Journal of Metals, Materials and Minerals, 35(4), e2248, 2025

Enhancing efficiency and productivity of microbial fuel cells for optimization of wastewater treatment with bioenergy production

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Received date:
20 December 2024
Revised date:
7 August 2025
Accepted date:
16 September 2025

Keywords:
Environment;
Health;
MFC;
Organic;
Electrode

Abstract

The primary objective is to remove the pollutants from the wastewater streams and reintegrate them into the water cycle, therefore safeguarding the environment and promoting public health. The utilization of membrane fuel cells (MFCs) enables the conversion of organic molecules present in wastewater into electrical energy, while concurrently generating potable water. To optimize the process, it is essential to understand the parameters that affect the performance and the relevant techniques such as electrode materials, microbial collectives, and operating settings to improve the efficiency and performance of MFC. Thus, it aims to present novel ideas that support the real-world deployment of MFCs as a key technology for circular economy and sustainability.

1. Introduction

Wastewater treatment is an issue of worldwide significance due to its containment of valuable resources such as organic matter, nutrients like nitrogen and phosphorus, and use of thermal energy [1]. The implementation of suitable technologies has the potential to convert wastewater treatment into an environmentally conscious method. Thus, wastewater's increasing acknowledgment as a feasible energy and resource source has spurred the development of energy-efficient technologies including anaerobic digestion (AD), activated sludge process, and fuel cells. Meanwhile, chemical, and thermal energy are the two types of energy found in domestic wastewater sources. Nitrogen (N) and phosphorus (P) are examples of nutritional molecules, and carbon (expressed as chemical oxygen demand, or COD) makes up about 26% of the chemical energy [2]. Because 74% of this energy potential is thermal, exploiting it might make wastewater treatment energy-producing or energy-independent. The AD procedures recover methane (CH_4) or H_2 from wastewater to partially offset treatment energy use, although they are more complex [3]. The Microbial Fuel Cell (MFC) technology offers new opportunities for sustainable wastewater treatment by directly extracting electrical energy, achieving excellent effluent quality, minimizing environmental impact, and enabling real-time monitoring [4]. The practical implementation of MFC technology has not yet been completed because of significant obstacles in the areas of cost, system development, and energy recovery. Reevaluating the problems and the approach's viability is required to determine whether the expected benefits can be realized [5]. Its goal is to solve significant issues including whether it is feasible to operate MFCs sustainably for the treatment of wastewater [6]. It also seeks to stimulate additional thought and conversation about suitable solutions for the best possible use of this particular technology.

2. Fundamentals of microbial fuel cells

The MFCs are bio-electrochemical systems that use microorganisms' metabolic activity to clean wastewater while also generating sustainable electricity. Their operation is based on the ability of exoelectrogenic microbes to oxidise organic substrates and transmit electrons to an electrode under anaerobic conditions [7]. Understanding the fundamental concepts, operating procedures, and structural components of MFCs is critical for assessing their efficiency in energy recovery and wastewater treatment. This part discusses the fundamental principles that underpin electron and proton generation, followed by a full description of the structural elements, electrochemical reactions, and operational characteristics that enable efficient MFC operation [8].

2.1 Basic working mechanism of microbial fuel cell

At the heart of the MFC process are special microbes, often called exoelectrogens, which have the unique ability to transfer electrons outside their cells during metabolism [9]. The typical MFC setup has two separate chambers: an anode and a cathode, divided by a proton

exchange membrane (PEM). The anode side is kept oxygen-free because the electricity-producing bacteria only works under anaerobic conditions. When organic substrates such as acetate or glucose are introduced into the anode chamber, microbes break them down for energy. This breakdown produces electrons, protons (H^+), and carbon dioxide as a byproduct. For example, the oxidation of acetate follows the reaction:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 8e^- + 9H^+$$

These electrons do not just float away; they need to get to the anode. Some bacteria, like Geobacter and Shewanella, pass electrons directly to the electrode using conductive pili (often called nanowires) or surface proteins like cytochromes. Others, such as certain Bacillus species, use small redox-active molecules (like flavins or pyocyanin) as shuttles to carry electrons from the cell to the electrode surface. This difference defines two main pathways: direct and mediated electron transfer [9].

Once the electrons reach the anode, they travel through an external circuit to the cathode, this movement is the electric current that can power small devices. Meanwhile, the protons generated during substrate oxidation move across the PEM to the cathode chamber. The membrane plays a key role here: it allows protons through but blocks oxygen from entering the anode and prevents fuel from leaking into the cathode side, helping maintain stable performance [10]. On the cathode side, oxygen, usually from the air, acts as the final electron acceptor. It combines with the incoming electrons and protons to form water:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

This completes the circuit and sustains continuous power generation. Because the system relies on organic matter as fuel, MFCs offer a dual benefit: they treat wastewater by breaking down pollutants while simultaneously generating clean electricity [11]. The fundamental operation of a dual-chamber MFC is illustrated in Figure 1. At the anode, microbes break down organic materials, releasing protons and electrons. Protons then move across a membrane to decrease oxygen at the cathode, producing both water and electricity at the same time.

2.2 Operation and structural components of MFCs

An MFC uses microorganisms to convert organic molecules into electricity in an anaerobic anode chamber and an aerobic cathode chamber separated by a proton exchange membrane (Figure 2). Microorganisms in the anode chamber oxidize organic effluent molecules, producing protons (H^+) and electrons (e^-). chemical energy propels these bacteria to break down complex chemical molecules [13]. The proton exchange membrane in the anodic compartment transports MFC protons to the cathodic chamber. Oxygen is the main electron acceptor and electrochemical reaction with oxygen produces water from protons and electrons. An external electrical circuit transports electrons from the anode oxidation process to the cathode [14]. Electricity from the MFC powers devices and stores energy in batteries or capacitors via electrons crossing the circuit [15].

Electrodes in the anode and cathode chambers, a PEM, and substrates that fuel microorganisms make up a microbial fuel cell. MFC exoelectrogenic microorganisms transform substrates into energy [10]. The PEM completes the circuit by combining hydrogen ions with electrons to generate water and carbon dioxide. For efficient, sustainable operation, the PEM separates the anode and cathode chambers, minimizing substrate and oxygen crossover, preventing unwanted ion transfer, and thereby increasing Coulombic efficiency [16]. Figure 3 illustrates this configuration, where microorganisms at the anode oxidize substrates to release electrons and protons. The electrons pass through an external circuit to produce electricity, while the protons migrate through the PEM to the cathode, where oxygen serves as the terminal electron acceptor and combines with electrons and protons to form water [17]. This schematic highlights the coordinated role of the anode, cathode, PEM, and catalyst layers in maintaining bio electrochemical reactions within the MFC.

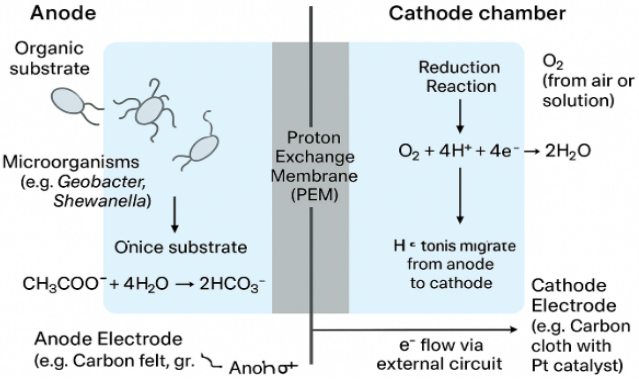

Figure 1. Schematic Representation of a Dual-Chamber MFC (Adapted from [12]).

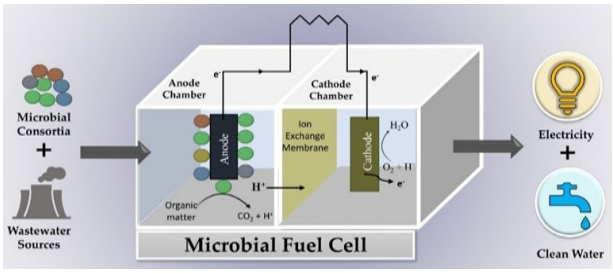

Figure 2. Membrane Electrode Assembly and proton exchange in MFC (Adapted from [11]).

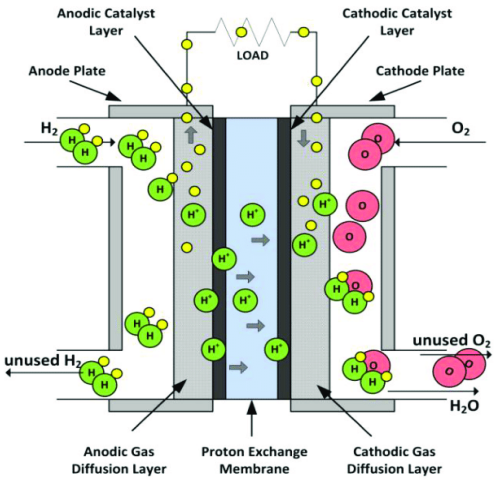

Figure 3. Typical Structural Configurations of an MFC (Adapted from [18]).

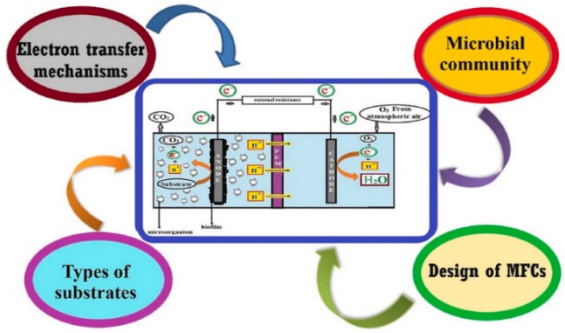

Figure 4. Determinants of MFC performance in wastewater treatment.

3. Determinants of microbial fuel cell function and efficiency

MFCs represent a promising step toward sustainable wastewater treatment, offering solutions to pressing energy, environmental, and resource challenges. By capturing the chemical energy stored in organic matter, MFCs can significantly reduce the energy demands of conventional treatment plants, particularly by minimizing or eliminating aeration, which typically accounts for 50% to 60% of total energy use in activated sludge systems [18]. Unlike traditional methods that consume energy, MFCs turn wastewater into a fuel source, enabling simultaneous pollutant removal and electricity generation.

The efficiency of MFCs depends on multiple interrelated factors spanning microbial, material, operational, and design domains. As illustrated in Figure 4, the performance of MFCs is governed by four major determinants: (i) electrode material properties, (ii) microbial community composition and electron transfer mechanisms, (iii) operational conditions such as substrate type, pH, and hydraulic retention time, and (iv) system configuration and scale [19]. This schematic framework highlights the necessity of a holistic perspective for enhancing both energy recovery and wastewater treatment efficiency.

Electrode material is a central determinant, as the anode serves as the solid interface for microbial colonization and extracellular electron transfer (EET). Physicochemical properties such as surface area, conductivity, and biocompatibility strongly influence performance [20,21]. Recent advancements, such as carbon dot (CD)-modified anodes and graphene oxide–zeolite composites, have enhanced microbial adhesion and reduced internal resistance, achieving power densities up to 661.1 $mW \cdot m^{-2}$ [22].

Microbial electron transfer pathways, whether direct electron transfer (DET) via nanowires and cytochromes or mediated electron transfer (MET) through redox shuttles like flavins, are equally crucial. Exoelectrogenic species such as Geobacter sulfurreducens and Shewanella oneidensis dominate DET processes [23], while biosurfactants such as sophorolipids can enhance MET efficiency [24]. Importantly, recent studies also show that biofilm thickness and morphology influence charge transfer; optimal thickness (50 μm to 70 μm) maximizes current output, whereas excessively thick layers hinder proton diffusion [25].

Operational parameters such as substrate type, concentration, hydraulic retention time (HRT), and external resistance further regulate MFC performance. While longer HRTs maximize organic removal, shorter HRTs have been linked to higher peak power densities [26], reflecting a trade-off between treatment efficiency and energy recovery. Oxygen availability at the cathode is another determinant; excessive oxygen crossover into the anode inhibits exoelectrogens and reduces Coulombic efficiency, while insufficient cathodic oxygen stifles current generation [27,28].

System configuration and scale also play defining roles. Single-chamber designs are cost-effective but prone to oxygen crossover, while dual-chamber MFCs with PEMs allow better control of environmental conditions but add complexity [29,30]. Moreover, scale directly affects power density: smaller reactors with high surface-area-to-volume ratios achieve power densities above 1,500 $mW \cdot m^{-2}$ whereas larger systems often fail to exceed 300 $mW \cdot m^{-2}$ due to mass transfer limitations and increased internal resistance [31,32]. Cascaded or stacked units can extend treatment capacity, though they often suffer from downstream substrate depletion and pH imbalance; optimized flow and reconfiguration strategies are being explored to overcome these limitations [31,33].

Despite such challenges, innovations in materials science and synthetic biology are pushing boundaries. Graphene-based anodes, conductive nanomaterials, and 3D-printed porous electrodes have demonstrated significant gains in current density [34]. Likewise, engineered microbial strains with enhanced cytochromes or conductive pili have improved electron transfer and stability [35]. Integration with capacitors or supercapacitors further extends applicability, enabling small-scale power storage for sensors or low-energy devices [29].

Together it provides a unifying framework for understanding the determinants of MFC efficiency. Each branch, materials, microbes, operational conditions, and system design, represents a modifiable lever for optimization [36]. The interplay between these factors underscores that improving MFC performance requires integrative strategies rather than isolated interventions. Continued advances in nanomaterials, bioengineering, and design optimization hold promise for transforming MFCs into scalable, energy-positive solutions for wastewater treatment.

4. Factors influencing MFC performance and efficiency

The performance of MFCs is strongly influenced by specific operational and environmental factors that can be tuned to achieve higher efficiency in practical applications. Real-world optimization requires careful adjustment of these parameters based on experimental data and system-specific constraints. Such optimization extends beyond flow management and system configuration; it demands a multi-scale approach that integrates physical design, chemical modification, and molecular-level biological control to enhance electron transfer, microbial activity, and interfacial efficiency.

Physical strategies include reducing electrode spacing to minimize ohmic losses, optimizing reactor geometry (e.g., serpentine flow channels), and employing 3D-printed porous electrodes to improve substrate distribution and biofilm development [34,37]. In cascaded systems, staged feeding and flow redistribution prevent fuel depletion in downstream units, ensuring more uniform performance across modules [33].

At the chemical level, cathode catalysts such as Fe-N-C or activated carbon enhance the oxygen reduction reaction, while anode surface functionalization with conductive nanomaterials, such as CDs [22], graphene/polyaniline composites [38], and graphene oxide-zeolite hybrids [34], improves biocompatibility and electron transfer kinetics.

Notably, nitrogen-doped graphene nanosheets (NGNS), synthesized via plasma-enhanced chemical vapor deposition (PE-CVD), have been demonstrated as efficient metal-free anode catalysts, achieving a maximum power density of 1008 mW·m^{-2} through enhanced conductivity, defect-rich structures, and durable hydrophobic properties [39]. Buffering the electrolyte to maintain optimal pH (7 to 9) stabilizes microbial activity and prevents proton accumulation [40].

Molecular and biological strategies focus on tailoring the microbial community. By enriching exoelectrogens like Geobacter and Shewanella promotes DET via nanowires and cytochromes [41], while the addition of redox mediators (e.g., neutral red) or biosurfactants like sophorolipid enhances MET in mixed cultures [24]. Lin *et al.* [42] demonstrated that graphene/polyaniline-modified anodes not only increase power output but also shape the exoelectrogenic population structure, favoring high-performance biofilms. Similarly, Paul *et al.* [34] reported enhanced MFC performance using graphene oxide-zeolite composite anodes, highlighting the synergy between material design and microbial ecology.

Beyond bacterial exoelectrogens, algal strains are also emerging as promising candidates for bioelectrogenesis. For instance, Scenedesmus sp. SB1 has been shown to produce sulphated pectin exopolysaccharides with favorable structural and thermal stability, while exhibiting superior photo electrogenic activity under optimized culture conditions compared to conventional media. This dual role of extracellular polymeric substance (EPS) production and electrogenicity underscores the potential of algae in advancing photosynthetic microbial fuel cells [25]. Furthermore, biofilm engineering, controlling thickness (ideally 50 μm to 70 μm) and extracellular polymeric substance EPS composition, directly affects charge conduction [43].

The integration of these multi-scale strategies is essential for advancing MFCs from laboratory curiosities to practical, scalable systems. Table 1 provides a comparative summary of the major MFC configurations reported in the literature, highlighting their electrode compositions, internal resistances, and observed power and current densities [4]. This concise overview allows readers to assess how structural and material choices influence MFC performance, bridging the gap between mechanistic understanding and practical implementation

The following subsections examine how key operational variables, HRT, substrate characteristics, pH, temperature, external resistance, and scaling strategies are optimized within this broader framework to balance wastewater treatment and power generation.

4.1 Hydraulic retention time (HRT)

HRT plays a critical role in determining both organic matter degradation and bioelectricity output. Ye *et al.* [26] investigated a dual-chamber MFC treating municipal wastewater and observed that COD removal remained consistently above 80% across HRTs ranging from 6 h to 24 h. However, maximum power density was achieved at shorter HRTs (6 h to 12 h), suggesting that faster flow rates enhance substrate availability for electroactive bacteria despite reduced contact time. This highlights a fundamental trade-off: longer HRT improves treatment completeness, while shorter HRT can boost power output by maintaining a higher concentration gradient of organic substrates. In cascaded MFC systems, Walter *et al.* [33] found that downstream units often suffer from fuel depletion due to prior substrate consumption, emphasizing the need for staged HRT design or flow redistribution to maintain uniform performance across modules.

HRT is a critical parameter in the design and operation of MFC reactors. It significantly impacts energy requirements, which directly correlates with the performance of the MFC. The Hydraulic HRT of wastewater is a critical parameter as it influences the efficiency of substrate utilization and determines the duration microorganisms are retained within the system. This duration quantifies the time that wastewater remains within an MFC. The influence of HRT on the power output of continuous-flow MFCs is significant, as changes in HRT directly affect both the composition and abundance of bacteria present in the bioreactor [26]. Extended HRTs typically result in improved substrate utilization; nevertheless, this frequently necessitates an increase in system volume [53].

Table 1. Comparison of Different MFC Configurations and Reported Performance.

MFC Configuration	Anode / Cathode material	Internal resistance [Ω or Ω·m^{-2}]	Max power density	Max current density	Ref.
Two-chamber MFC stack with Cu wire connection	Graphite granules	6.5 (s), 1 (p)	308 (s), 263 (p) W·m^{-3}	0.085 (s), 0.425 (p) A·m^{-3}	[44]
Bipolar two-chamber MFC stack with Ti plate wiring	Ti plates	1.2 mΩ·m^{-3} (s)	144 W·m^{-2} (s)	2.8 A·m^{-2}	[45]
Two-chamber MFC stack with Cu wire	Graphite	11.5 Ω·m^{-2} (s), 1 Ω·m^{-2} (p)	0.11 (s), 0.13 (p) W·m^{-2}	0.098 (s), 0.381 (p) A·m^{-2}	[46]
Two-chamber MFC stack	Carbon cloth	–	2.22 (s), 1.98 (p) W·m^{-2}	16.9 (s), 4.45 (p) A·m^{-2}	[47]
Single-chamber MFC stack	Carbon fiber veil	–	0.97 W·m^{-2} (p)	~7.1 A·m^{-2} (p)	[31]
Tubular single-chamber MFC stack with Ti wire	A: Graphite felt / C: Carbon fiber cloth	10 Ω to15 Ω (p)	67.5 (s), 175.7 (p) W·m^{-2}	0.128 (s), 0.675 (p) A·m^{-2}	[48]
Tubular single-chamber MFC stack with Ti wire	A: Graphite felt / C: Metal catalyst	800 (s), 15 (s–p)	4.1 (s), 6.0 (s–p) W·m^{-2}	2.1 (s), 13.8 (s–p) A·m^{-2}	[49]
Cascade single-chamber 3D-printed MFC stack	Carbon veil	–	–	–	[50]
Horizontally stackable single-chamber MFC with Ti wire	A: Carbon brush / C: Carbon mesh	2.3 × 10^{8} Ω·m^{-2}	0.116 W·m^{-2}	0.435 A·m^{-2}	[51]
Bipolar plate single-chamber MFC stack	Graphite felt / Graphite plate	634 Ω	0.023 W·m^{-2} (s)	0.037 A·m^{-2}	[52]

Notes: (s) = single measurement / standard conditions; (p) = pilot-scale or other reported conditions. – indicates data not reported.

In addition to this, the HRT is responsible for determining the value of shear stress, which has a direct impact on the development of biofilm on a surface. The optimal HRT is the time that is compatible with the amount of time required to create live bacteria. Therefore, while employing MFC systems, it is crucial that the value of HRT be measured precisely. Research examining the impacts of HRT on nutrient recovery in MFCs revealed that altering the HRT from 0.35 days to 0.69 days had a negligible influence on COD removal rates, which consistently surpassed 92%. The nutrient recovery rate exhibited slight fluctuations, ranging from 80% to 90% as HRT increased. Nonetheless, maximum power production decreased as HRT increased, with the minimum output measured at 510.3 mV at an HRT of 0.35 days. The results demonstrate that a laboratory-scale double-chamber microbial fuel cell, using municipal wastewater as a substrate, can efficiently accomplish substantial organic matter removal, nutrient recovery, and electricity production at an optimised HRT [26].

4.2 Substrate type and concentration

The choice of substrate directly influences microbial activity and electron yield. Simple substrates like acetate are commonly used in laboratory studies due to their rapid biodegradability and well-understood oxidation pathways. However, for practical wastewater treatment, complex substrates such as synthetic wastewater, pig slurry, and lignocellulosic waste offer more realistic and sustainable feedstocks. Takeuchi *et al.* [54] demonstrated that a MFC using Cellulomonas fimi with cellulose as fuel generated a maximum power output of 38.7 $mW \cdot m^{-2}$, confirming the feasibility of converting non-soluble organic matter into electricity. Another study using synthetic wastewater and pig slurry showed that changes in substrate composition significantly affected both power density and microbial community structure in a double-chamber MFC [54]. However, excessively high substrate concentrations can lead to overgrowth of non-exoelectrogenic microbes or accumulation of inhibitory metabolites, increasing internal resistance and reducing Coulombic efficiency [55]. Therefore, maintaining an optimal substrate loading rate is essential for stable and efficient operation.

4.3 Key factors influencing overall performance

The performance of MFCs, which employ microorganisms to transform organic matter into electricity, is greatly influenced by several characteristics that define their efficiency. Improved optimization of these parameters is essential for maximizing the efficiency and scalability of MFC. The performance of MFCs depends on microbial activity, electron transfer, and system parameters. The following three thematic groups organize the critical factors for clarity:

4.3.1 Electrode properties and electron transfer

The efficiency of MFCs critically depends on the interface between microbes and electrodes, where the biochemical energy from organic substrates is converted into electrical energy. Electrode materials influence microbial adhesion, biofilm development, and electron transfer rates, while the mechanisms by which microbes transfer electrons, either directly or via soluble mediators, determine the overall power output. Optimizing these properties is therefore essential for enhancing current generation, system stability, and scalability. This section discusses the key electrode characteristics and electron transfer pathways that govern MFC performance.

(a) Electrode material

One of the biggest challenges in scaling up MFCs is their relatively low power output. While MFCs are praised for their simple design, mild operating conditions, and potential for sustainable wastewater treatment, their practical application has been limited by inefficiencies in electron transfer between bacteria and electrodes [56]. A major factor influencing this process is the choice of electrode materials, especially the anode, which serves as both a habitat for electroactive microbes and a conductor for the electrons they produce [20].

Traditional anodes made from carbon cloth, graphite, or carbon felt are widely used due to their conductivity and chemical stability. However, many of these materials have smooth, hydrophobic surfaces that limit bacterial attachment and slow down electron transfer [21]. To overcome this, researchers have explored surface modifications that improve biocompatibility and reduce resistance at the microbe-electrode interface [57].

Recent advances focus on nanomaterial-based modifications, which can dramatically boost performance. For example, CDs, tiny, carbon-rich nanoparticles, are gaining attention for their high biocompatibility, chemical stability, and ability to facilitate electron movement. When used to coat anodes, oxygen-functionalized CDs increase surface hydrophilicity and conductivity, allowing more bacteria to attach and transfer electrons efficiently. In one study, MFCs with CD-modified anodes achieved a maximum power density of 661.1 ± 42.6 $mW \cdot m^{-2}$ and an open-circuit voltage of 534.5 ± 6.4 mV, significantly outperforming unmodified controls [57].Other promising composites include graphene-polyaniline coatings, which not only enhance conductivity but also shorten the microbial acclimation period. One such modified anode (reduced graphene oxide/polyaniline/carbon cloth) increased power output by nearly 1.9 times compared to plain carbon cloth, while also extending bacterial adaptation time by about 2.4 times [38].Similarly, hybrid materials like graphene oxide-zeolite-clay composites (GZMA) applied to carbon felt have shown even greater gains, delivering over 3.5 times higher power density than untreated carbon felt anodes [34]. These porous, biocompatible structures provide more surface area for biofilm growth and better mass transfer of nutrients and protons.

Innovations in fabrication techniques are also making a difference. For instance, 3D-printed porous carbon electrodes offer a structured, customizable architecture that supports dense microbial colonization. With optimized pore sizes (e.g., 300 μm), these 3D electrodes have demonstrated output voltages as high as 1256 mV and power densities reaching 233.5 $mW \cdot m^{-2}$, outperforming conventional 2D designs [34]. Building on these advances, recent work has shown that biochar derived from neem wood, when modified with $NiFe_2O_4$ nanorods and poly(3,4-ethylenedioxythiophene) (PEDOT), can function as a binder-free, free-standing anode, enabling compact biofilm formation, continuous electron conduction pathways, and superior catalytic activity. This nanocomposite anode achieved a peak power density of 1200 $mW \cdot m^{-2}$, underscoring the promise of waste-derived, structurally stable, and

cost-efficient electrode materials to address the durability and scalability challenges of MFCs [58]. Figure 5 illustrates the progression of anode materials in microbial fuel cells, from conventional carbon-based substrates (e.g., carbon cloth and graphite) to advanced nanostructured composites and engineered 3D architectures. The trend highlights a shift toward materials that not only enhance conductivity and microbial adhesion but also improve structural stability and scalability. The PEDOT/$NiFe_2O_4$/biochar composite represents one such innovation at the frontier of this progression.

Overall, the development of advanced electrode materials, especially those incorporating nanomaterials or engineered 3D structures, is key to unlocking the full potential of MFCs. By improving electron transfer kinetics, microbial loading, and system stability, these innovations pave the way for more efficient, scalable, and sustainable bioenergy systems.

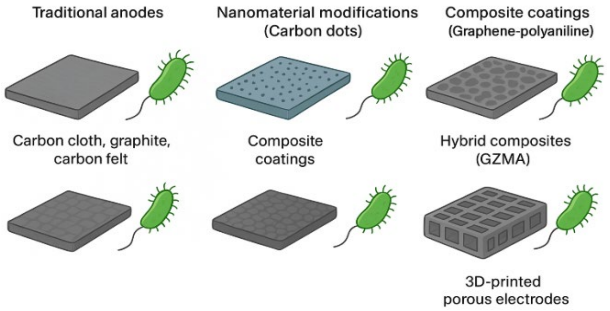

Figure 5. Progression of anode materials for microbial fuel cells (Adapted from [12]).

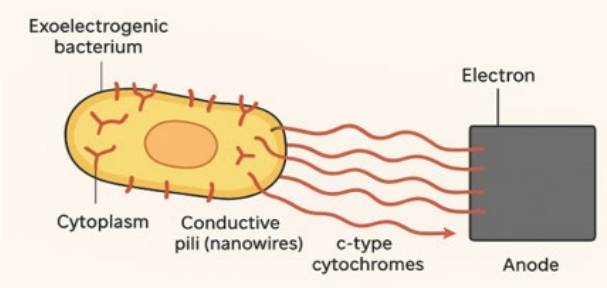

Figure 6. Schematic representation of DET in exoelectrogenic bacteria (Adapted from [64]).

(b) Electron transfer mechanisms

In microbial fuel cells, bacteria generate electricity by breaking down organic matter, but the electrons they produce are trapped inside their cells. Since the electrode is a solid surface outside the cell, one of the biggest challenges is getting those electrons across the cell membrane to the electrode.

Bacteria have evolved two main ways to do this. The first is DET, where microbes use built-in biological wires, like conductive pili (nanowires) or special proteins (such as c-type cytochromes) in their outer membrane, to pass electrons straight to the electrode [59]. This method doesn't need any extra chemicals and is common in well-studied exoelectrogens like *Geobacter* and *Shewanella*.

The second way is MET, where bacteria release small redox-active molecules, such as flavins, pyocyanin, or quinones, that act as shuttles. These molecules pick up electrons from the cell and carry them to the electrode [60]. Some of these mediators are naturally produced by the bacteria themselves, and their effectiveness depends on their ability to move freely, interact with the electrode, and transfer electrons with minimal energy loss.

For either method to work, the electron carrier, whether it's a protein or a soluble molecule, must be electrochemically active and have a favorable redox potential, ideally close to that of the metabolic reactions inside the cell. Over time, researchers have found that many bacteria use a mix of both strategies, depending on environmental conditions and electrode materials.

Understanding these electron transfer pathways is key to improving MFC performance. By choosing the right microbes, modifying electrode surfaces, or even enhancing natural shuttle production, scientists can boost power output and make MFCs more efficient for real-world applications like wastewater treatment and biosensing.

(c) Direct electron transfer (DET)

Some bacteria, called exoelectrogens, can transfer electrons directly to electrodes without chemical shuttles, process known as DET. They use biological structures like conductive pili (nanowires) and outer-membrane c-type cytochromes to move electrons from inside the cell to the anode [9]. *Geobacter sulfurreducens* is the best-studied example, with around 110 genes coding for c-type cytochromes, highlighting their role in electron transport [23].

Even in other species like *Desulfovibrio desulfuricans*, DET is effective, achieving a current density of 233 $mA \cdot m^{-2}$ on treated graphite felt, thanks to surface cytochromes [61]. In thick biofilms, only bottom-layer cells touch the electrode, so upper layers use conductive pili networks to pass electrons down. These nanowires (especially type IV pili) show metal-like conductivity, similar to carbon nanotubes [23].

This direct transfer extends across species through direct interspecies electron transfer (DIET). For instance, *Geobacter sulfurreducens* and *G. metallireducens* exchange electrons via pili and cytochrome OmcS during ethanol metabolism, forming electrically conductive aggregates [62]. Similar syntrophic interactions occur in mixed communities, like between *Clostridium* (fermenter) and sulfate-reducing bacteria (*Desulfovibrio*, *Aeromonas*), where electrons are passed directly to the electrode, boosting current generation [63].

These natural electrical connections make DET a powerful mechanism for improving MFC efficiency. The Figure 6 illustrates how exoelectrogenic bacteria transport electrons from the cytoplasm to the anode through specialized conductive structures. Electrons generated during microbial metabolism are transferred across the cell envelope via c-type cytochromes and extended to the extracellular environment through conductive pili (nanowires). These nanowires directly connect the bacterial cell to the anode surface, enabling efficient electron flow without the requirement of soluble mediators. This mechanism underpins the fundamental bio electrochemical interactions in MFCs, facilitating direct energy recovery from microbial metabolism.

(d) Mediated electron transfer

Some bacteria use soluble molecules to shuttle electrons from inside the cell to the electrode, a process called MET. These molecules,

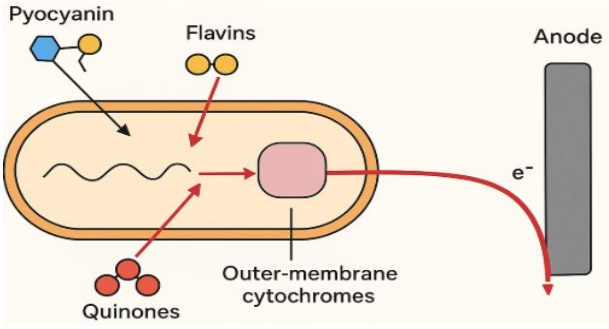

Figure 7. Schematic representation of MET in electroactive bacteria (Adapted from [66]).

such as pyocyanin, flavins, and quinones, act as mobile carriers that transport electrons across the cell membrane and deliver them to the anode. In some cases, these shuttles are naturally produced by the bacteria themselves. For example, both *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* release riboflavin, which binds to outer-membrane cytochromes and plays a key role in extracellular electron transfer [65]. Figure 7 provides a schematic representation of this process, illustrating how intracellular metabolites such as pyocyanin, flavins, and quinones transfer electrons to outer-membrane cytochromes, which then deliver the electrons to the anode. This highlights the dynamic role of redox-active mediators in bridging the intracellular electron flow with extracellular electron acceptors.

4.3.2 Substrate and operational parameters

The availability of fuel and the way an MFC is operated critically determine its efficiency and stability. Substrate concentration, composition, and loading rate influence microbial activity, biofilm growth, and the overall electricity generation. Similarly, operational settings, such as external resistance and oxygen levels, control electron flow and power output. Optimizing these parameters ensures that microbes can effectively convert organic matter into electricity while minimizing losses due to competing reactions or suboptimal conditions. This section discusses the key substrate and operational factors that govern MFC performance.

(a) Substrate loading rate

The substrate loading rate greatly affects MFC performance. At optimal levels, it boosts microbial activity and ionic strength, improving power output and organic matter breakdown [67]. However, excessive substrate increases internal resistance and promotes competing microbes like methanogens, which consume substrates and protons but do not produce electricity, reducing current despite higher COD removal [55]. Pre-treating complex substrates (e.g., potato pulp) enhances availability and power generation, showing that balanced loading and proper feed preparation are crucial for efficient MFC operation [68].

(b) External resistance:

An MFC can adjust its current flow and power output through the use of an external load or resistance that is coupled to the MFC [91]. It is of the utmost importance to ascertain the optimal resistance, which is reliant upon the particular configuration and operational conditions of the MFC. By matching the exterior resistance of the MFC to the internal resistance of the cell, it is possible to optimize power generation, which will increase the cell's overall efficiency [69]. Research on the impacts of external resistance indicated that an increase in external resistance resulted in a loss in power. Hysteresis was detected as a result of changes in microbial diversity inside the anode. In the first phase of increasing external resistance, the peak power output, 1.69×10^{-3} mW, was achieved with a 2,700 Ω load. Nonetheless, by reducing the external resistance, the peak power of 1.27×10^{-3} mW was achieved with a 2,200 Ω load. The effluent COD reduction occurred with an increase in external resistance, indicating that wastewater treatment improved at higher external resistance levels [70]. External resistance must be matched to the internal resistance of the MFC to achieve maximum power transfer. Jadhav and Ghangrekar [55] showed that varying external loads significantly impact current generation and substrate utilization. Too low or too high a resistance results in suboptimal electron flow, reducing overall efficiency. Dynamic adjustment of external circuits based on real-time performance can help maintain peak power output under fluctuating conditions.

(c) Oxygen availability

To enable electron transfer and microbial respiration through the anode chamber, MFCs usually operate in anaerobic conditions. Conversely, the oxygen level in the cathode chamber is crucial for the operation of cathodic processes. Precise regulation of oxygen levels is crucial to avoid its infiltration into the anode chamber, which can negatively impact the operation of the MFC [27]. The concentration of dissolved oxygen is influenced by the microalgae present in the cathode chamber and the mechanical aeration applied. CO_2 additionally contributes indirectly to this process. Insufficient dissolved oxygen concentration limits the availability of electron acceptors at the cathode, thereby hindering electricity production. In a cathode lacking supplementary aeration, the rate of dissolved oxygen consumption typically exceeds the rate of dissolved oxygen dissolution from the air. Conversely, elevated dissolved oxygen concentrations facilitate the diffusion of oxygen from the cathode chamber to the anode chamber, driven by osmotic pressure and oxygen gradients. This phenomenon negatively affects the growth of anode microorganisms and disrupts electron release at the anode, ultimately leading to a decrease in power density [28]. In one experiment, it was demonstrated that raising the quantity of dissolved oxygen in the cathode chamber from 7.8 $mg \cdot L^{-1}$ to 9.5 $mg \cdot L^{-1}$ resulted in a 53.4% drop in the power density [71].

4.3.3 Environmental conditions

Environmental factors, such as pH and temperature, strongly influence microbial physiology, biofilm stability, and MFC performance. Matsena *et al.* [40] reported that power output, voltage stability, and bacterial growth peaked at pH 9 in a dual-chamber MFC, with methanogenesis suppressed between pH 7 and pH 9, favouring electroactive bacteria over competing anaerobes. Similarly, Mahmood *et al.* [72] found optimal performance in a palm oil mill effluent-fed MFC at anodic pH 7 to pH 9, indicating that slightly alkaline conditions enhance proton transfer and biofilm stability. Temperature also affects

metabolic rates, with most MFCs performing efficiently between 25°C and 35°C, aligning with mesophilic microbial activity [73].

(a) Wastewater characteristics

Organic feedstock or wastewater used in MFCs can exhibit significant variations in material content and type. A comprehensive knowledge of these variations is crucial for the efficient construction and management of an MFC. The distinctive chemical properties of the feedstock significantly influence its interaction with the microbial populations and the overall energy conversion process. By analysing these characteristics, engineers may tailor the MFC system to better effectively manage the feedstock, ensuring optimal efficiency and performance. This information enhances the versatility of the MFC setup and the system's ability to effectively handle various types of organic matter [74].

(b) pH

The generation of protons is essential for electrochemical processing in MFCs, and it is significantly influenced by pH levels in both the cathode and anode chambers. The Nernst equation elucidates an inverse relationship between pH and power output in MFC, indicating that an increase in pH leads to a drop in power production due to the reduced rate of oxygen reduction at elevated pH levels. The pH also affects electrically active biofilms on the anode by modulating the metabolic pathways of these microorganisms [75]. It has been shown that microbial enzymes work best at a neutral pH. When the pH is too high or too low, it causes biofilms to not work as well and, as a result, the power production drops. The primary causes of this are the alterations in proton shuttling, ionic concentration, membrane potential, and cytosolic pH. Anodic marine consortia in double chamber MFC were the subject of an investigation on the impact of pH changes. According to their research, the efficiency of MFCs degrades when the anodic pH moves away from neutrality. The growth of a thicker biofilm with maximum power density was considerably enhanced by anodic pH values between 8 and 10, which are considered alkaline circumstances. In contrast, pH levels below 5.5 and above 10 showed a significant decline in MFC performance [76]. Another study used anaerobic digester waste as an inoculum to determine pH's effect on double chamber MFC's anaerobic microbial consortia Power density, output voltage, and bacterial growth were highest at pH 9. The research also found that suppressing methanogenesis by altering substrate pH between pH 7 and pH 9 stabilises maximal power output [40]. Other studies examined how anodic pH affects electricity generation in double chamber MFC utilising palm-oil empty fruit bunch. The research found highest power between pH 7 and pH 9 [72].

(c) Temperature

Temperature is a significant component in microbiological activities. It influences the kinetics and thermodynamics of metabolic processes, hence affecting the overall performance of MFCs. It also has an impact on the preservation and development of microbial communities, since each community has its own ideal temperature. Temperature influences the establishment of stable electrically active biofilms. Studies have revealed that an increase in temperature generates an increase in power densities, most likely due to the rise in microbial metabolism up to an optimal limit of each kind of cell [77]. An investigation into the structure of microbial communities was carried out at lower temperatures. The findings of this investigation revealed that mixed culture dominating strains of *Pseudomonas* and *Geobacter* were detected. Temperature was shown to have an influence on the production of biofilms as well as the performance of electrocatalytic systems. The research examined the impact of temperatures ranging from 5°C to 45°C, with the reported highest power density of 881 $A \cdot cm^{-2}$ occurring at 35°C [78]. The impact of temperature, salinity, and pH on MFC performance was the subject of an additional investigation. At a pH of 7, the columbic efficiency (CE) was at its peak. As the temperature rose from 24°C to 35°C, both CE and power density improved [79].

4.4 Challenges in scaling and cascading MFCs

Scaling MFCs from lab-scale reactors to practical installations introduces significant engineering challenges. Ieropoulos *et al.* [31] observed a decline in power density when moving from single units to stacked configurations, primarily due to increased internal resistance and uneven distribution of substrate and protons. In cascaded systems, sequential connection often leads to diminishing returns, as downstream units receive depleted influent with limited fuel value [33]. To address this, researchers have explored strategies such as periodic reversal of electrode roles, modular designs with independent feeding, and integration of energy storage elements to stabilize output [31,33]. These approaches aim to improve longevity, manage loading imbalances, and enhance overall system resilience.

In conclusion, while the fundamental principles of MFC operation are well established, achieving consistent and scalable performance depends on precise control and adaptive management of operational parameters. These optimization strategies are crucial for advancing MFC technology beyond bench-scale demonstrations toward real-world implementation in wastewater treatment facilities.

5. Optimization strategies and practical implementations of MFCs

MFCs hold transformative potential, not just as devices that generate electricity from organic waste, but as systems capable of turning waste-water treatment into an energy-producing process. While earlier sections have detailed the technical factors affecting MFC performance, this section emphasizes how these elements converge in real-world implementations and outlines strategies for sustainable, scalable outcomes.

5.1 Pilot-scale studies in wastewater treatment

Pilot studies are essential to bridge laboratory findings with field-scale realities. For instance, Ieropoulos *et al.* [31] deployed a stack of ceramic MFCs at a domestic wastewater site, sustaining a power output of 200 $mW \cdot m^{-2}$ to 300 $mW \cdot m^{-2}$ over six months while achieving over 80% COD removal. Similarly, a South Korean pilot employing tubular single-chamber MFCs achieved 75% to 85% organic removal

and generated sufficient power to operate sensors, demonstrating feasibility in decentralized wastewater management [75].

Successful deployment often depends on integration with other treatment systems. MFCs coupled with constructed wetlands, anaerobic digesters, or microalgae bioreactors can boost treatment efficiency while enabling multi-stage energy recovery [5,80]. It is found that coupling with algae not only supplies oxygen to the cathode but also removes nutrients and CO_2, thereby enhancing both water quality and system performance [81].

5.2 Long-term stability and durability

Long-term operation remains a critical challenge due to electrode fouling, microbial shifts, and material degradation. However, recent progress demonstrates improving stability. It was found only a 12% drop in power density after 180 days using graphene-modified electrodes, while cascaded systems maintained over 70% of their initial output after a year through recirculation and electrode reversal [82].

Durability is further supported by smart engineering strategies such as anti-fouling cathodes, modular unit design for easier maintenance, and adaptive control systems that respond to variable influent conditions. Optimization of operating parameters, including external resistance, electrode spacing, and substrate concentration, also plays a pivotal role in sustaining performance. Systematic experimentation and monitoring under real wastewater conditions are essential for identifying configurations that maximize both treatment efficiency and energy recovery [83].

5.3 Microbial re-wiring and synthetic biology approaches

The most exciting frontier lies in engineering the biological engine of MFCs. Advances in synthetic biology now allow the "rewiring" of microbes for enhanced electroactivity. Engineered *Shewanella oneidensis* with overexpressed cytochromes (MtrC, OmcA) produced up to 40% more current [84], while CRISPR-edited *Geobacter sulfurreducens* displayed improved pili conductivity and biofilm stability [85]. Moreover, quorum-sensing engineered microbial consortia have been designed to self-regulate biofilm growth and electron shuttle production, enhancing electron transfer and system stability [86].

A critical aspect of this progress lies in understanding and improving biofilm formation mechanisms in electroactive microorganisms, since EPS directly influence both biofilm development and electron transfer. Strategies that combine genetic engineering of biofilm-related genes with optimized culture conditions and operation parameters can significantly enhance electrocatalytic rates, leading to higher current densities and improved COD removal in MFC-based wastewater treatment [87].

Beyond targeted strain modification, omics-based tools, including metagenomics, transcriptomics, and proteomics, are providing insights into functional genes and pathways in electroactive biofilms [88]. These approaches enable selective enrichment or genetic tailoring of exoelectrogenic communities for higher efficiency [89]. A recent pilot integrating waste-derived carbon anodes [15] with an engineered Bacillus strain [90] demonstrated 88% COD removal and a stable 968 $mW \cdot m^{-2}$ over 100 days, highlighting the potential of synergizing material innovations with microbial engineering.

6. Conclusions and future directions

MFCs represent a promising dual-function technology, simultaneously addressing the urgent need for sustainable wastewater treatment and renewable energy generation. Recent advances have moved MFCs beyond laboratory-scale studies toward practical deployment, with pilot-scale demonstrations showing consistent COD removal efficiencies above 75% and measurable power generation sufficient for small-scale applications. Long-term operational strategies, including electrode material innovations, anti-fouling designs, and adaptive recirculation systems, have proven effective in maintaining performance stability over extended periods. Equally transformative are developments in microbial engineering, where CRISPR-based genome editing, synthetic biology, and quorum-sensing regulation are enabling the design of highly efficient, resilient electroactive consortia.

Looking ahead, future research should focus on four critical priorities: (i) integration of MFCs with renewable energy systems such as solar and algal bioreactors to maximize synergistic benefits; (ii) scaling up through diverse pilot implementations in domestic and industrial wastewater treatment to validate performance under real conditions; (iii) long-term durability testing to overcome challenges of electrode degradation, biofilm evolution, and system fouling; and (iv) microbial community engineering guided by multi-omics tools to optimize electron transfer pathways and stability. Collectively, these efforts will accelerate the transition of MFCs from experimental devices to commercially viable systems, capable of contributing significantly to the circular bioeconomy and the global sustainability agenda.

Acknowledgement

This study was supported by C.V. Raman Global University (CGU) and Bhubaneswar Institute of Technology (BIT), Bhubaneswar, for carrying out the research work

Data Availability

All data generated or analyzed during this study are included in this manuscript.

Author contributions

All authors performed the following tasks: Conceptualization, conduct experiments and collected data, analyzed sample and Data, Supervision, Writing draft manuscript and editing, Review and editing, Funding acquisition, Project administration, and Funding resources.

Funding

The article received no funding from any sources.

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

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