Revolutionizing Bioenergy: The Microalgae-Microbial Fuel Cell Frontier

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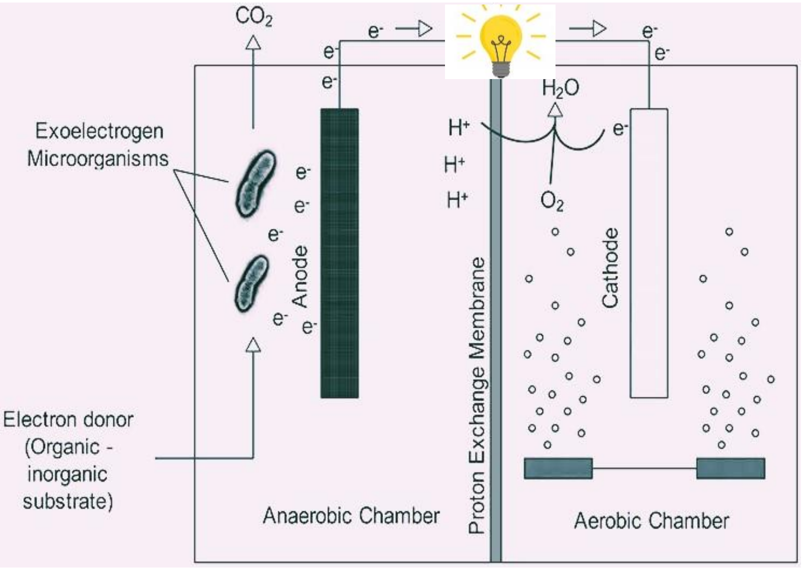
Graphical abstract



The fundamental design of MFCs comprises four key elements: the anode (electron-producing component), cathode (electron-receiving component), proton exchange membrane (PEM), and external electrical circuit. In this setup, the anode and cathode represent the primary chambers of the MFCs, which are divided by the PEM. The external electrical circuit connects the cathode and anode to facilitate the transfer of electrons.

Abstract

Microalgae-Microbial Fuel Cell (M-MFC) technology stands out as a highly promising innovation at the nexus of renewable energy and environmental conservation. This cutting-edge approach utilizes microorganisms, including bacteria and algae, to convert the chemical energy present in wastewater into electricity, addressing both wastewater treatment and electricity generation. M-MFC relies on microorganisms to convert chemical energy, utilizing components

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readily available in wastewater, making it a sustainable energy source with considerable potential. Beyond its eco-friendly electricity generation, M-MFC offers cost-effective electricity production, alleviating expenses associated with wastewater treatment and overall electricity consumption. In this comprehensive review, we explore the intricate bio-electrochemical mechanism of M-MFC, shedding light on recent developments and applications. The discussion encompasses crucial factors influencing M-MFC performance, and its essential elements and functions. This review examines the MFC system, particular M-MFCs, with focus attention to the functions of key elements such as the anode, cathode, and microorganisms. Additionally, it delves into the material design and configuration of M-MFCs. Furthermore, the review addresses current issues and limitations related to M-MFC systems.

Abbreviations: *MFC, Microbial fuel cell; M-MFC, Microalgae assisted microbial fluid cell; COD, Chemical oxygen demand;* CO_2*, Carbon dioxide; PEM, Proton exchange membrane; Cu, Copper; Pt, Platinum; LCA, Life cycle assessment; GHG, Greenhouse gas.*

Keywords*: Microalgae-Microbial Fuel Cell (M-MFC) Biotechnology, Bioelectricity; Biomass; Lipid; Biodiesel, Wastewater treatment,* CO_2 *sequestration.*

1.0 Introduction

Human civilization currently faces a critical dual challenge: diminishing energy resources and escalating environmental degradation. This situation arises from the rapid depletion of fossil fuels and growing concerns about global climate change, both of which are closely linked to the extensive use of conventional fuels. Industrialization and population growth have significantly escalated fossil fuel consumption, leading to severe environmental pollution, threats to biodiversity, increased CO_2 *emissions, and exacerbation of global warming. These developments have resulted in a variety of adverse effects, including floods, wildfires, hurricanes, and* *disruptions to ecosystems. Compounding the energy crisis is the rising global energy demand alongside diminishing fossil fuel reserves. Thus, there is an urgent need for sustainable and economically viable renewable energy sources. Researchers are actively seeking solutions to these challenges, as highlighted in studies by (Temper et al., 2020) and (Zabed et al. 2020), aiming to mitigate environmental impacts while meeting growing energy demands.*

Significantly, the concentration of carbon dioxide in the atmosphere has increased notably due to fossil fuel combustion, rising from 388.5 ppm in 2009 to 409.95 ppm in 2019, indicating a concerning surge within just ten years (Elshobary et al., 2021). Despite this, global society remains heavily reliant on fossil fuels for energy provision and electricity generation (Holechek et al., 2022). Consequently, exploring alternative clean energy sources is crucial to ensuring energy security and reducing carbon dioxide (CO_2) emissions. Various clean energy technologies, such as solar, hydro, wind, biomass, tidal, geothermal, and wave power, have been rapidly advancing. However, a key challenge associated with these renewable resources is the efficient storage and transportation of the energy they generate, despite their environmental advantages over fossil fuels (Oyekale et al., 2020).

Photosynthesis involves converting solar energy into chemical energy, with algae demonstrating the highest conversion efficiencies among photosynthetic organisms, reaching up to 9% (Xie et al., 2022). Microalgae offer numerous advantages over conventional photosynthetic plants, including rapid growth, lack of resource competition, and adaptability to non-arable land. While typical photosynthetic plants achieve energy conversion efficiencies ranging from 4.6% to 6%, microalgae have shown efficiencies of up to 9% (Zabed et al., 2020). Moreover, microalgae excel in capturing CO_2 and removing nutrients from wastewater, and they hold significant promise for bioengineering applications (Arun et al., 2020). Photosynthesis begins with the absorption of light photons and ends with converting carbon into various compounds, such as *carbohydrates, lipids, and proteins, along with the release of oxygen. Therefore, utilizing living algae or algal biomass for energy production presents a viable and promising strategy.*

Recent progress has brought attention to electrochemical energy storage devices like micro-supercapacitors, supercapacitors, two-dimensional materials, and bioelectricity devices for their high-power density, quick charge/discharge rates, and extended lifespans (Jiang et al., 2020) (Ren et al., 2020). Bioelectricity devices, in particular, offer a potentially sustainable solution by generating electricity from organic matter through various biological processes, thus simultaneously addressing wastewater treatment and CO_2 *sequestration (Das et al., 2023). Recent research has explored leveraging photosynthesis to produce bioelectricity, hydrogen, and other biofuels as alternatives to fossil fuels (Elshobary et al., 2021).*

Microalgae possess qualities that make them well-suited for use in Microbial Fuel Cells (MFCs) and as potential biomass sources. The combination of microalgae with MFCs has attracted attention because microalgae can serve as both oxygen generators and electron acceptors in the cathode chamber (Nawaz et al., 2022).

The integration of microalgae with Microbial Fuel Cells (MFCs) shows great potential in tackling energy shortages and maintaining water quality (Jaiswal et al., 2020). Microalgae-assisted MFCs are considered effective solutions for generating electricity, removing pollutants, and simultaneously treating wastewater, as demonstrated in Table 1. This table illustrates the capabilities of microalgae-assisted MFCs in addressing challenges related to energy and water quality, providing solutions for power generation, pollutant removal, and wastewater treatment. Scientists have been intrigued by the potential of microalgae to aid MFCs due to their enhanced ability to generate electricity, treat wastewater efficiently, and produce biofuels from microalgal biomass (see Table 1) (Monika et al., 2022). Table 1 highlights the potential of microalgae in assisting Microbial Fuel Cells (MFCs) due to their enhanced capacity for electricity generation, efficient wastewater treatment, and biofuel production. It provides key parameters such as *internal diameter (ID), external diameter (ED), length (L), width (W), height (H), chemical oxygen demand (COD), total nitrogen (TN), total phosphorous (TP), and working volume (WV) for understanding the characteristics and performance of MFCs utilizing microalgae. Researchers are intrigued by the ability of microalgae to contribute to electricity generation and biofuel production, making them a valuable resource in sustainable energy applications.*

The findings presented in Table 1 indicate significant promise for microalgal-assisted MFCs in both wastewater treatment and electricity production. The noteworthy removal of Chemical Oxygen Demand (COD), substantial reduction in nutrients, and high-power densities are encouraging outcomes. Nevertheless, it's important to acknowledge variations in results and assess the practical scalability and cost-effectiveness for real-world applications.

Latest developments in microbial fuel cells (MFCs) have greatly expanded the potential for generating bioelectricity through microbial metabolism. Electroactive microorganisms like bacteria and yeast participate in biocatalytic reactions within MFCs to generate pure bioelectricity (Garbini et al., 2023). Although bacteria are commonly used in MFCs, many are inefficient at producing electrical current and may require significant feeding and efficient electron acceptors, which can be expensive (Kumar et al., 2023). In this regard, highly bioactive microalgae that produce oxygen present a promising alternative to bacteria-assisted MFCs. On the cathodic side of MFCs, oxygen acts as a continuous electron acceptor, while photosynthesis supplies energy for current generation on the anode side.

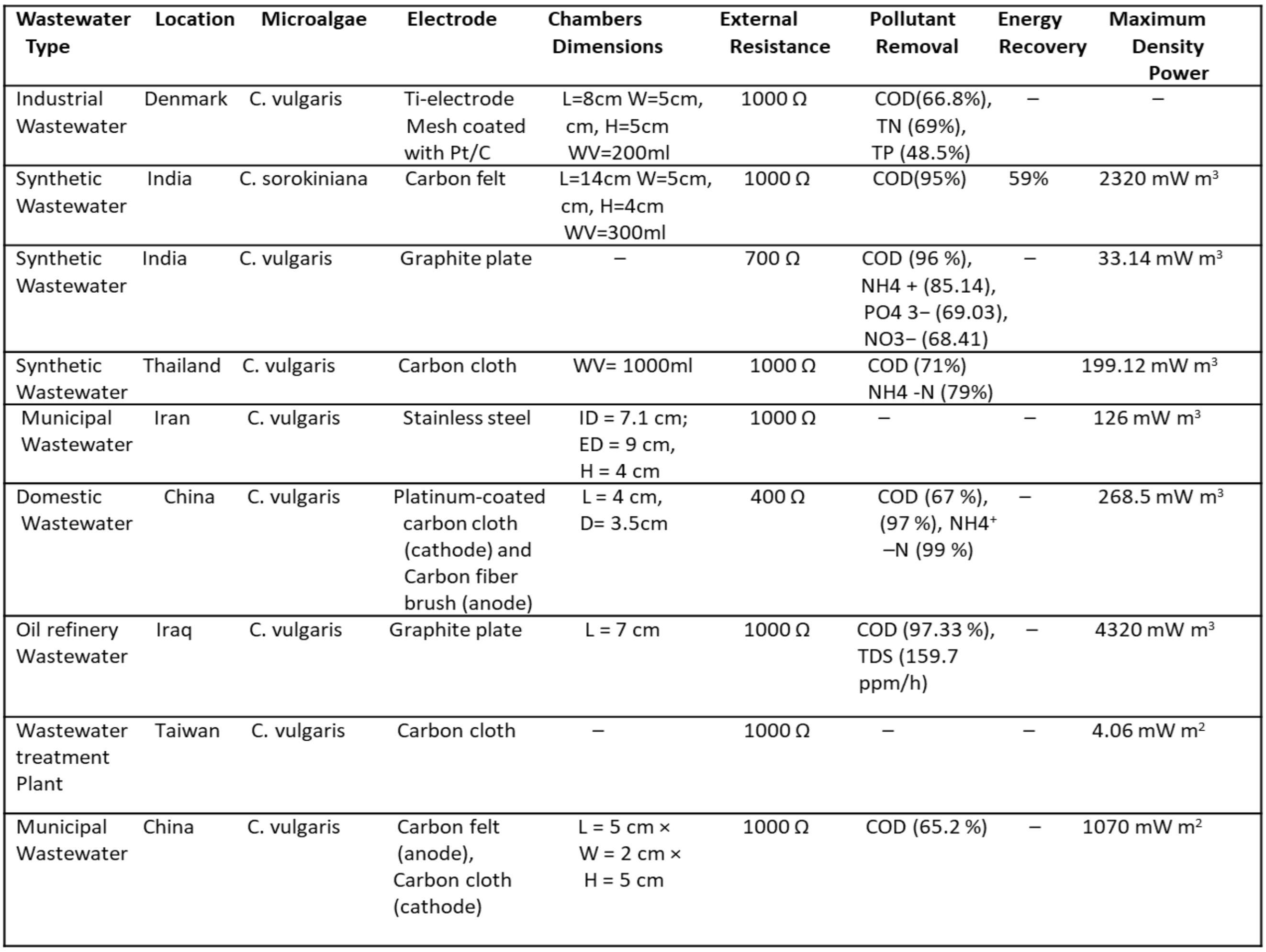
Table 1 highlights the significant potential of microalgae in boosting electricity generation in MFCs. Researchers have observed improved capacity for electricity production when microalgae are integrated into MFC systems, showcasing the promising role of these organisms in enhancing bioenergy generation. The table underscores the efficiency of microalgae in wastewater treatment within MFCs. Microalgae demonstrate the ability to effectively treat wastewater by utilizing nutrients *and organic matter present in the water, offering a sustainable solution for environmental remediation while simultaneously generating biofuels. The findings presented in Table 1 emphasize the importance of further exploring the potential of microalgae in MFC technology. Future research efforts should focus on optimizing the integration of microalgae in MFC systems to maximize electricity generation, improve wastewater treatment efficiency, and advance biofuel production capabilities. Overall impact, the insights from Table 1 underscore the transformative impact of microalgae on revolutionizing bioenergy generation through MFCs. By harnessing the unique capabilities of microalgae, researchers can drive innovation in sustainable energy production and environmental remediation, paving the way for a greener and more efficient bioenergy landscape.*

Electrochemical microalgae have shown more favorable outcomes in terms of electricity generation and energy consumption compared to bacteria (Sharma et al., 2022).

Recent advances in MFCs have brought forth innovative opportunities for integrating microalgae, enabling the use of algal biomass for electricity generation (Kannan et al., 2021). While most laboratory-scale photobioreactors typically rely on artificial lighting, such as fluorescent lamps, to meet the light requirements of microalgae, this practice escalates operational costs and perpetuates reliance on fossil fuels. However, this constraint may be overcome by tapping into the bioenergy potential of microalgae in alternative applications (Jaiswal et al., 2020).

In recent years, numerous studies have investigated the use of microalgal-assisted MFCs for both wastewater treatment and simultaneous electricity generation. Drawing on data collected over the past five years, this research offers a comprehensive overview of the current status of microalgal-assisted MFCs. Previous reviews have predominantly focused on system configurations, including single-chambered and double-chambered setups, as well as microbial electrolysis cells (Monika et al., 2022).

***Table 1**: Researchers have been captivated by the potential of microalgae to assist MFCs because of their improved capacity for electricity generation, efficient wastewater treatment, and production of biofuels from microalgal biomass (Monika et al., 2022).*



Wastewater Type	Location	Microalgae	Electrode	Chambers Dimensions	External Resistance	Pollutant Removal	Energy Recovery	Maximum Density Power
Industrial Wastewater	Denmark	C. vulgaris	Ti-electrode Mesh coated with Pt/C	L=8cm W=5cm, cm, H=5cm WV=200ml	1000 Ω	COD(66.8%), TN (69%), TP (48.5%)	–	–
Synthetic Wastewater	India	C. sorokiniana	Carbon felt	L=14cm W=5cm, cm, H=4cm WV=300ml	1000 Ω	COD(95%)	59%	2320 mW m^3
Synthetic Wastewater	India	C. vulgaris	Graphite plate	–	700 Ω	COD (96 %), NH4 + (85.14), PO4 3– (69.03), NO3– (68.41)	–	33.14 mW m^3
Synthetic Wastewater	Thailand	C. vulgaris	Carbon cloth	WV= 1000ml	1000 Ω	COD (71%) NH4 -N (79%)		199.12 mW m^3
Municipal Wastewater	Iran	C. vulgaris	Stainless steel	ID = 7.1 cm; ED = 9 cm, H = 4 cm	1000 Ω	–	–	126 mW m^3
Domestic Wastewater	China	C. vulgaris	Platinum-coated carbon cloth (cathode) and Carbon fiber brush (anode)	L = 4 cm, D= 3.5cm	400 Ω	COD (67 %), (97 %), $NH4^+$ –N (99 %)	–	268.5 mW m^3
Oil refinery Wastewater	Iraq	C. vulgaris	Graphite plate	L = 7 cm	1000 Ω	COD (97.33 %), TDS (159.7 ppm/h)	–	4320 mW m^3
Wastewater treatment Plant	Taiwan	C. vulgaris	Carbon cloth	–	1000 Ω	–	–	4.06 mW m^2
Municipal Wastewater	China	C. vulgaris	Carbon felt (anode), Carbon cloth (cathode)	L = 5 cm × W = 2 cm × H = 5 cm	1000 Ω	COD (65.2 %)	–	1070 mW m^2

Note: ID = internal diameter, ED = external diameter, L = length, W = width, H = height, COD = chemical oxygen demand, TN = total nitrogen, TP = total phosphorous, WV = working volume, TDS= total dissolved solid.

This review examines the MFC system, focusing on both traditional MFCs and M-MFCs, with particular attentions to the functions of key elements such as the anode, cathode, and microorganisms. Additionally, it delves into the material design and configuration of M-MFCs. Furthermore, the review addresses current issues and limitations related to M-MFC systems.

1.1 Conventional/Traditional Microbial Fuel Cell (MFC) System

Conventional/Traditional Microbial Fuel Cells (MFCs) offer technological solutions that simultaneously generate electricity and treat wastewater. In these systems, microorganisms convert the energy from organic matter in wastewater into electricity. The use of MFCs presents several distinct advantages, including (a) reduced sludge formation compared to aerobic activated sludge treatment and anaerobic digester technology, (b) elimination of energy input for aeration in open-air cathode or mono-chambered configurations, (c) adaptability and resilience to varying operating conditions, (d) direct conversion of substrates into electricity, (e) minimal gas treatment requirements due to CO_2*-rich off-gas, and (f) particular suitability for regions lacking electricity infrastructure (Ardakani et al., 2020).*

An MFC operates similarly to a battery, comprising substrate, anode, and cathode electrodes, and typically employs substances such as acetate, glucose, or wastewater. Within the MFC, a proton exchange membrane (PEM) separates the two compartments (as seen in Figure 1). The cathode is equipped with catalysts to enhance its efficiency, while the anode contains either wastewater or a medium enriched with organic substances. Bacterial colonies are cultivated under anaerobic conditions, using acetate, glucose, or wastewater as their primary substrates, and adhering to the negatively charged anode electrode. The bacteria metabolize the organic matter, releasing energy in the form of protons and electrons, which are transferred to the anode electrode via the PEM. The electrons travel through the external circuit to the cathode electrode (Saravanan et al., 2022). Within the cathode chamber, the electron combines with oxygen and a proton, leading to the formation of water.

Previous research building on the importance of the external electrical circuit in facilitating electron transfer, further research can explore novel circuit designs to minimize internal resistances and maximize power output in MFC systems (Nawas et al., 2020).

A recent study by Shabani and his colleagues in 2022. They conducted a critical review on proton exchange membrane (PEM) applied in the current Microbial fuel Cells (MFC) technology. They have emphasized the critical role of PEMs in separating the anode and cathode chambers to maintain proton transport. Integrating insights from these studies into the design of selective and efficient PEM materials can enhance proton conductivity and overall MFC efficiency. Microbial Fuel Cells (MFCs) offer dual advantages by generating sustainable electricity and providing an eco-friendly solution for wastewater treatment. Through the utilization of microorganisms, MFCs convert organic matter into clean, renewable electricity while concurrently breaking down pollutants in wastewater. This not only mitigates the environmental impact of wastewater discharge but also reduces operating and maintenance expenses, rendering MFCs a cost-effective and scalable technology for various applications (Anh et al., 2022; Shahjalal et al., 2021).

Notwithstanding their benefits, MFCs come with limitations. They often produce low power densities, which restricts their suitability for high-demand electricity applications. As noted by Hassan and colleagues in 2023, despite numerous advancements, the widespread adoption of Microbial Fuel Cell (MFC) technology for power generation still faces significant challenges.

One major challenge is the relatively modest power output observed in larger systems. For instance, a 90-liter reactor with a cathode specific area of 6 m^2 m^{-3} *achieves only about 1 W* m^{-3}*. This limitation hinders the scalability of MFCs for large-scale wastewater treatment, necessitating the use of a greater number of reactors with smaller individual volumes. Treatment rates can be slow, especially with larger wastewater volumes, resulting in prolonged retention times. MFC performance is also sensitive to environmental conditions, requiring careful maintenance of microbial communities. Commercial adoption and standardization practices are still evolving, presenting challenges for widespread implementation. Factors such as the physical space required, initial investment, and technical expertise can serve as barriers,* *particularly in constrained environments. Researchers are working to address these limitations to enhance MFC effectiveness in sustainable energy and wastewater treatment (Ahmed et al., 2022).*

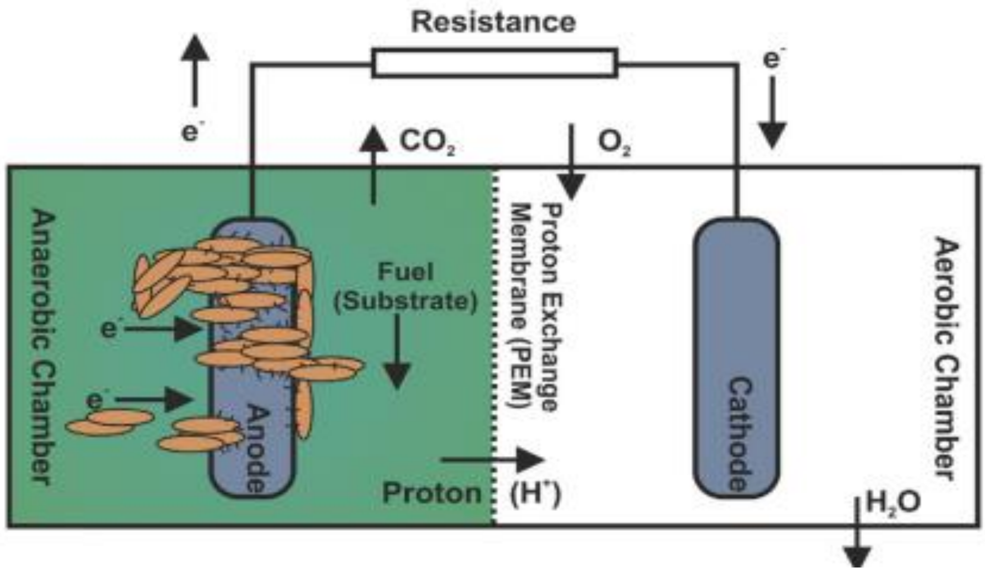


Figure 1: *Schematic of a typically employed two-chamber microbial fuel cell highlighting the various electrochemical and electro-microbiological processes.*

1.2 The Developing Integration of Microalgae in MFCs

Microalgal-assisted Microbial Fuel Cells (MFCs) are an advanced innovation within Bioelectrochemical Systems (BES). This innovative approach utilizes microalgae as oxygenators, significantly reducing operational costs by eliminating the need for external aeration. Cultivating microalgae in MFCs can be achieved through two primary methods: introducing live microalgae and using deceased biomass as a substrate. When live microalgae are incorporated into the cathode, they provide a source of oxygen, which serves as an electron acceptor (Sarma et al., 2023). This dual functionality of microalgae in MFCs not only enhances the efficiency of the system but also integrates the advantages of renewable biomass utilization and wastewater treatment.

Without the assist of microalgae, the Microbial fuel cell (MFCs) are technologies that can both generate electricity and remediate wastewater. This energy produced of organic matter present in wastewater is converted into electricity by microorganisms. The cathode receives the protons and electrons generated at the electrode as a consequence of microbial metabolism. Protons pass via a separator, often a proton exchange membrane, while electrons move through an external circuit. At the cathode, platinum is typically used to catalyse the reduction of ambient oxygen, which results in the formation of water when mixed with protons. In bio-electrochemical systems, a substrate is an essential component, and its type as well as concentration are important factors affecting the composition of a microbes and, consequently, output power (Prathiba et al., 2022). The use of simple organic compounds such as acetate is common because of their high-power output and ease of manipulation. To generate a significant amount of electricity, however, we need to investigate feed-stock options that are not only significantly more affordable but also widely available. In this particular setting, the biomass of microalgae can be put to use. The streams are polluted as a result of microalgae. They are the most important contributors to eutrophication. When wastewater is treated around the world, a significant amount of microalgae biomass is collected as a byproduct. Human health may be in danger if microalgae are discharged directly into the sewer system. Thus, microalgae must be removed from water bodies and thrown away. Biomass from microalgae is used to make biofuels. However, producing biofuels from microalgae biomass is currently not economically feasible due to high costs of cultivation, harvesting, and extraction, along with low biomass productivity. The energy balance is unfavorable, and significant technological and infrastructural advancements are required. Additionally, microalgae biofuels face competition from cheaper fossil fuels and other renewables (Subhash et al., 2022). Research and development are needed to improve efficiency and reduce costs. Microalgae can be utilised as a substrate alternatively in MFC. This strategy combines the production of electricity and trash treatment. Therefore, *using microalgae has two advantages: it reduces pollution and serves as a fuel for MFC. Microalgae biomass has a high concentration of proteins (32%) and carbohydrates (51%), both of which can be easily broken down by electro-gens to produce electricity (Shahid et al., 2020). In 2024 Vennila and colleagues used Chlorella vulgaris (a microalga) powder as a substrate and attained the maximal power density at 0.98 W/m*^{2} *(277 W/m*^{3}*).*

In the past, expensive catalysts (such Pt or CuO) have been utilized to improve the cathode's performance. A suitable level of dissolved oxygen can be added to water and circulated as an alternative. The amount of dissolved oxygen present should be equal to the amount of oxygen created by the catalysts. Nevertheless, the ongoing pumping of water increases the operating costs of the MFC. A possible strategy to boost cathodic performance involves substituting catalysts for photosynthetic microalgae species. As microalgae develop, oxygen is released, acting as a terminal electron acceptor. Carbon fixation is a benefit of employing microalgae as a cathode. Then, CO_2 *is produced by the electrogens during MFC operation. At the cathode, microalgae use* CO_2 *as a source of carbon and encourage growth.*

M-MFCs have the following benefits over other bioenergy production technologies: (a) Immediate energy produced out of a substrate, (b) efficiency at room temperature, (c) no need for an external energy source, (d) dependable baseload power, (e) affordable feedstock storage, and (f) low environmental impact are all characteristics of this technology (Kumar et al., 2023). However, it has some limited drawbacks such as using cost expensive material such as platinum (Pt) at cathode chamber (Boas et al., 2022). A suitable anode electrolyte for hydrogen production in a Microbial Electrolysis Cell (MEC) should have high ionic conductivity and low resistance to enhance efficiency, be biocompatible to support microbial growth, maintain chemical and thermal stability, provide essential nutrients, and be cost-effective and readily available. Examples include phosphate buffer solution (PBS) for its buffering capacity and support of microbial activity, and sodium bicarbonate solution for its cost-effectiveness and pH *maintenance (Rossi et al., 2021). Using a suitable anode electrolyte, such as phosphate buffer solution (PBS) or sodium bicarbonate solution, can lead to high current density production and optimum coulombic efficiency in a microbial electrolysis cell. These electrolytes enhance ionic conductivity and support microbial growth, which facilitates efficient electron transfer. This results in higher current densities and improved coulombic efficiency, as the microbes more effectively convert organic matter into electrical energy (Rossi et al., 2021). The ability of MEC to reach high power densities is also predicted to depend on production of a conductive biofilm matrix that facilitates rapid rate of electron transfer between the microorganism and electrode (Mier et al., 2021). Anode performance is still not sufficient to enable commercial consideration of this system. A power density of 1 kW/m*^{3} *has been proposed as a target sufficient to support application development. This is the utilisation of microalgae in MFCs is among the most promising approaches. Among other aspects, microalgae can be utilised as a substrate at the anode to extract nutrients or to capture the* CO_2 *produced in the cathode.*

There are a variety of benefits to using photo-bioreactors, which have been described in the literature (Benner et al., 2022). They grow a lot of biomass, are excellent for outdoor cultivation, and have a large surface area exposed to light. Additionally, they ease control and lessen the possibility of contamination. Photo-bioreactors, however, have a number of drawbacks, including the high costs of operation and the output capital needed. They are intricate systems that require protection against oxygen buildup, biofouling, and shear stress-induced cell damage. Furthermore, the type of bioreactor chosen relies on the microalgae strain, the location, the size of the space provided, and the type of the desired product (Benner et al., 2022) (Behera et al., 2022).

1.3 The Roles of Key Components in MFCs and M-MFCs

A typical MFC designed for power generation comprises both an anode chamber and a cathode chamber, with a proton exchange membrane (PEM) positioned between them. Each chamber is equipped with two distinct types of electrodes, specifically the cathode and the anode. Various components of the M-MFC are illustrated in Figure 3. The figure illustrates various components of Microalgae Microbial fuel cell (M-MFC) system.

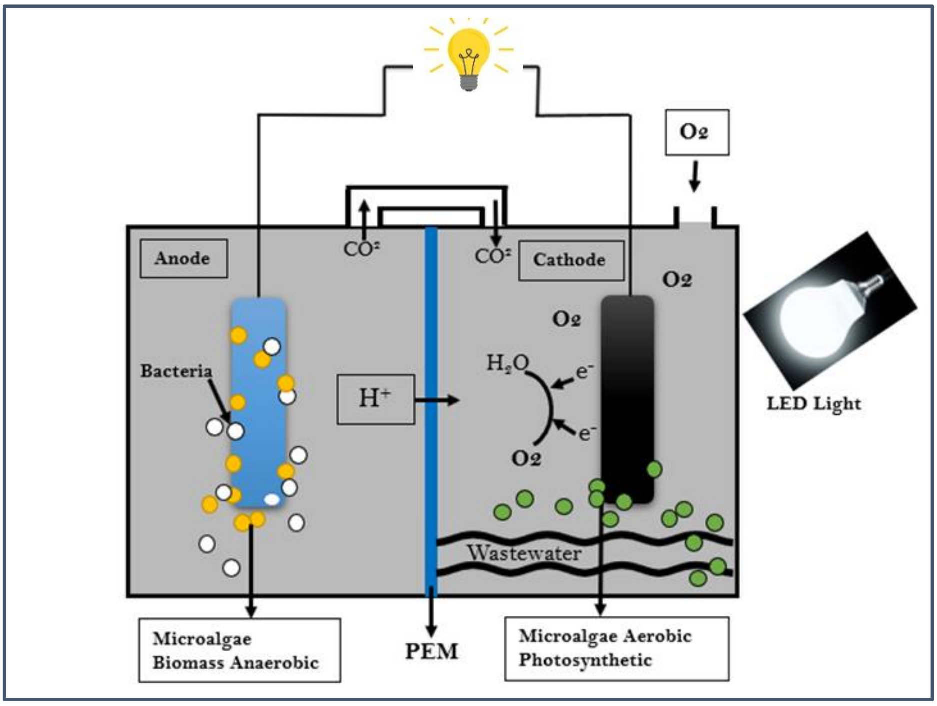


Figure 3: Microalgae-Microbial Fluid Cell (M-MFC)

1.3.1 The Role of the Anode

In a traditional Microbial Fuel Cell (MFC), the anaerobic anode chamber is constructed to receive organic compounds or substrates that undergo microbial oxidation, typically sourced from various organic wastes or wastewater. The main goal is to facilitate microbial metabolism of these organic compounds, leading to the production of electrons by exoelectrogenic microorganisms. The anode chamber serves a crucial role in supporting the growth and activity of microorganisms capable of transferring electrons to the anode electrode, forming the basis of *the electrochemical process. The electrons generated during microbial metabolism are then channelled through an external circuit from the anode to the cathode, where they take part in the reduction reaction. In the MFC system, microorganisms residing on the anode produce electrons through the consumption of organic matter. These electrons subsequently travel to the anode via self-generated mediators or nanowires.*

Previous studies, such as those by Dwivedi and colleagues (2022), have highlighted the significance of the anode in MFCs, emphasizing its role as the primary site for microbial oxidation of organic compounds. Understanding the microbial community dynamics at the anode interface, as explored in these studies, sheds light on electron transfer mechanisms and bioelectricity generation efficiency (Dwivedi et al. 2022).

In contrast, the anode chamber of a Microalgae-assisted Microbial Fuel Cell (M-MFC) introduces a unique approach. In this system, dead microalgae biomass serves as a substrate in the anode chamber. The microbial oxidation of this dead microalgae biomass becomes the primary mechanism for electron generation. This distinctive feature allows for a dual-source of electrons within the anode chamber, with both organic substrates and dead microalgae contributing to the overall electron flow.

In 2022, Prathiba and his colleagues conducted a comprehensive review on the effectiveness and processes of using microalgal biomass for anaerobic respiration in a double-chamber MFC. They chose Chlorella regularis as the model microalgae due to its widespread occurrence in aquifers. Initially, they assessed the components of C. regularis to determine its suitability as an anolyte. They then analyzed the electrochemical performance of the MFC, using C. regularis as the sole electron donor (Prathiba et al., 2022). The study demonstrates the potential of using microalgal biomass waste (Chlorella regularis) as a bioresource for bioelectricity production in a microbial fuel cell (MFC). The algal biomass, rich in proteins and carbohydrates, served as an effective electron donor, achieving a power density of 0.86 W/m^2 and a coulombic efficiency *of 61.5%. By enhancing the biomass concentration, power density increased to 1.07 W/m² with a COD removal of 65.2%, comparable to commercial acetate-fed MFCs. The superior electrochemical performance is attributed to the diverse microbial community and complex biomass composition, suggesting a viable strategy for utilizing microalgal biomass in bioelectricity generation.*

The major differences between a typical MFC anode chamber and an M-MFC anode chamber lie in the substrate used, the dual-source of electrons, and the additional functionalities, such as wastewater treatment and lipid production, embedded in the innovative M-MFC system. This approach showcases the integration of both dead and live microalgae for a comprehensive and sustainable Microbial Fuel Cell system (Table 2).

Table 2 shows MFC converts organic matter into electricity using microorganisms. This process involves the microbial oxidation of organic substrates present in the wastewater, leading to electron generation and subsequent electricity production. While M-MFC utilizes microalgae to generate electricity through a combination of photosynthesis and microbial metabolism. Microalgae serve as electron donors and oxygen producers, contributing to bioelectricity generation in a sustainable manner. MFC capable of remediating wastewater by utilizing microorganisms to break down organic pollutants and contaminants present in the water, offering a dual benefit of electricity generation and wastewater treatment. However, M-MFC offers simultaneous wastewater treatment and electricity generation by leveraging the unique properties of microalgae to remove pollutants while generating bioelectricity, showcasing a more integrated and efficient approach to environmental remediation.

MFC relies on external aeration or oxygen supply to facilitate microbial metabolism and electron transfer processes within the cell. M-MFC the microalgae provide oxygen through photosynthesis, reducing the dependency on external aeration and enhancing the sustainability of the system by utilizing natural oxygen production mechanisms.

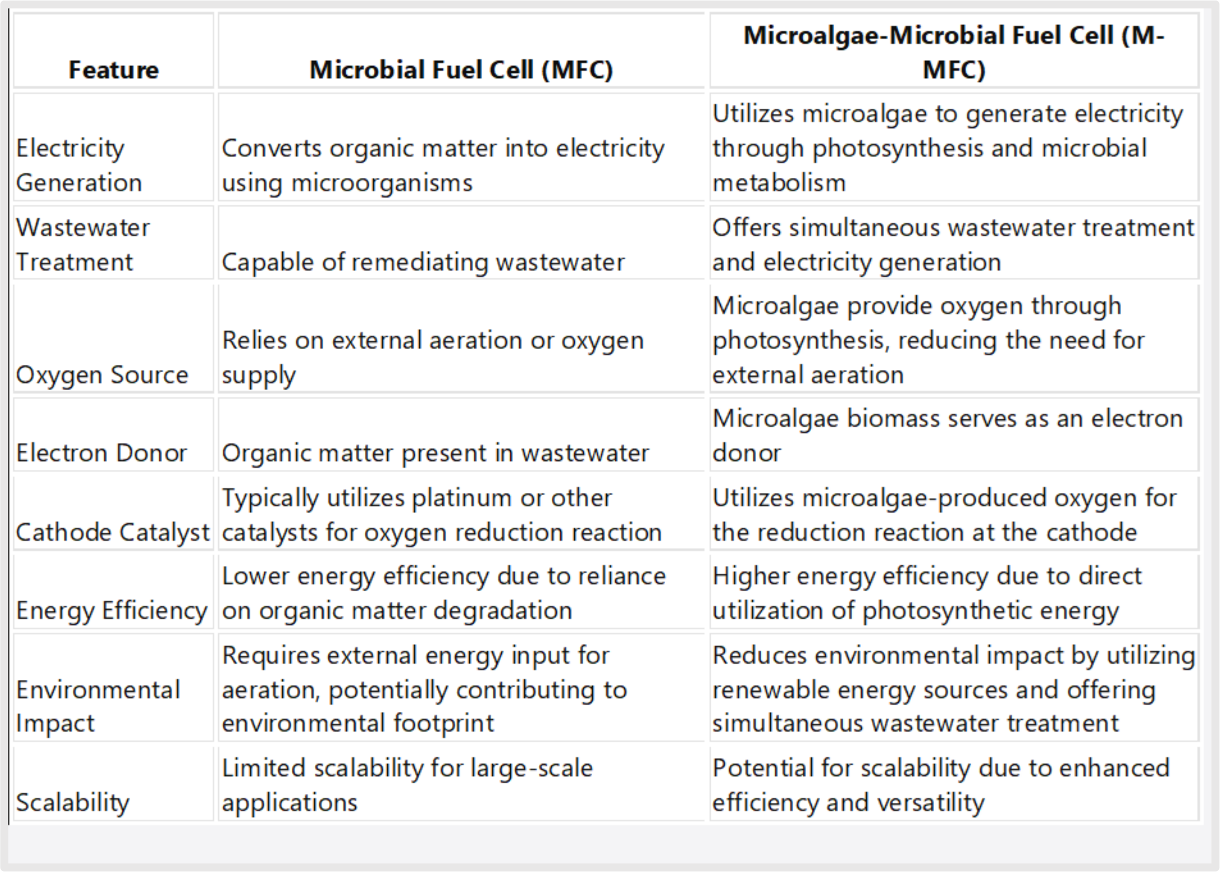
Table 2 also shows MFCs typically utilizes catalysts like platinum (which is very expensive) for the oxygen reduction reaction at the cathode to enhance reaction rates and overall MFC performance. Hence, M-MFCs benefits from the inherent properties of microalgae as electron donors and oxygen producers, reducing the reliance on external catalysts and promoting a more environmentally friendly approach to bioelectricity generation.

By comparing these key features between traditional MFCs and innovative M-MFCs, it becomes evident that the integration of microalgae in MFC technology offers unique advantages in terms of sustainability, efficiency, and dual-purpose functionality for both electricity generation and wastewater treatment.

Exoelectrogens, predominantly bacteria, play a pivotal role in generating electrical energy by oxidizing organic substances and transferring the resulting electrons to an external electron acceptor. The movement of electrons from the anode chamber to the cathode is facilitated by an external circuit. Exoelectrogens are also responsible for proton production, and the transfer of protons from the anode to the cathode through the proton exchange membrane (PEM) depends on charge mobility and differential charge (Elshobary et al., 2021).

Enhancing the microbial electron transfer rate at the anode can be achieved through various approaches, including optimizing cell design, electrode materials, and the introduction of electron mediators. The anode material must possess specific characteristics to support the formation of an active biofilm. A biofilm is an extracellular polymeric substance (EPS), typically enclosed in a self-produced polymeric matrix primarily composed of polysaccharides. This term is commonly used to describe a surface-attached microbial community. As the anode surface substrate, the conductive properties of the biofilm matrix allow electrons to efficiently reach the anode. This conductive biofilm matrix becomes an integral part of the anode and is often referred to as the biofilm anode.

***Table 2**: Comparison between Microbial Fuel Cells (MFCs) and Microalgae-Microbial Fuel Cells (M-MFCs)*



Feature	**Microbial Fuel Cell (MFC)**	**Microalgae-Microbial Fuel Cell (M-MFC)**
Electricity Generation	Converts organic matter into electricity using microorganisms	Utilizes microalgae to generate electricity through photosynthesis and microbial metabolism
Wastewater Treatment	Capable of remediating wastewater	Offers simultaneous wastewater treatment and electricity generation
Oxygen Source	Relies on external aeration or oxygen supply	Microalgae provide oxygen through photosynthesis, reducing the need for external aeration
Electron Donor	Organic matter present in wastewater	Microalgae biomass serves as an electron donor
Cathode Catalyst	Typically utilizes platinum or other catalysts for oxygen reduction reaction	Utilizes microalgae-produced oxygen for the reduction reaction at the cathode
Energy Efficiency	Lower energy efficiency due to reliance on organic matter degradation	Higher energy efficiency due to direct utilization of photosynthetic energy
Environmental Impact	Requires external energy input for aeration, potentially contributing to environmental footprint	Reduces environmental impact by utilizing renewable energy sources and offering simultaneous wastewater treatment
Scalability	Limited scalability for large-scale applications	Potential for scalability due to enhanced efficiency and versatility

When exoelectrogens are employed in a continuous MFC process, carbon materials like cloth, fibers, or veils become an excellent choice for the anode due to their porous characteristics. This type of anode substrate allows for the efficient distribution of the substrate throughout the entire provides an ideal environment for respiration, the microbial community forms a biofilm with a thickness of over 30 mm. While the underlying cells are limited in their access to carbon/electron cell through advective transport. However, the presence of non-permeable electrodes, such as rods or graphite plates used in biofilm formation, can result in a thinner cell structure. This, in turn, leads to a reduction in power generation and a lower metabolic rate (Yaqoob et al., 2021). A polymer material like polyaniline or polytetrafluoroethylene (PTFE) with substantial *conductivity can also serve as a favored anode electrode. Research conducted by Radha and colleagues (2022) demonstrated that incorporating carbon nanotubes into the electrode structure in MFC could enhance both electron transfer feasibility and increase the electrode surface area (Radha et. Al., 2022).*

1.3.2 The Role of the Cathode

The electrons from the anode Chamber are transferred through an external circuit to the cathode, allowing reduction reactions to take place, usually facilitated by a cathodic catalyst. In certain MFC setups, such as those with a two-chamber design, a membrane (such as a cationic, anionic, or ultrafiltration membrane) is positioned between the anode and cathode to prevent electrical short-circuiting and reduce oxygen infiltration to the anode-respiring bacteria (ARB) (Kumar et al., 2023).

Previous research by Senthilkumar et al. (2020) has delved into enhancing cathode efficiency through the use of specific catalysts and continuous oxygen supply. By building upon these findings, further investigations can optimize cathodic reactions, improving overall MFC performance and electricity generation.

The M-MFC system extends its functionality beyond electricity generation. In the MFC system, dead microalgal biomass is used for electron production in the anode chamber, while live and fresh microalgae are incorporated in the cathode chamber. This dual-function approach allows the microalgae to generate oxygen in the cathode and serve as a substrate in the anode (Kumar et al., 2023). This innovative integration of dead and live microalgae in separate chambers enhances the complexity and versatility of the system. In the cathode chamber of the Microalgae-assisted Microbial Fuel Cell (M-MFC) system, the introduction of live and fresh microalgae serves a dual purpose, showcasing a novel approach to enhance oxygen availability and electron acceptance. The key function of the live microalgae lies in its ability to undergo photosynthesis, *a crucial biological process that harnesses light energy to convert carbon dioxide and water into organic compounds and oxygen (Elshobary et al., 2021). During photosynthesis, live microalgae absorb light energy through pigments such as chlorophyll. This energy is then utilized to drive a series of biochemical reactions, resulting in the production of carbohydrates and the release of oxygen. In the context of the M-MFC system, the oxygen generated through photosynthesis becomes a valuable asset in the cathode chamber. Firstly, the oxygen produced by live microalgae in the cathode chamber acts as an electron acceptor during the reduction reaction. While conventional Microbial Fuel Cells (MFCs) often employ expensive platinum-based materials as electron acceptors in the cathode, the M-MFC system leverages the naturally occurring oxygen produced by photosynthetic microalgae. This substitution not only reduces the reliance on costly materials but also aligns with the principles of sustainability by utilizing the inherent capabilities of live microorganisms. Moreover, the introduction of live microalgae in the cathode chamber provides a dynamic and self-sustaining mechanism. As the microalgae continuously undergo photosynthesis, they contribute to the consistent generation of oxygen, creating a favorable environment for the reduction reaction to occur. Sustained oxygen production is essential for maintaining efficient electron flow within the M-MFC system, ensuring a steady and reliable bioelectricity generation process. The utilization of live microalgae in the cathode chamber not only addresses the need for an electron acceptor but also introduces a holistic approach to energy generation. By integrating the natural photosynthetic capabilities of microalgae, the M-MFC system showcases a cost-effective and environmentally friendly alternative to traditional MFC cathodes, which often rely on expensive platinum-based catalysts. This innovative synergy between live microalgae and microbial processes demonstrates the potential for sustainable and economically viable Microbial Fuel Cell technology (Yadav et al., 2020).*

Establishing an appropriate cathode configuration is a critical factor in enhancing bioelectricity generation and advancing MFC technology from pilot-scale applications to industrial-scale implementation. The cathode material plays a pivotal role in determining the power output of the MFC due to its high redox potential and efficient proton capture capabilities.

Common materials used for cathodes include carbon paper, fiber, granular graphite, copper (Cu), and platinum (Pt). Platinum, in particular, is employed in the cathode chamber to increase the reaction rate and reduce the activation energy of cathodic reactions in MFC. This innovative use of platinum has shown promise in improving MFC performance (Asiri et al., 2020).

Initially, the use of platinum as a cathode material resulted in high electricity generation. Given the high cost of platinum, various efforts have been made to explore alternatives and reduce the overall cost of MFC by substituting platinum with more economical materials such as activated carbon (AC) powder, due to its low cost of \$1.4 kg^{-1} *compared to Pt (\$625 g*$^{-1}$*) and metal–organic framework (MOF) which is composed of a metal ion and an organic linker, alternative metals such as Fe, Co, and Ni were also used as catalysts material (Koo et al., 2021).*

1.3.3 The Role of the Microorganism

Exoelectrogens are a group of microorganisms, primarily bacteria, that are instrumental in the operation of Microbial Fuel Cells (MFCs). Their defining characteristic is their ability to release electrons as a byproduct of metabolizing organic compounds. This unique trait enables them to participate in the generation of electrical current within the MFC.

Exoelectrogens are typically situated in the anode chamber of the MFC, where they engage in the oxidation of organic substrates and transfer the resulting electrons to the anode electrode. This electron transfer process is at the heart of electricity production in MFCs (Kakar et al., 2022).

This group of microorganisms is quite diverse, encompassing various species, including Geobacter, Shewanella, and Rhodoferax, among others. Each species may possess distinct attributes that impact their performance within MFCs. Exoelectrogens often form biofilms on the anode electrode's surface. These biofilms act as conductive pathways, enhancing the efficiency of electron transfer by facilitating the movement of electrons to the anode (Naaz et al., 2023).

Exoelectrogens display an ability to thrive in different environmental conditions, making them adaptable to various organic substrates and wastewater types. This adaptability is advantageous for MFC applications across diverse settings. Ongoing research endeavors focus on gaining a deeper understanding of exoelectrogens, their mechanisms of electron transfer, and methods to optimize their performance in MFCs. Genetic engineering and biofilm engineering are among the strategies explored to enhance the efficiency of electricity generation.

Apart from their role in electricity generation, exoelectrogens also hold potential for bioremediation by breaking down organic pollutants in wastewater.

While exoelectrogens show promise in MFCs, challenges remain, such as improving their metabolic rates and enhancing overall electron transfer efficiency. Researchers are actively investigating ways to enhance their performance and reduce the costs associated with MFC technology. Exoelectrogens are a critical component in the operation of MFCs, and ongoing research endeavors seek to unlock their potential for sustainable electricity generation and wastewater treatment (Elshobary et al., 2021).

1.4 Material Design and Configuration in M-MFCs

With or without the use of polymer electrolyte membranes, one or two chambers are frequently assembled in traditional MFC technology to produce electricity thru a chemically specified substrate (such as a solution of glucose or acetate, for example) (PEM) (Allam et al., 2020), *(Selvaraj et al., 2020). The two chambers namely an anode and cathode. In two-compartment MFCs, the anodic and cathodic chambers are joined in an H-shape, with an Ultrex or Nafion PEM salt bridge complete the circuit and maintaining the device's electrical neutrality. Operationally, the cathodic chamber receives continual oxygen supply while the anodic chamber is home to the organic substrate and sludge, with the exchange membrane supporting ionic transfer (Senthilkumar et al., 2020). The anode, cathode, and membrane surface areas are crucial in determining how much electricity the MFC can produce. H-shaped 2-compartment MFCs are mainly exclusively used in laboratory research due to their weak power densities and large internal resistances (Boas et al., 2022).*

Integrating microalgae in Microbial Fuel Cells (MFCs) involves a meticulous selection of methodologies to optimize bioelectricity generation and environmental applications. The following paragraphs delve into the detailed analysis of these methodologies and the rationale behind choosing specific microalgae strains for the anode and cathode chambers:

In the integration of microalgae in MFCs, the selection criteria for microalgae species play a crucial role in determining the overall performance of the system. Factors such as growth rate, lipid content, nutrient requirements, and tolerance to environmental conditions are essential considerations when choosing microalgae strains for the anode and cathode chambers. For instance, microalgae species with high lipid content are preferred for the anode chamber to enhance electron production through microbial metabolism. In contrast, microalgae with efficient photosynthetic capabilities are selected for the cathode chamber to facilitate oxygen production and sustain electron flow within the MFC system.

Moreover, the choice of electrode materials in MFCs significantly influences the interaction between microalgae and bacteria, impacting electron transfer processes. Conductive materials with high surface area, such as carbon-based electrodes, promote microbial adhesion and electron transfer efficiency. By selecting appropriate electrode materials tailored to the *metabolic activities of the chosen microalgae strains, the MFC system can achieve enhanced bioelectricity generation and overall performance.*

Operational parameters, including temperature, pH, light intensity, and nutrient availability, are meticulously controlled to create an optimal environment for microalgae growth and electron transfer processes in MFCs. Fine-tuning these parameters based on the metabolic activities of the specific microalgae strains in the anode and cathode chambers ensures maximum bioelectricity generation efficiency. By understanding the intricate interplay between microalgae species, electrode materials, and operational conditions, researchers can design MFC systems that harness the full potential of microalgae-microbial interactions for sustainable bioenergy production and environmental remediation.

However, in the case of M-MFC, the cathode chamber is maintained by microalgal organisms, which necessitate the presence of carbon dioxide (CO_2), water, and temperature for the purpose of growth and the production of fundamental nutrients. The cathode chamber receives the created carbon dioxide along with the anode electrolyte. Microalgae are biodegradable because they consume carbon dioxide, store it in their cells, and then release oxygen more quickly. After that, oxygen combines with the hydrogen ions (H+) that were produced at the anode and then travels through the PEM membrane to the cathode, where it forms fresh water.

Regarding the choice of material, anodic materials are recommended for their high conductivity, great chemical stability, and good biocompatibility (Halim et al., 2020). Despite being benchmarked for its conductivity; copper's antibacterial property makes it a less than ideal anodic material. A good substitute for copper used for anodic reasons in MFCs is stainless-steel mesh because it is non-corrosive and less hazardous. The remarkable flexibility and plasticity of carbon, which can be functionalized as crushed graphite plates, granules, rods, fibres, or glassy carbon, makes it one of the most ideal anodic possibilities (Yadav et al.; 2021).

Several variables, such as the number of electrons present, the kind of receiver, the presence of protons, the activity of the catalyst, and the electrode layout, can have an impact on the performance of the MFC cathode. The cathode chamber is sometimes refereed as an anaerobic chamber, highlighting the crucial function of oxygen in it. Most scientists agree that O_2 serves as an electron acceptor, drawing and consuming the electron produced in the anode chamber prior interacting with the H+ that has migrated from the separating membrane (Tang, 2022) (Tiquia-Arashiro et al., 2020) . Only water is produced as a result of such a procedure, demonstrating its advantageous impacts on the environment (Wang et al., 2022). To enable oxygen replenishment and improve MFC activity, some designs position one side of the cathode in the cathode chamber whereas the other makes contact with air above the surface. The choice of cathode material is dependent on the material's availability and oxidation potential. For a stabilised MFC performance, the non-toxicity of such chosen material as well as its oxygenated equivalents is also crucial.

An MFC's usual structure consists of an electrode distance, a cationic membrane formed of ceramic or clay ware material, and two chambers: an anode and a cathode (Patel et al., 2022). For the purpose of producing electricity in MFCs, depending on the properties of the electron acceptor, microorganisms' biopotential, which is fuelled by metabolic and physiological activities, is responsible. The separation of the chambers while maintaining the anaerobic environment on the anodic side is made possible in large part by the membrane for proton exchange or other similarly functioning membranes (Das et al., 2020). In laboratory MFC research, Ultrex CMI-7000 membranes and Nafion are frequently used; the latter is more cost-effective. In the meantime, it verified that polymer inclusion membranes (PIMs) based on ionic liquids are compatible with MFCs technology (Ebrahimi et al., 2021). Porous clay materials, including such innovative porous clay earthenware (NCE), showed better power outputs when employed as a separator in comparison to such polymer electrolyte membranes (PEM). A typical *reactor in microbial electrochemical technology (MET) features an anode and cathode separated by a barrier, usually a proton exchange membrane (PEM) like Nafion 117. Daud and colleagues in 2022 demonstrated that a new porous clay earthenware (NCE) was created to replace the costly PEM. The NCEs were made using raw clay powder and starch powder as a pore-forming agent in varying amounts (10 vol%, 20 vol%, and 30 vol%). The mixture was ball-milled, hydraulically pressed into green ceramic pellets, and sintered at temperatures up to 1200 °C. The MFC–NCE with 30 vol% starch powder achieved the highest power density of 2250 ± 21 mW/m² (6.0 A/m²), an internal resistance of 75 ± 24 Ω, and a coulombic efficiency (CE) of 44 ± 21% in batch mode operation. In contrast, the MFC–PEM configuration produced the lowest power density, CE, and the highest internal resistance, with values of up to 1350 ± 17 mW/m² (3.0 A/m²), 23 ± 15%, and 326 ± 13 Ω, respectively* (Daud et al., 2020).

Ionic liquid had a beneficial role in moving the activated proton through the separator, as evidenced by the observed positive association between the quantity of immobilised ionic liquid membranes and the overall power production of MFCs.

1.5 Current Issues & Limitations Related to M-MFC System

The relationship between microalgae and bacteria isn't limited to particular species, making it challenging to identify unique metabolites for each species. In a combined system, identifying and choosing microalgal strains with specific biochemical compositions is essential for estimating the potential for power generation and recovering valuable products (Jadhav et al., *2019).*

Many electroactive microorganisms haven't been cultured yet, making it difficult to understand their metabolic processes and how they cycle nutrients. In the cathode chamber, when microalgal cells grow too much, they block light, which slows down their own growth. The *changes in metabolism and the interactions between bacteria and microalgae over time would alter the in situ conditions of the culture (Jadhav et al., 2019).*

Sometimes, when microalgae and bacteria are grown together, the microalgae can increase the pH and salinity of the culture, which might slow down the growth of electroactive bacteria. It's better to use a mix of different microbes in the anode chamber of Microbial Fuel Cells (MFCs) because they are more resilient to stress and can adapt to different nutrients. These MFCs work by using reactions in the mitochondria of microalgae cells. If we improve the ability of microalgae to turn sunlight into energy through genetic changes, it can help overcome some limitations. Overall, the ability of microalgae and bacteria to handle stress in these MFCs can lead to more electricity and biomass for making biofuels (Mekuto et al., 2020).

Using ion-exchange membranes or separators like proton exchange membranes (PEM) poses practical limitations for the widespread adoption of microalgal-assisted MFCs technology due to its high cost and increased internal resistance. PEM is commonly used in MFCs because it offers relatively good conductivity to cations and low internal resistance compared to other separators. PEM membranes can be categorized based on materials or pore size, including cation exchange membranes (CEM), anion exchange membranes (AEM), bipolar membranes (BPM), microfiltration membranes (MFM), and ultrafiltration membranes (UFM). Nafion and Ultrex membranes are popular choices in MFC systems due to their excellent proton selectivity. Additionally, PEM membranes can transport cations such as Na^+*,* K^+*,* NH^{4+}*,* Ca^{2+}*, and* Mg^{2+}*. However, finding an efficient membrane material at a lower cost remains a challenge for scaling up MFCs (Nunoz et al., 2021).*

Some studies have explored cost-effective alternatives to traditional membranes in Microbial Fuel Cells (MFCs), such as using materials like glass fiber or removing the membrane altogether. Low-cost ceramic materials, including clayware and coconut shell, have shown promise in enhancing power generation by improving proton movement and biofilm thickness. While *membraneless microalgal-assisted MFCs have been investigated, they tend to exhibit lower power densities due to challenges in electron and proton transfer. Moving forward, focusing on suitable membrane configurations, utilizing low-cost materials as separators, and enhancing the electrochemical activity of microorganisms through genetic modifications hold potential for scaling up microalgal-assisted MFCs and improving their efficiency (Nunoz et al., 2021).*

However, the M-MFC system does face challenges. Despite the enhanced electron generation, power output levels may still face limitations, potentially impacting the system's suitability for high-demand electricity applications. Environmental sensitivity, particularly in maintaining the optimal conditions for the microbial community, could present operational challenges (Monika et al., 2022).

The commercial adoption and establishment of standardized practices for M-MFCs are still evolving, presenting challenges regarding technology maturity and widespread adoption. Spatial constraints for larger M-MFC systems, coupled with the initial investment and technical expertise required, may hinder their feasibility, particularly in urban or space-limited environments.

To sum up, M-MFCs show significant promise for sustainable energy generation and environmental applications. However, addressing challenges related to power output, environmental sensitivity, technology maturity, and spatial constraints is essential to fully realize the potential of this innovative technology. Ongoing research efforts are vital for overcoming these limitations and advancing the effectiveness of Microalgae-assisted Microbial Fuel Cells.

1.6 Conclusion

In conclusion, this study unveils the innovative potential of integrating live and dead microalgae within Microbial Fuel Cells (MFCs) to achieve a symbiotic synergy with far-reaching implications. The dual-chamber approach, leveraging dead microalgae in the anode and live *microalgae in the cathode, has demonstrated remarkable outcomes in sustainable bioelectricity generation and environmental applications.*

The findings underscore the significance of deceased microalgae biomass as a valuable substrate, enhancing microbial metabolism and electron generation in the anode chamber. Simultaneously, live microalgae, through their photosynthetic activity in the cathode chamber, contribute to efficient oxygen production, thereby facilitating the reduction reaction and sustaining electron flow within the M-MFC system.

The symbiotic integration of microalgae not only advances bioelectricity generation but also extends the functionality of the M-MFC system to environmental applications. Preliminary results suggest a promising potential for wastewater treatment, emphasizing the versatility of this integrated approach beyond conventional MFC paradigms.

The sustainability impact of the M-MFC system is evident, with reduced reliance on traditional electron acceptors and the prospect of simultaneous electricity generation and environmental benefits. The novel combination of live and deceased microalgae within MFCs opens avenues for further exploration in the realms of sustainable energy and environmental remediation.

As the research community seeks viable solutions for the intersection of energy and environmental challenges, the symbiotic synergy presented in this study offers a paradigm shift in Microbial Fuel Cell technology. This investigation serves not only as a milestone in understanding the capabilities of Microalgae assisted MFCs but also as an inspiration for future endeavors in sustainable bioenergy and environmental applications.

In essence, the integration of live and deceased microalgae within Microbial Fuel Cells emerges as a promising avenue, presenting opportunities for advancements that align with the broader goals of achieving sustainable and multifunctional energy systems.

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