane split the anode and cathode compartments (Fig. **5**). The double chamber has the drawback of low power generation because of high internal resistance and complicated design. However, one has better control for different reactions in the double-chamber MBFCs as various environments can be sustained in separate chambers. In Double-chamber MBFC, electrons travel a longer distance as the anode and cathode are separated into two different sections. Due to this parameter, an increase in the internal resistance and a decrease in the efficiency can be observed in this type of MBFC. The internal resistance can be minimized if the electrode is clamped to the membrane [32].

3.1.1. Up-flow MBFC

It comprises an anion exchange membrane and graphite electrodes that can be operated ina continuous batch or fed-batch processes (Fig. **6**). To obtain a high mass transfer rate and efficiency, they are operated in fed-batch operations. On the other hand, in the continuous mode process, it was observed that the reduction in hydraulic retention time enhanced bioenergy production. Jang *et al*. have reported an up-flow MBFC, where they made the MBFC on plexiglass and separated it into two compartments [33]. One was filled with glass wool and another with glass beads. The cylindrical setup contains an anode compartment at the bottom where feed is supplied, and at the cathode, the effluent stream continuously passes and discharges from the topside. In this process, oxygen penetration is a disadvantage from the cathodic chamber to the anodic chamber. Oon *et al*. have developed a hybrid design where bioenergy production of 6.12 mW/m^2 and wastewater treatment operated simultaneously [34].

3.1.2. Stacked Microbial Biofuel Cells

In stacked MBFCs, the chambers are connected in parallel or series, as we see in electrical circuits. It was observed that both parallel and series connectivity have advantages like the high currents were obtained in parallel, and high voltages were obtained in series connectivity. The production of bioenergy and chemical oxygen demand (COD)

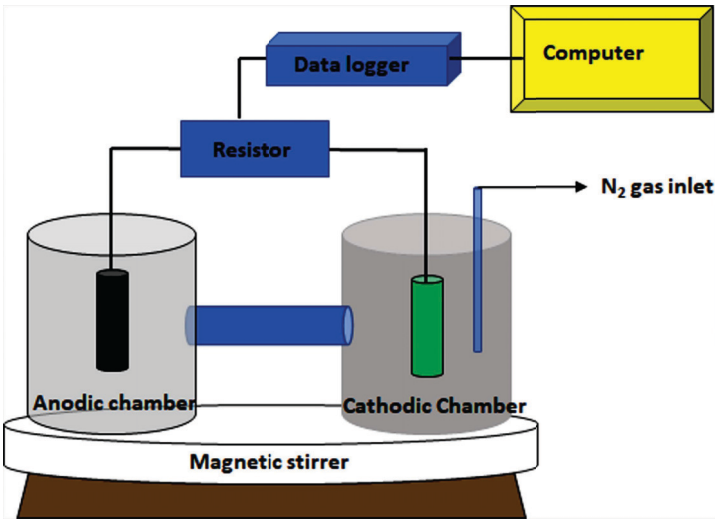

Fig. (5). Double chamber MBFCs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

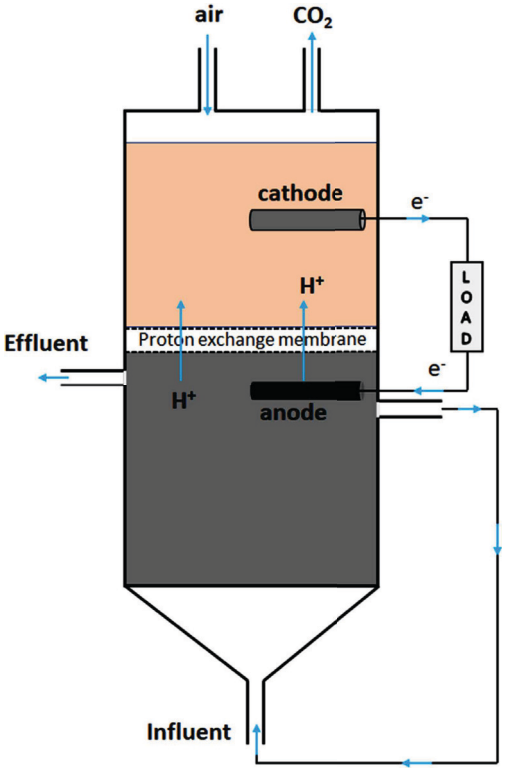

Fig. (6). Up-flow microbial fuel cell. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

removal was directly proportional to the surface area of the electrodes, as shown in Fig. (**7**) [35]. The major drawback of this type of MBFC is that it cannot be commercialized. Furthermore, the stacking can be arranged inhorizontal or vertical order. Few researchers used urine samples as the substrate for MBFC, and the experiment lasted 19 months. The whole setup was made of ceramic and was cost-effective. They checked the efficiency with varied designs like cascade, stacked, and individual, for which they obtained 75 mW, 21.4 mW, and 1.56 mW power output, respectively. The COD removal was high with three-module systems compared to conventional membrane MBFCs. Conversely, the traditional membrane MBFCs produced more power compared to stacked MBFCs. Kim *et al*. proposed a few modifications by regulating the voltage reversal and harmonizing the system kinetics using electroactive microorganisms [36].

3.1.3. Miniature MBFCs

Miniature MBFCs are designed micro or minutely to increase the surface area, decrease the distance between the electrodes, and have a quick reaction time, as in Fig. (**8**) [37]. Lorenza *et al*. have prepared a 3D-printed layer-by-layer prototype where the chamber was microscaled and acted as a biosensor for water quality monitoring [38]. Chouler *et al*. used urine as a substrate; increasing the electrode length by two times resulting in increased power density [39]. 3D electrodes in small-sized MBFCs retain a high volume ratio - surface area and increased mass transfer rate [40]. However, miniature MBFCs presume many difficulties with viscous solutions and substrate, irregular proton transfer, *etc*. [41, 42].

Different type of MFBCs have been considered, and Table **1** explain the advantages and disadvantages of the kinds of MBFCs based on their technology, operating conditions, and various factors [43-49].

The MBFCs have promising applications in wastewater treatment as it gives viable solutions for chemical oxygen demand (COD) removal and generation of electricity at the same time. The primary application of MFCs is the development of biosensors. MBFCs can also be utilized in biohydrogen and power production. Since MBFCs will be an appropriate choice for producing electricity from biomass, electricity production using microbes can be a dynamic form of bioenergy for the future [50]. Another application of MFCs, as pointed out by Bose, has to deal with biosensors for the online monitoring of organic matter and detection of water toxicity [51]. Studies have shown that the usual methods used to calculate the total organic content and biological oxygen demand in wastewater treatment are unsuitable for online screening and control of natural wastewater treatment.

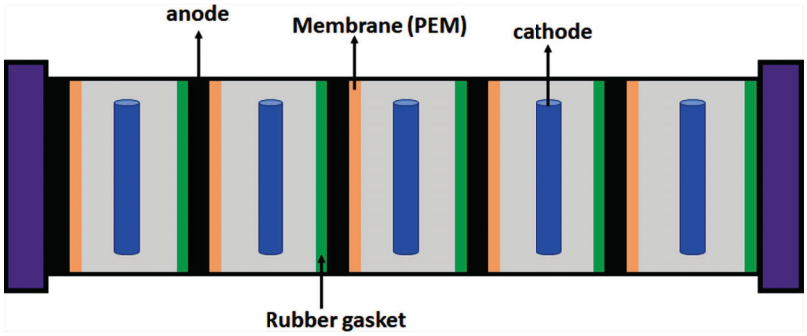

Fig. (7). Stacked microbial fuel cells. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

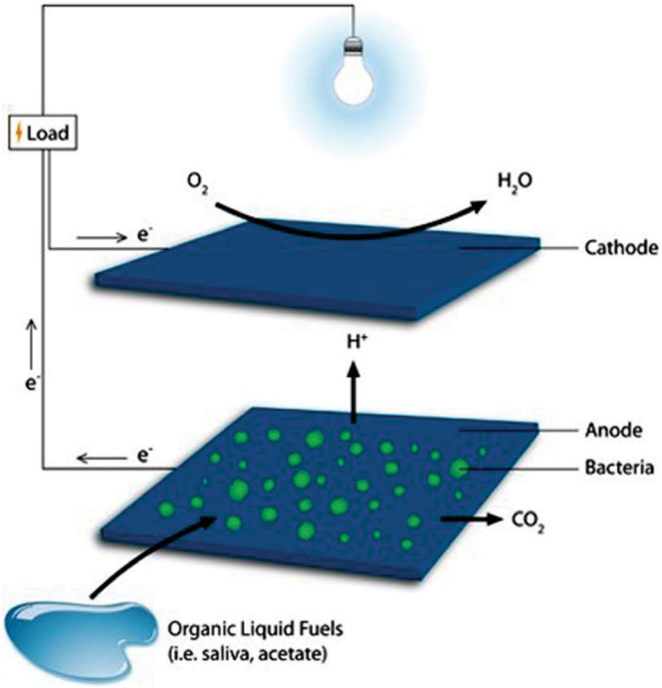

Fig. (8). Miniature microbial biofuel cell. Figure adapted from permission with [43] copyright open access. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Advantages and disadvantages of different types of MBFCs.

Types of MBFC	Advantages	Disadvantages	References
Single-Chambered	(a) Easy operation (b) Low-cost investment (c) High power output (d) Increased proton diffusion (e) Low internal resistance (f) Improved O_2 reduction rate (e) low electrode spacing	(a) Expensive maintenance (b) Membrane malfunction	[44-46]
Single-chambered mediator-less	(a) Low cost (b) Nontoxic (c) More O_2 diffusion rate	(a) Low efficiency	[45]
Double chambered	(a) Various operation conditions (b) High overall performance	(a) Constant replacement of solution at the cathode (b) Expensive setup (c) Membrane malfunction	[47]

(Table 1) contd…

Types of MBFC	Advantages	Disadvantages	References
Up-flow	(a) Superior loading capacity (b) Simple to scale-up (c) Continuous operations (d) More COD removal (e) Less clogging	(a) The long distance between the cathode and anode (b) High cost of fluid pumping	[48, 49]
Miniature	(a) Enhanced power (b) Low fluid volume (c) Fewer electrode sizes (d) Long-term applicability (e) High electrode surface-area	(a) Not suitable for wastewater treatment (b) Low loading rate	[43, 49]
Stacked	(a) High power density (b) Continuous operation (c) High loading rate (d) High voltage (e) Easy fabrication	(a) Low loading rate (b) Low efficiency (c) Voltage reversal	[49]

To detect the toxic content in the water necessary for providing safe water for human, animal, and crop consumption, MBFCs can act as a possible biological oxygen demand sensor because of the linear correlation of MBFC with the strength of organic matter in wastewater. The main drawback of using MBFC technology is associated with insufficient power output. Secondly, the issue of the high cost of electrode materials, membranes, and cathode catalyst poses a further limitation to the technology. Providing an electrode material of high surface area to improve the power output is a direction this technology should focus on in the future. Doing this in the absence of polymer electrolyte membrane (PEM) in futuristic MBFCs (at a large scale) can make the technology more economical.

MBFC technology uses the same biomass in anaerobic digestion technology in many cases for energy production. MBFCs can convert biomass at a temperature below 20 °C and with low substrate concentration, which tends to be problematic for methanogenic digesters in both technologies. As a result of the over-reliance on biofilms for mediators less electron transport is associated with MBFCs, which is a disadvantage to the technology, anaerobic digesters such as the upflow anaerobic sludge capacity reduce or eliminate this transport. By reusing the microbial consortium without cell immobilization in an anaerobic digester, the MBFC technology can coexist with anaerobic digestion in the coming days. Bose reported that the main application of MBFCs is in generating electricity [51]. Some examples of MFC performance for electricity from the literature are presented in Table **2** [52-54].

3.2. Enzymatic Biofuel Cell

The Enzymatic biofuel cell is a specific type of fuel cell which uses enzymes as a catalyst instead of noble metals to oxidize fuel. EBFC is known for its inexpensive ingredients and fuel. EBFCs have a similar operating protocol to conventional fuel cells, except that the biological catalyst (enzyme) catalyzes the half-cell reaction [55]. The EBFCs use enzymes to transform chemical energy into electrical energy through biochemical reactions. The operational procedure is similar to a traditional fuel cell. In place of microbes, enzymes or catalysts are used (Fig. **9**).

The EBFCs are further categorized into two based on electron transfer. Direct electron transfer (DET) and mediator electron transfer (MET). DET is the transfer of electrons between the electrode and the substrate without an external mediator, and MET is the transfer of electrons in the presence of a mediator, as in Fig. (**10**) [5]. In the DET, the enzymes are immobilized at the electrode, which is the complete biochemical activity the enzymes could exploit. Thus, providing enhanced efficiency and fewer voltage losses. The redox mediator is employed in the MET of BFCs to transport the electron between the enzyme's active site and the electrode. The MET pathway was observed to provide improvised output power for the BFCs by facilitating rapid electron transfer and enhancing electrically conductive. The MET biofuel cells have drawbacks such as cell voltage loss and less stability. The fuel concentration is the critical parameter for accurate EBFC processes. A high concentration of substrate is the rate-determining step in EBFC operation [56].

EBFC is considered an attractive eco-friendly technology, owing to its unique features like portability, the potential to yield sustainable and renewable energy, and easy miniaturization [57]. EBFCs can produce electric power from various organic substrates and can be operated at room temperature. Moreover, they have exceptional intrinsic properties like good catalytic activity, mild operating conditions like neutral pH, ambient temperature, and binding to a specific substrate [58]. EBFCs are mainly used in implantable

Table 2. Common materials used for MBFC.

Cathode Material	Advantages	Disadvantages	References
Graphite and carbon	Used mostly as an anode	A poor catalyst for oxygen reduction	[26]
Platinum	Excellent catalyst ability	Expensive	[26]
Manganese oxide	High power density	Short longevity	[52]
Lead oxide and cobalt complex	High power density	Short longevity	[52]
Proton-exchange Membrane			
Nafion	Excellent ionic conductivity and allows ion transfer	Oxygen leakage from the cathode	[53]
Anode Material			
Stainless steel	Low cost and high conductivity	Low power production and poor microbe attachment	[53]
Carbon paper	High conductivity	Expensive and low-specific area	[53]
Graphite rod	Defined surface area and high conductivity	Low strength	[53]
Graphite fiber brush	High conductivity and porosity	Expensive	[53]
Carbon cloth	Large specific area and Flexible	Brittle	[53]
Graphite granules	High porosity, low cost, more surface area	High contact resistance	[53]
Conductive polymer	Large surface area	Low conductivity	[53, 54]

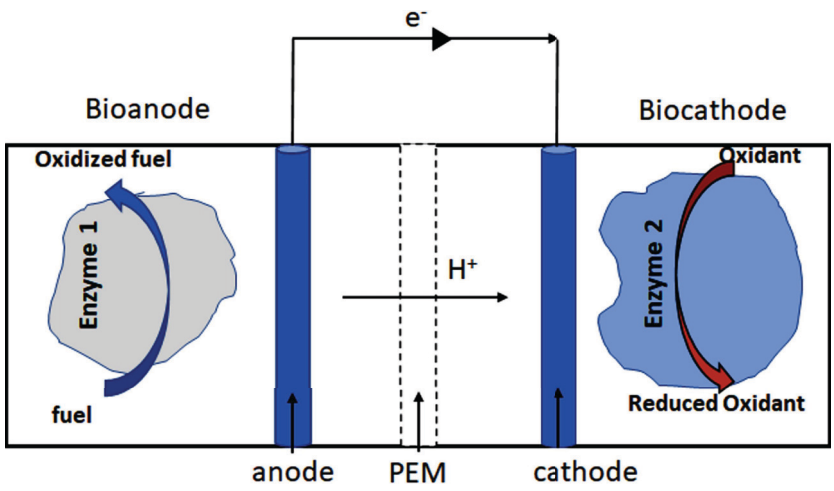

Fig. (9). Schematic representation of EBFC device. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

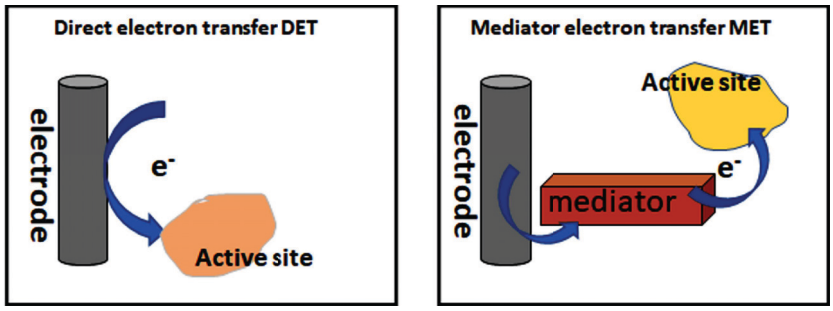

Fig. (10). DET and MET representation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

biomedical devices such as biosensors as they can use the biofluids present in living organisms to generate electric power [56].

3.3. Abiotic Fuel Cells

In abiotic fuel cells, the catalysts used are abiotic and solid-state materials. These systems can work even at high temperatures and pH conditions. However, it results in a low current density. (a) nature of the metal catalyst, (b) pH and concentration of glucose solution, and (c) operating condition of FCs are the factors that affect the cell performance [59, 60]. The nature of a metal catalyst means the shape, size, metal composition, and ionomers that conduct electricity between the electrode and the metal catalyst [61].

4. STRATEGIES AND CATALYTIC CHARACTERISTICS FOR EFFICIENT BIOFUEL CELLS

Significant work has been carried out in EBFC development [3, 7, 55, 62-64]. Many researchers have worked on electrode materials [65-67], bioelectrocatalysis [12, 68], enzyme immobilization [69-71], *etc*. There are still some areas where development must be focused on, like power density, energy output, operational stability, voltage output, *etc*. Hydrogen fuel is one of the most sought energy fuels in recent times. It is a clean fuel produced from water splitting or biomass. It can also be employed in BFCs incorporated with hydrogenase enzymes [67, 72-75]. The storage of hydrogen safely is one of the prime types of research and highly focused areas these days. Formic acid, a stable carrier of hydrogen, is used to power EBFCs because of its low toxicity, controlled flammability, and high volumetric capacity [76]. Methanol is another alternative fuel easily transported and sufficiently accessible but has severe toxic disasters if consumed. It has a three-fold higher energy density compared to formic acid. Ethanol can also be an alternative to hydrogen but was less explored. Glycerol is another possibility as an energy source. It has low flammability, low vapor pressure, and low toxicity and is obtained as a byproduct of biodiesel production [77].

Furthermore, power output is one of the challenges in BFCs. Four methods monitor the voltage-current curve or polarization profile and the respective power output. (i) by varying resistance measurement of currents and voltages by connecting the EBFC to a resistor, (ii) potentiostatic discharge: recording various currents generated by applying various voltages, (iii) potentiodynamic discharge rates: it records the voltage-current response when a meager sweep rate (less than 1 mV/s) is reached, (iv) galvanostatic discharge: EBFC discharge voltage records at various currents. Of these, (i), (ii), and (iv) are widely applied for long-term testing [78].

4.1. Employment of Nanomaterials in EBFCs

Porous carbon and gold nanomaterial electrodes have been employed vastly to increase the current density [67, 79, 80]. Conducting materials with a high surface-to-volume ratio allows extra enzyme loading capacity [81]. Porous 3D nanomaterials accompanied by metal electrodes provide good conductivity, chemical stability, and biocompatibility, fabricated through dealloying [82-84], anodization [85], diazonium grafting [86-88], electropolymerization [89, 90]. The following table (Table **3**) represents the wastewater treatment source and efficiency through MBFC [91-100].

4.1.1. 2D-Nanocomposites-based BFCs

Developments in nanotechnology and nanoscience have demanded scientists to explore micro-and nanomaterials for electrodes. Since the beginning of this century, the search for new nanomaterials in the field of electrodes has taken a fast pace [32]. It was discussed earlier that the smaller the unit, the increased surface area, high enzyme loading capacity, high crystallinity, efficient energy storage, improved catalytic activity, good adsorption efficiency, the superior potential for durability, storage, recovery, recycling, and reusability, thus improving the overall efficiency of BFCs [101]. Most nanomaterials have been reported utilizing carbon, precious metals, and inorganic metals for BFCs.

The efficiency of BFCs has improved with the use of nanomaterials in immobilization methodology. The movement of electrons from the active location of the enzyme to the surface of the electrode is the rate-limiting step in determining the efficiency of BFCs [102]. This can be overpowered by the inclusion of nanomaterials into the BFC mechanism. Thus, researchers have recently paid much attention to developing carbon-based inorganic nanoparticles and metallic nanomaterials [103, 104].

4.1.2. Carbon-based Nanomaterials

Carbon with an amorphous texture has shown good conductivity, increased porosity, and massive surface area, making it a suitable electrode material. With the advancement in the field of nanotechnology, various carbon-based nanomaterials (CBNMs) have been explored, like porous carbon [105, 106], carbon nanotubes [107, 108], and graphene [109, 110]. They showed promising results in various applications of BFCs. When carbon nanomaterials are employed in BFCs, they offer high conductivity because of the reduced weight and size of the electrode and are suitable for implant applications. Despite excellent applications, biosafety measures for long-term use are still in question because of incomplete toxicity studies at the cellular level [111, 112]. Another drawback is the adsorption of enzymes on the carbon substrate leads to low utilization of enzymes for effective contribution to the chemical reactions [113]. Some of the carbon-based biofuel cells applied in the literature are:

4.1.2.1. Graphene

In 2004, Novoselov prepared graphene in a single layer for the first time through the exfoliation of graphite [114]. Graphene is a 2D single flat layer arranged in a honeycomb pattern as sp2 hybridized carbon atoms with a bond length of 1.43 Å and bond angle of 120^{o}. It is the thinnest material with a theoretical thickness of 3.35 Å [115-119]. It is one of

Table 3. Treatment of wastewater through specialized MBFCs.

Wastewater Substrate Source	Inoculum Source	MBFC Efficiency	Power Density	References
White wine lees	Denitrification wastewater tank	• Single chambered • 90 % COD removal • 15 % CE	262 mW/m^2	[91]
Red wine lees	Denitrification wastewater tank	• Single chambered • 27 % COD removal • 9 % CE	111 mW/m^2	[91]
Dairy industry	Activated sludge	• No mediator membrane and catalysts • 90.5% COD removal, • 37.5% CE	621.1 mW/m^2	[92]
Chocolate industry	Anaerobic sludge	• Single-chambered • 3% COD removal	22.898 W/m^2	[93]
Tannery industry	Anaerobic sludge	• Single compartment • 88% COD removal	7 mW/m^2	[94]
Palm olein oil industry	Pseudomonas aeruginosa ZH1	• Two chambered • 3% COD removal	451.3 mW/m^2	[95]
Human urine	E. coli	• Double chambered • 46% SCOD removal	93 mA/m^2	[96]
Paper recycling	LZ-P1 strain	• Dual chambered • 0.9% COD removal • 8.72% CE	44.05 mW/m^2	[97]
Swine industry	Swine	• Two-step MBFC • 72% SCOD removal	33.3 mW/m^2	[98]
Rubber industry	Sludge	• Ceramic separated • 9.77%COD removal	3.26 μW/m^3	[99]
Wood industry	Municipal wastewater	• Two chambered. • 66% COD removal	14 mW/m^3	[100]

CE - coloumbic efficiency

the strongest materials with a tensile strength of 130 GPa and a light weight of 0.77 mg/m^2. It has high flexibility with a large surface area of about 2630 m^2/gm. Despite large work carried out by various researchers on graphene exploring green energy, most of them are at the base level and cover research on supercapacitors and fuel cells [120]. Most of the electrochemical application work was carried out in the graphene family with fluorographene, reduced graphene oxide, graphene oxide, graphane, graphing, and graphene [121]. Of these, GO and rGO are promising materials for preparing 2D and 3D nanomaterials [122, 123]. In BFCs, graphene materials are graphene hydrogel, graphene aerogel, graphene foam, *etc*.

Graphene doped with heteroatoms shows high catalytic activity compared to Pt over carbon in various graphene composites with different metal oxides and conducting materials. The graphene sheets show irreversible agglomeration because of strong pi-pi interactions and Vander Waal forces [124]. 3D graphene offers excellent flexibility, high strength, and a large surface area compared to 2D graphene materials. 3D materials with an extensive surface area are a suitable choice for microbial fuel cells because of more chances of microbes' attachment to the surface. Some approaches to growing 3D graphene materials from GO include microbial reduction, chemical reduction, chemical vapor deposition, hydrothermal reduction, electrochemical reduction, *etc*. The critical factor governing the BFC performance is the electron transfer efficiency between electrodes and biocatalysts [26].

4.1.2.2. 2D Graphene Materials

These materials are highly chosen because of their high electrical conductivity and surface area. It was reported that

2D graphene helped the *E. coli* attachment on stainless steel mesh. It witnessed an increase in power density by 17 times compared to the normal SS electrode [125]. Positive-charged film of poly (3,4-ethylene dioxythiophene) (PEDOT) was applied to an anode made of graphene film with carbon paper coating to increase the negative-charged bacterial interaction with the anode. Compared to conventional carbon paper electrodes, the current in this system was enhanced by seven times, and a 15 times increase was observed in the maximum power output [126]. In another approach reported by Wang *et al*., 2D graphene film was used in making multilayered electrodes. It was exploited as a spacer between different multilayered films. This multilayered electrode assembly was achieved because of π-π or electrostatic interactions. At 0.48 V in a phosphate buffer solution comprising 30 mM glucose and 10 mM NAD^+, the glucose and oxygen BFC exhibited a maximum power density of about 22.50 $\mu W/cm^2$. In an open circuit, they exhibited a voltage of 0.69 V [127].

By reducing the fuel crossing across the liquid and gas graphene membranes without disturbing proton conduction, they enhanced the fuel cell efficiency. Fang *et al*. formed graphene/ hexagonal boron nitride (hBN) on the electrode and membrane assembly through the chemical vapor deposition technique. They observed that the graphene barrier allows only protons to permeate and restricts the passage of methanol, thereby improving the efficiency of methyl alcohol fuel cells. The dense lattice framework of 2D graphene was the reason for such selective permeation, which also blocked proton transfer [128].

4.1.2.3. 3D Graphene Materials

The enhanced surface area and high volume/ surface ratio of 3D graphene attracted many researchers to use these materials for electrode substrates. These factors enable biocatalysts' immobilization or bacterial colonization on the electrode. 2D graphene showed improved electrode efficiency compared to the conventional electrode. However, 2D graphene still has some limitations, for example in 2D graphene with SS mesh electrode, only the graphene located on the anode exterior surface is exposed to bacterial suspension and not the interior anodic region. When 3D graphene is prepared, an additional surface is created either in porous form or hollow cavities that enable total graphene utilization, further enhancing the FC performance [129]. 3D graphene structures are categorized into graphene gels and foams. Additionally, gels are categorized as hydrogels and aerogels. In hydrogels, the space inside the graphene crosslinks is filled with water, and on the other hand, in aerogels, the space is filled with air which can be removed by freeze-drying.

Xie and his group incorporated graphene foams into SS electrical collectors to enhance their electrical conductivity. A 14-fold increase in maximum power density was generated with a 3D graphene material anode when assessed with a plain anode [130]. Wang *et al*. prepared a 3D reduced graphene oxide-based nickel foam anode. This material provided a large surface area for microbial growth and efficient diffusion of culture media. This technique facilitates large-scale electrode preparation. They observed that the reduced graphene oxide-based nickel foam showed high efficiency than plain nickel foam and conventional electrodes. Some researchers coated graphene with hydrophilic polyaniline to enhance the hydrophilicity, allowing bacteria to adhere to the surface. They also observed that the bacteria attached deeply to the 3D electrode. They also observed a 4-fold enhancement in maximum power density with these 3D graphene/ polyanilines compared to carbon cloth [131].

These 3D graphene structures have advantages over other graphene structures, as they allow to make of lightweight FCs with improved performance. Macroporous substrates can be achieved with this 3D material by freeze casting or drying techniques. A group prepared 3D-chitosan with vacuum-striped graphene to obtain superior porous materials. These hollow porous substrates allowed the bacteria to grow colonies in the porous region and increased electron transfer rates [132].

4.1.3. Inorganic Nanomaterials

The unique properties of polysulfones demand their usage in nanoparticle preparation. They possess properties like high mechanical, chemical, and thermal stability. They also withhold strong acidic conditions at a low cost. They also have the desired film-forming capacity [133, 134]. These inorganic materials, when molded into membranes, decrease the swelling and prolong the degradation of sulfonic endings at high temperatures. Generally, inorganic materials are used as fillers in the nano substrate, improving the required properties [135, 136]. Aluminum oxide [137], silicon oxide [138], ferric oxide [139], and titanium oxide [140] are a few inorganic fillers used in nanocomposite materials. Of these fillers, titanium oxide and silicon oxide materials are extensively studied and are functionalized between polymer matrices [141, 142].

4.1.4. Metallic Nanomaterials

Metal nanoparticles have attracted the attention of researchers for their usage in BFCs because of their excellent catalytic properties and high conductivity. They act as carriers by transporting electrons between the solid substrate and enzymes, enhancing biocatalytic activity [143]. Some researchers have used gold nanoparticles to prepare mediatorless enzymatic BFCs and biosensors for fructose and glucose oxidation [70, 144, 145]. Moreover, platinum also possesses high conductivity, catalytic properties, and biocompatibility. Sugar/ O_2 EBFCs and amperometric glucose sensors are a few good examples of incorporating platinum nanoparticles into biomolecules [146-148]. The main drawback of these metallic nanoparticles is the cost, which can be balanced by using them in low amounts.

5. POTENTIAL APPLICATIONS OF BIOFUEL CELLS

Biofuel cells have potential applications in energy harvesting devices, specifically for personal and biomedical applications as shown in Fig. (**11**) [149]. Recently, BFCs

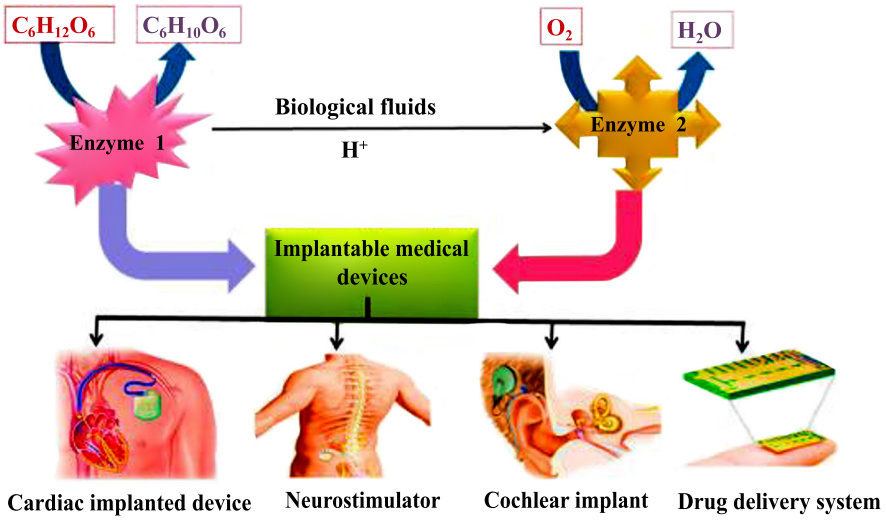

Fig. (11). Targeted application of BFCs in implantable biomedical devices. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

have garnered massive attention as an alternative approach for green sources for numerous wearable and implantable applications [150-152]. Biofluids such as tears and blood comprise beneficial metabolites like lactate and glucose, functioning as electrical energy generators. The recent advancement in nanoscale technologies gave new opportunities to develop compact BFCs for implantable biomedical devices. BFCs have vast applicability in biomedical devices like glucose biosensors, gastric stimulators, brain neurotransmitters, drug delivery systems, and variable sensors [19, 153, 154]. There is increasing use of BFCs in pacemakers to generate electricity even though more attention is required to address weak responses leading to infection and low lifetime and efficient use of BFCs in pacemakers [155-157]. Kulkarni *et al*. developed a novel enzymatic glucose BFC that detects glucose levels with high sensitivity near 86.42 Hz/cm^2. These self-powered power sensors based on BFCs offer good performance over old glucometers and continuous glucose monitors [158].

Since biofuel cells may be able to function in living systems, they may be able to draw the oxygen and fuel they need for operation from their surroundings. This opens up a wide range of potential implanted medical devices using biofuel cells as power sources. For instance, a biosensor for glucose has been created that produces an electrical current using an anode based on glucose oxidase and a cathode based on cytochrome c. This procedure can be applied to a biosensor to measure glucose concentration in the range of 1-80 mM. It has also been designed to detect lactate [159]. Power sources for medication delivery systems are another potential application for small fuel cells, and biofuel cells have already been designed to be small enough for this usage [160, 161].

Many fuel cells have been demonstrated to produce energy by oxidizing substances in wastewater streams. This process can be used for two beneficial purposes: (a) to remove organic chemicals from the waste stream and (b) to generate electricity. According to a recent study, a town of 150,000 people's wastewater can produce up to 2.3 MW of power (assuming 100 percent efficiency), while a power of 0.5 MW might be more practical. It should be noted in this context that wastewater treatment in a microbial fuel cell can remove up to 80% of the chemical oxygen demand, and the electricity produced by this method could possibly be used locally to power additional wastewater treatment. The review's economic assessment [162] demonstrates the application's potential. However, it is heavily reliant on localized power prices.

The idea of creating robots that can "live off the land" by using biofuel cells to produce their energy and the difficulties that must be addressed have already been covered [163]. The "Slugbot," which, as its name suggests, hunts slugs, is a classic illustration [164]. Although the "Slugbot" has a rechargeable battery, after it has captured a slug, it keeps it in a holding tank until the battery starts to run low. After transferring the slugs, the "Slugbot" returns to a microbial fuel cell and uses the energy generated by their "digestion" to recharge its batteries (www-robotics.usc.edu). Another "gastropod" with the name "Chew-Chew" has also been created, and it can "feed" by consuming meat to continue operating.

6. CHALLENGES

Microbial fuel cells have been in use to generate energy. It has been employed on a microscale successfully. However, it has some drawbacks to apply for commercial applications, as it produces low power output, durability, and stability for long-term usage, proton exchange or cation exchange, membrane durability, substrate feed continuity, the strength of electrodes, cost of the setup, *etc*., require improvement. Setting up a new MBFC is thirty times more expensive than the conventional activated sludge process [165]. Carbon paper and carbon cloth electrodes are efficient electrodes and are commonly used. However, their scale-up at a commercial scale is expensive, and further design for a large scale is challenging. Jaiswal has reported that the structural construction of microalgal FC requires a high cost, and the pH mem-

brane employed in this setup reduces the voltage and power output [166]. Few limitations are noted in the MBFCs, such as internal resistance, the distance between the electrodes, the exchange of ions through the cation exchange membrane, or the proton exchange membrane. These factors pose a massive challenge in setting up biofuel cells for long-term function.

BFCs are miniature devices that produce micropower. Their application in commercial devices is questionable. The cost of generating electricity using BFCs is not yet comparable to the traditional sources of power generation which cannot be regarded as a significant contribution to power generation [167]. For commercialization, BFCs must overcome specific challenges such as low manufacturing costs, long-term operational stability, *etc*. EBFCs have low mediator stability, high potential losses, and lower open circuit potential [19].

The enzymes are loaded on the electrode surfaces in high quantities to improve the catalytic activity of enzymes and faster electron transfer between the electrode and enzyme. This will eventually lead to improved current density and power. The contribution of material engineering and genetic modification of enzymes will increase enzymes' stability and provide optimistic attachment sites on the surface of the electrode [19]. The enzymes are grouped in reaction pathways such that the traveling of the reaction intermediates is decreased. This prevents the formation of undesirable toxic intermediates and competes for side reactions [168].

7. FUTURE PERSPECTIVES

Biofuel cell methodologies are transcribing the world as they promote the production of bioenergy and treat wastewater at the same time. The technology and design setup broaden the range of wastewater treatment from ultra-pollute to less polluting and bioenergy production. The power output through this technology is still less, which can be improved (a) by adequately designing the setup by keeping the internal resistance low, (b) by increasing the transfer of electrons by implementing nanoparticles, and (c) by using genetically modified microbes that produce more electricity and increase conductivity [169] (d) pretreating the feed to decrease the biofilm formation time [170] (e) MBFCs stacking and design can be optimized to avoid short-circuiting, and voltage reversal (f) integration of MBFCs with newer treatment technology can speed up the process with improved efficiency [171].

CONCLUSION

The increasing energy demand for portable electronic devices drives interest in sustainable power generation. Several miniaturized biofuel cells can be used for this in which ubiquitous solutions, like glucose or lactate, can be utilized. Biofuel cells, a form of renewable energy, have received considerable attention. Biological organic matter can be oxidized directly in these cells to produce electricity. The conversion of energy in BFCs is highly determined by the area of the electrode that is effective as a catalyst. Real-world applications require high energy conversion efficiency, low cost, long-term stability, ease of production, and high reproducibility. Biofuel cell technology can also be applied in wastewater treatment, water desalination, bioremediation, biosensor development, power production, microbial carbon capture, and biohydrogen production. This process generates electricity through wastewater treatment, thus completing the tasks of water recycling and creating renewable energy. The generation of electricity by applying microbes will recycle the waste without harming the environment. The investment for new BFCs and their maintenance cost is relatively large compared to the energy output. However, the environmental impact is friendly despite the energy production capacity, which can be improved with further research and development. By overcoming the challenges like low power density output, COD removal, contaminant degradation, and energy production without CO_2 emission, the BFCs technology can ramp up future perspectives and preserve the earth for survival.

LIST OF ABBREVIATIONS

BFCs	=	Biofuel Cells
DET	=	Direct Electron Transfer
EBFCs	=	Enzymatic Biofuel Cells
NEBFCs	=	Non-Enzymatic Biofuel Cells

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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