

Review

Microalgae-Assisted Microbial Fuel Cell for Treatment of Difficult Waste Streams

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Abstract: Microalgae microbial fuel cells (pMFCs) are distinguished by their ability to combine waste utilization with the simultaneous recovery of energy and valuable materials. The generation of high current density is linked to the efficient electron transfer to the anode via the anodic biofilm and the high photosynthetic activity of the microalgae cultivated in the cathode chamber. This review explores the impact of wastewater type on energy production and wastewater treatment. Additionally, it discusses the challenges related to microalgae growth in the cathode chamber, the necessity of aeration, and the sequestration of carbon dioxide from the anode chamber. The efficiency of microalgae in utilizing nutrients from various types of wastewater is also presented. In conclusion, the comparison between wastewater treatment and energy balance in pMFCs and conventional wastewater treatment plants is provided. On average, MFCs consume only 0.024 kW or 0.076 kWh/kg COD, which is approximately ten times less than the energy used by activated sludge bioprocesses. This demonstrates that MFCs offer highly efficient energy consumption compared to traditional wastewater treatment systems while simultaneously recovering energy through exoelectrogenic, bioelectrochemical processes.

Keywords: microalgae; wastewater; power density



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1. Introduction

The escalating global energy demand and the depletion of fossil fuel reserves are driving an energy crisis, compelling scientists and researchers to seek renewable energy sources that are both environmentally sustainable and economically viable. Alongside the energy crisis, challenges such as increasing urbanization, overpopulation, and rapid industrialization have exacerbated environmental pollution, posing significant threats to human, animal, and plant life.

Microbial fuel cells (MFCs) represent an innovative technology that harnesses the energy stored in organic compounds, converting it into electrical energy through microbial metabolism [1]. A typical MFC consists of two chambers, an anode and a cathode, separated by a semi-permeable proton exchange membrane (Figure 1). In the anode chamber, organic substrates are oxidized by microbial consortia acting as specific biocatalysts. This oxidation results in the transfer of electrons to a proximate electrode and the simultaneous release of protons into the solution under anaerobic conditions. The electrons are then transported to the cathode via an external circuit, generating electricity due to the potential difference between the electrodes. Concurrently, protons migrate through the semi-permeable membrane to the oxygen-containing cathode. At the cathode, a chemical or microbiological reduction process occurs, where protons combine with electrons and oxygen to form water [2].

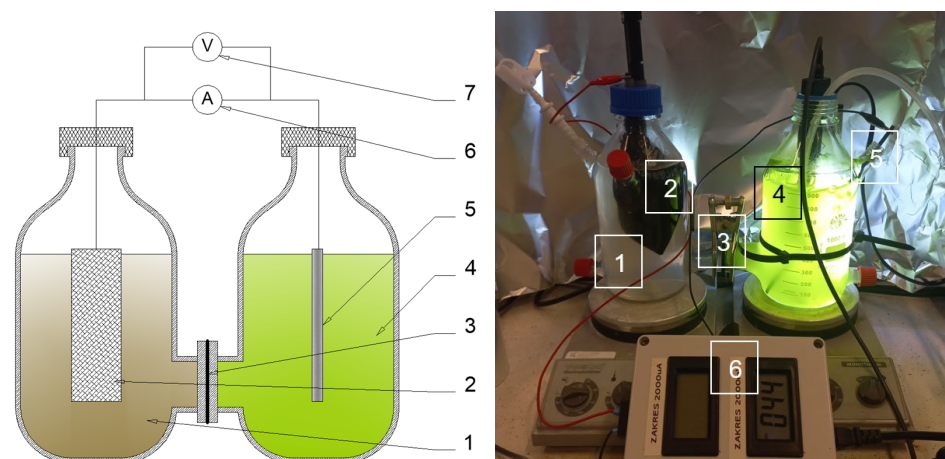


Figure 1. The scheme and photo of the pmFC: 1, anode chamber; 2, carbon felt anode; 3, membrane Nafion; 4, cathode chamber; 5, carbon cloth cathode; 6/7, ammeter/voltmeter (scheme: author Marcin Zieliński original published in [3] reused under the Creative Commons CC BY 4.0 license; photo: author Paulina Rusanowska).

The generation of energy in MFCs is facilitated by microorganisms, primarily bacteria, which oxidize substrates to carbon dioxide, releasing protons and electrons. These bacteria colonize the anode surface as a biofilm, which is a critical feature enabling direct contact between bacterial cells and the electrode surface. The biofilm-forming bacteria can transport electrons extracellularly, bypassing exogenous electron acceptors and directly transferring them to the anode [4].

The fundamental mechanism of electricity generation in MFCs is rooted in the microbial transfer of electrons from a reduced donor with a low electrochemical potential to an acceptor with a higher potential, such as oxygen [5]. During catabolic processes, bacteria generate electrons stored intracellularly as intermediates like NADH. Redox mediators in the solution penetrate microbial cells, competing with oxygen to oxidize these electrons and subsequently diffusing back to the cell's outer envelope, where they transfer electrons to an extracellular acceptor [6]. In the anode compartment of the MFC, which lacks oxygen, the presence of the electrode influences bacterial metabolism. When the anode's potential exceeds that of alternative electron acceptors in the substrate, such as sulfides, it becomes the preferred electron acceptor. This selection enhances bacterial energy yield, steering their metabolism toward electrogenesis [7]. However, under anaerobic conditions, some microorganisms in the anode compartment may engage in alternative metabolic pathways, including fermentation, methanogenesis, or the use of non-electrogenic electron acceptors, which do not contribute to energy production [8].

The efficiency of MFCs is a critical factor in their development as a competitive technology for renewable electricity generation. A primary challenge lies in engineering cells capable of producing substantial electricity from industrial waste. Typically, the performance of MFCs is quantified in terms of power density (e.g., mW/m^2) or current density (e.g., mA/m^2) relative to the surface area of the anode. Additionally, various parameters characterize MFC efficiency, such as Coulombic efficiency, which measures the ratio of electrons transferred to the anode relative to the theoretical electron yield of the substrate and substrate bioconversion rates [9]. Another important biological parameter is the chemical oxygen demand (COD), which depends on the abundance of bacterial cells, the kinetics of microbial processes, and the availability of organic biomass in the fuel [10].

In the 1990s, MFC systems were only capable of generating very low power outputs, which were typically below $0.1 \text{ mW}/\text{m}^2$ [11]. These limited values were primarily attributed to the cell configuration, electrode materials, and high internal resistance. Subsequent

advancements in MFC technology have significantly increased power outputs, reaching up to 3600 mW/m^2 in some cases [10]. However, it is important to note that these higher values were achieved using pure, homogeneous substrates such as glucose or acetate, which simulate organic compounds in liquid waste. In contrast, when MFCs are fueled with real wastewater containing mixed organic compounds, the power outputs are considerably lower, typically ranging from 6.8 to 420 mW/m^2 [12].

Expressing MFC efficiency in terms of Coulombic efficiency reveals that efficiencies of 80–99% can be achieved with pure substrates, whereas the use of brewery wastewater reduces this parameter by up to fourfold [13]. For maximum theoretical energy yield, the supplied substrate must be fully oxidized to CO_2 with effective electron transfer to the electrode. However, the heterogeneous composition of real wastewater makes achieving high efficiency challenging. Typical COD removal efficiencies in MFCs range between 40% and 60%, whereas efficiencies as high as 90% have been observed in MFCs operated with glucose [14]. Despite substantial progress, achieving the theoretical maximum voltage of a single MFC (1.14 V) remains elusive with current single-cell outputs limited to 0.3–0.7 V [15,16]. Enhancing MFC performance remains a critical research focus with the ultimate goal of creating systems that maximize energy output while minimizing energy input.

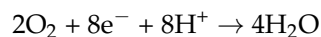
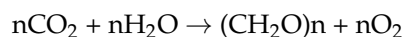
Numerous factors—biological, physical, and chemical—affect MFC performance. These include mass transfer rates within the biofilm, microbial substrate oxidation processes, electron transfer mechanisms, and reduction reactions at the cathode. Among these, electron transfer efficiency and the biological activity of the microbial community are the most extensively studied aspects [17]. The energy output of an MFC is primarily determined by the type and density of microbial cells forming the bioanode consortium [3].

To enhance the economic feasibility of MFCs, oxygen-producing organisms can be introduced into the cathode chamber as an alternative to mechanical aeration. Various microorganisms have been utilized in photosynthetic MFCs, including phototrophic bacteria, algae, and plants [3,18]. Plant-assisted MFCs harness energy from rhizodeposits secreted by plant roots, as well as from the decomposition of plant biomass, utilizing these as substrates for electroactive bacteria [19]. The key mechanisms driving energy generation in plant-assisted MFCs are rhizodeposition and the photosynthetic pathway [18]. The power density of 675 mW/m^2 was obtained with *Spartina anglica* on nitrate less and ammonium rich medium [20]. Like plants, cyanobacteria and microalgae contain two light-absorbing photosystems, enabling them to convert light energy into chemical energy. During photosynthesis, they utilize water as an electron donor, producing ATP, NADPH, and molecular oxygen. Microalgae-assisted MFCs are particularly well suited for wastewater treatment, CO_2 capture, and controlled biofuel production. However, they require frequent harvesting and maintenance. In contrast, plant-assisted MFCs provide a more passive and self-sustaining approach, making them ideal for wetland environments and long-term applications. Nevertheless, their power output is generally lower compared to microalgae-based MFCs [20].

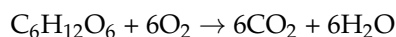
Initially, microalgae were integrated with MFCs in a two-step process, where the effluent from the MFC was treated anaerobically before being directed to a photobioreactor [21]. This approach significantly improved wastewater treatment efficiency, with COD, total phosphorus (TP), and ammonium nitrogen (N-NH_4) removal rates of 77.9%, 23.5%, and 97.6%, respectively, in MFCs without photobioreactor treatment, increasing to 99.3% and 99% for TP and N-NH_4 , respectively, after photobioreactor treatment with mixed microalgal cultures [22]. However, more recently, photosynthetic microalgae have been directly integrated into the cathode chamber of MFCs to enhance oxygen availability and achieve self-sufficiency. This integration improves oxygen reduction at the cathode,

as evidenced by a 32–34.5% increase in power density compared to mechanically aerated systems [3,23]. Microalgae also form biofilms on the cathode surface, further enhancing oxygen reduction rates [24].

Cathodic reactions are as follows:



During the light phase, microalgae photosynthesize to produce oxygen and biomass, whereas respiration occurs during the dark phase:



Additionally, as mentioned above, microalgae contribute to the removal of inorganic pollutants, such as nitrogen and phosphorus, which are prevalent in wastewater from agricultural and domestic sources. They can also serve as electron acceptors in the cathode, covering potential windows for oxygen ($E_0 = +820$ mV) and nitrate ($E_0 = +430$ mV), enabling simultaneous bioelectricity generation and pollutant reduction [25–27]. Furthermore, microalgae-based MFCs offer opportunities for producing high-value by-products, including biomass, lipids, and pigments, with diverse applications [22,28,29].

Moreover, as can also be seen in the first equation, the use of microalgae not only facilitates bioelectricity generation but also plays a crucial role in carbon sequestration by actively capturing and utilizing CO_2 during photosynthesis. This process contributes to reducing greenhouse gas emissions while simultaneously supporting biomass growth, which can further be harnessed for biofuel production or other valuable bioproducts. Promising results of research on the fixing of carbon dioxide in technological systems used for the cultivation of microalgae biomass caused it to currently be one of the potential solutions to reduce the emission of this greenhouse gas [30,31]. Ambient CO_2 levels are generally low (approximately 0.036%) when compared to industrial areas, which release large amounts of flue gases into the atmosphere. Microalgae are able to fix CO_2 from a range of sources, such as the atmosphere, industrial exhaust gases (flue and flaring gas) as well as in the form of soluble carbonates (NaHCO_3 and Na_2CO_3) [32]. Given the increasing levels of environmental pollution, particularly due to industrial emissions and fossil fuel combustion, integrating microalgae into microbial fuel cells presents a sustainable approach to mitigating CO_2 accumulation in the atmosphere while promoting cleaner energy solutions.

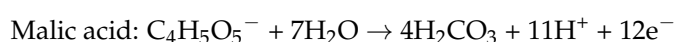
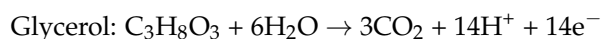
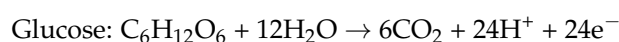
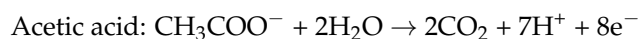
This review first presents the potential use of pMFCs for treating various types of waste streams. It then identifies the environmental parameters of their performance and the microorganisms used in these systems. Finally, the advantages of the presented technology are briefly discussed.

2. Types of Wastewater

The quality of the substrate is a critical determinant of MFC efficiency. Theoretically, less complex substrates yield higher power outputs. It is well documented that MFCs powered by wastewater generate significantly less energy compared to those fueled by pure substrates. However, enriching wastewater with acetate has been shown to enhance MFC efficiency, as the energy output correlates positively with the proportion of readily biodegradable fractions in the wastewater. Moreover, the presence of inhibitory chemicals in industrial wastewater can further reduce efficiency, as observed in comparisons with pure substrates [33]. The mode of substrate supply also influences MFC performance.

Batch-fed systems often promote the growth of microorganisms that produce soluble redox mediators, whereas continuously fed systems favor biofilm-forming species capable of direct electron transfer to the electrode [34].

Most exoelectrogens are capable of metabolizing simple carbon sources such as acetate, lactate, citrate, and methanol to generate electricity in MFCs [35,36]. In contrast, the direct utilization of complex carbon sources, cellulose, and starch in MFCs has been infrequently reported [37,38]. Among these, acetate is the simplest and demonstrates a higher power density compared to glucose and domestic wastewater [35]. Depending on the specific substrate present in the wastewater, various oxidation reactions may occur at the anode:



Recent research has increasingly focused on the use of real wastewater as a substrate source due to its highly variable composition. The type and complexity of substrates significantly influence the efficiency of power generation in MFCs, as more complex substrates are less accessible and degradable by anaerobic microorganisms, resulting in reduced energy output. The nature and composition of the substrate play a crucial role in determining the efficiency of the pMFC. In pMFCs, diverse substrates were treated in the anode and cathode chambers (Table 1). The review focused more on complex waste streams, but aspects related to easily degradable ones were also not omitted.

Table 1. Examples of types of wastewater treated in pMFCs.

Type of pMFC	Type of Wastewater	COD Removal	Additional Info	Power Density	Reference
Upflow membrane-less MFC with photobioreactor	Domestic wastewater (238 mg/L)	77.9%	N-NH ₄ removal 99.0%; TP removal 99.3%	481 mW/m ³	[22]
Tubular with blue-green algae	Domestic sludge	78%		114 mW/m ²	[39]
Double chamber with <i>Chlorella vulgaris</i>	Domestic wastewater	19.45%	CO ₂ sequestration	5600 mW/m ³	[40]
Double-chamber pMFC of anaerobic bacteria and <i>Chlorella vulgaris</i>	Chocolate factory wastewater	Anode 71%, cathode 78.6%	Removal of 73% of the blue dye brl in the anolyte; biodepuration of 95% of fats and oils in the catholyte in 32 d	327.67 mW/m ²	[41]
Double-chamber pMFC with <i>Tetraselmis indica</i>	Pharmaceutical wastewater	90.3%	Nitrate reduction 81.6% phosphate reduction 94.9%	560.8 mW/m ²	[42]
H-type with <i>Scenedesmus abundans</i>	Pharmaceutical wastewater	97.24%		6180 mW/m ²	[43]
Double-chamber pMFC with <i>Oscillatoria</i> sp.	Diluted food wastewater (2500 mg/L)	55.1%		29.6 mW/m ²	[44]
Double-chamber pMFC with <i>Scenedesmus</i> sp.	Diluted food wastewater (2500 mg/L)	48.5%		23.5 mW/m ²	[44]
pMFC with <i>Saccharomyces cerevisiae</i> and <i>Spirulina platensis</i>	Cafeteria wastewater	40%		98 mW/m ²	[45]
H-type with <i>Klebsiella pneumoniae</i> FA2 and <i>Chlorella sorokiniana</i>	80% food wastewater in anode; 50% dairy wastewater in cathode		High lipid production obtained by microalgae	212 mW/m ²	[46]
Double chamber with <i>Chlorella</i> sp.	Aquaculture wastewater (N:P ratio = 3)	80.30%	N-NH ₄ removal 69.93%; TP removal 54.44%	102.37 mW/m ²	[47]

Table 1. Cont.

Type of pMFC	Type of Wastewater	COD Removal	Additional Info	Power Density	Reference
Double chamber with <i>Scenedesmus obliquus</i>	Aquaculture wastewater (COD = 113 mg/L; 50.5 mg NO ₃ /L; 12.1 mg PO ₄ ^{3−} /L)		Nitrate removal of 70.17%; TP removal of 88.33%-under blue light operation	530 mV	[48]
Double chamber with <i>Arthrospira maxima</i>	Anaerobic digester effluent (2.7 g COD/L)	63%		67 mW/m ²	[49]
Double chamber with <i>Arthrospira platensis</i>	Tapioca wastewater	67%		14.5 mW/m ²	[26]
Tubular double chamber with <i>Golekinia</i> sp.	Anaerobically digested effluent from kitchen waste	78%		2800 mW/m ²	[50]
Double chamber with <i>Synechococcus</i> sp.	Kitchen wastewater	73.50%		830 mW/m ²	[51]
Double chamber with <i>Chlorococcum</i> sp.	Kitchen wastewater	68.50%		604 mW/m ²	[51]
Double chamber with <i>Scenedesmus dimorphus</i>	Dairy wastewater	72%		380 mW/m ²	[52]
Double chamber with <i>Chlorella pyrenoidosa</i>	Swine wastewater		Lipid content in microalgae 37%; N-NH ₃ removal 56%	103 mW/m ³	[53]

Cellulose-containing wastewater presents a complex substrate for anaerobic treatment. Cellulose, a primary structural polymer in plant cell walls, is continuously synthesized through photosynthesis. Cellulosic waste biomass is a by-product of various industries, including forestry (e.g., leaves and grass) and agriculture (e.g., corn stover, rice husks, sugarcane bagasse, straw, and fruit or vegetable waste) as well as the wood and paper industries [54]. The degradation of complex substrates like cellulose in plant microbial fuel cells (pMFCs) initially relied on specialized microorganisms capable of secreting cellulase to facilitate electricity generation. For instance, power densities of 4.90 ± 0.01 mW/m², 38.70 mW/m², and 9.56 ± 0.37 mW/m² have been reported using strains such as *Enterobacter cloacae*, *Cellulomonas fimi*, and the facultative strain Lsc-8 (*Cellulomonas* genus), respectively, in the anode compartment [37,38,55]. However, incomplete cellulose degradation was observed due to the limited cellulase production by these microorganisms, indicating that cellulose is a less degradable substrate with restricted applicability in pMFCs.

Oily wastewater has also been treated in pMFCs. Various studies have explored potential solutions for the remediation of such challenging waste streams. Spent engine oil (SEO) is a major environmental pollutant containing toxic polycyclic aromatic hydrocarbons, heavy metals, and lubricant additives. The removal of SEO emulsified in water is particularly challenging and poses a significant threat to aquatic ecosystems [54]. In the pMFC configuration, oily wastewater was treated using microalgae. SEO degradation was conducted in the cathode chamber, whereas the anode chamber facilitated anaerobic degradation utilizing newspaper powder, dried microalgae biomass, and mixed fruit peel powder. Among these substrates, the pMFC employing newspaper powder achieved the highest power density with a maximum output of 34.88 ± 1.00 mW/m². Fruit peel and dried microalgae-based pMFCs exhibited comparable power densities of 15.57 ± 0.49 mW/m² (at 161.1 ± 2.54 mA/m²) and 16.00 ± 0.32 mW/m² (at 163.3 ± 1.66 mA/m²), respectively. The anode chambers demonstrated that chemical oxygen demand (COD) removal efficiencies ranging from 65% to 86% with the microalgae powder-based pMFC achieving the highest COD removal efficiency of 86.25%. Additionally, the cathode chambers exhibited oil and grease removal efficiencies of 42.3%, 37.7%, and 29.1% for pMFCs utilizing newspaper powder, fruit peels, and microalgae powder, respectively. Nutrient removal efficiencies in the cathode chambers were also significant, with nitrate–nitrogen (N-NO₃) removal reaching 76–80%, ammonium–nitrogen (N-NH₄) removal at 96–97%, and phosphate–phosphorus

(P-PO₄) removal at 90–94% across all pMFCs [54]. These findings indicate that challenging wastewater, such as SEO-contaminated water, can be efficiently treated in pMFCs, with effective treatment in the anode chamber playing a crucial role.

Additional studies have demonstrated the potential of pMFCs for the purification of bilgewater, which contains a mixture of fuel, oil, grease, detergents, and seawater. Single- and double-chamber pMFCs employing *Chlorella sorokiniana* have been investigated for this purpose [56]. The power density achieved in these setups exceeded that obtained for SEO treatment. In single-chamber pMFCs, power densities ranged from 71.3 to 83.4 mW/m², although the precise control of oxygen production was required through acetate supplementation and the optimization of light-dark cycles. The double-chamber pMFC exhibited superior power density, with an increase of up to 23.9% compared to the single-chamber system, due to the separate cathode chamber allowing the optimal utilization of O₂ as an electron acceptor. Both configurations effectively removed 67.2–77.4% of soluble chemical oxygen demand (SCOD) from synthetic bilgewater.

Furthermore, another oil-rich wastewater from the ghee industry, characterized by a COD of 8000 mg/L, has been successfully treated using pMFCs. This wastewater underwent sequential treatment in the anode and cathode chambers. Power and current densities of 3.33 mW/m² and 13.45 mA/m² were achieved, respectively, with a high COD removal efficiency of 96% [57]. These studies highlight the versatility of pMFCs in treating diverse complex wastewater streams, demonstrating their potential for sustainable wastewater management and bioelectricity generation.

Livestock wastewater contains organic matter, nitrogen, phosphorus, heavy metals, antibiotics, endocrine disruptors, microplastics, and antibiotic resistance genes [58]. Swine wastewater was treated in a pMFC with the cathode inoculated with *Arthrospira platensis* [59] and *Chlorella vulgaris* [60]. Both studies utilized diluted wastewater (with COD values of approximately 3000 mg/L and 1500 mg/L, respectively), which was initially treated in the anode chamber and subsequently in the cathode chamber by microalgae/cyanobacteria. Both studies achieved high efficiency in the removal of pollutant indicators.

Dairy wastewater has frequently been used as a substrate in pMFCs. Dairy wastewater has high organic content, and it is primarily composed of carbohydrates, proteins, and lipids [61]. *Scenedesmus dimorphus* was inoculated in the cathode chamber, while pre-treated anaerobic sludge from dairy wastewater was introduced into the anode chamber. In the conducted experiment, a power density of 360 mW/m³ was achieved, and the COD removal was 72% [52]. In another experiment, *Klebsiella pneumoniae* was utilized in the anode chamber with food wastewater, while *Chlorella sorokiniana* was used in the cathode chamber with dairy wastewater. The maximum power density achieved was 212 mW/m² [46]. Diluted food industry wastewater (COD of 2500 mg/L) was also used in the anode chamber of a pMFC with *Oscillatoria* sp. and *Scenedesmus* sp. in the cathode chamber. The maximum power densities obtained were 32.5 and 28.5 mW/m², respectively [44].

Another commonly used substrate in pMFCs is effluent from anaerobic digesters. Different types of anaerobic digester effluent have been evaluated, including those from the rubber, seafood, palm oil industries, and swine farms [62]. The maximum power density of 55.43 W/m³ was achieved using effluent from the rubber industry, which simultaneously removed COD, nitrogen, and phosphorus (72.4 ± 0.9%, 40.5 ± 0.8%, and 24.4 ± 1.5%, respectively). The authors proposed a system composed of four pMFCs with a photosynthetic-cathodic chamber connected in a vertical cascade, which could improve power density up to 116.9 ± 15.5 W/m³, sequentially treat wastewater, and also produce microalgal biomass (465 ± 10 g/m³) with a high lipid content (38.17 ± 0.01%). The anaerobic digestate from the rubber industry contained a high concentration of sulfate, which would increase the ionic strength and improve proton transfer, thereby reducing the

ohmic loss of the system. Although COD in the swine farm effluent was relatively higher than in other wastewater types, its complex organic substances could lead to an extremely slow hydrolysis rate, resulting in lower electricity production. As COD levels reach a high saturation point relative to the anode surface area, further increases in COD may not enhance electricity production. Anaerobically digested swine wastewater was directly used for cathode treatment with *Chlorella pyrenoidosa* [53]. This wastewater exhibited reduced COD levels (about 450–700 mg/L) and high nitrogen content (about TN 700–800 mg/L, ammonia nitrogen of 500–650 mg/L), making it ideal for direct utilization by microalgae. The obtained current density was 410 mA/m³, and the TN removal efficiency was 68% with a COD/TN ratio of 2.3.

Tempe wastewater has also been used in pMFCs though with different algae utilized in the cathodic chamber. One study used tempe wastewater along with *Cladophora* macroalgae and achieved a power density of 1293 $\mu\text{W}/\text{m}^2$ [59]. Similar results were obtained when a consortium of *Chlorella vulgaris* and *Arthrospira platensis* was used in the cathodic chamber [63].

Hydroponic wastewater from hydroponic agriculture contains high levels of nutrients caused by excessive dosing in hydroponic systems. This type of wastewater was also used in pMFC and achieved a maximum power density of 122.5 mW/m², a COD removal efficiency of 93.7%, nitrogen removal efficiency of 27.5% at the anode chamber, and N-NH₄ removal efficiency of 22.6%, along with a phosphate removal efficiency of 100% [64].

Aquaculture wastewater, in addition to feces and uneaten feed, contains phosphorus, nitrogenous compounds, and organic matter that, at high levels, disrupt the natural balance of aquatic ecosystems [65]. One study demonstrated that *Scenedesmus obliquus* can contribute to the removal of nutrients while producing added-value products (carbohydrates, proteins, lipids) that can be used as fish feed when using aquaculture wastewater [48].

Microalgal biomass can also be used as a substrate in the anode chamber, creating a closed-loop system. *Chlorella vulgaris* was used in this way, initially inoculated in the cathode, and the biomass, once cultivated and suspended in tap water to a COD value of 1100 mg/L, was introduced into the anode. The pMFC exhibited a COD removal rate of 75% and a power generation of 54.48 mW/m² [66].

The scientific studies presented above indicate that various types of wastewater, including those that are highly recalcitrant, can be treated in pMFCs. In some cases, a sequential treatment approach was applied, where a significant COD load was removed in the anode chamber, which was followed by nitrogen and phosphorus removal in the cathode chamber. However, in some studies, two separate wastewater streams were introduced into the anode and cathode chambers independently. The effectiveness of wastewater treatment in pMFCs largely depends on selecting appropriate system parameters, which will be discussed in the following section.

3. Influence of Environmental Parameters on pMFC Performance

pMFC are influenced by several technological parameters that can affect their overall performance in terms of power generation, efficiency, and sustainability. These parameters can be broadly categorized into environmental (i.e., light intensity, pH, temperature, CO₂ concentration), biological (microorganisms in anode and cathode chamber), and engineering factors (pMFC configuration and electrode) (Figure 2). This section will discuss the environmental parameters; the biological influence will be discussed in another section. The engineering factors were broadly discussed in the literature [67,68].

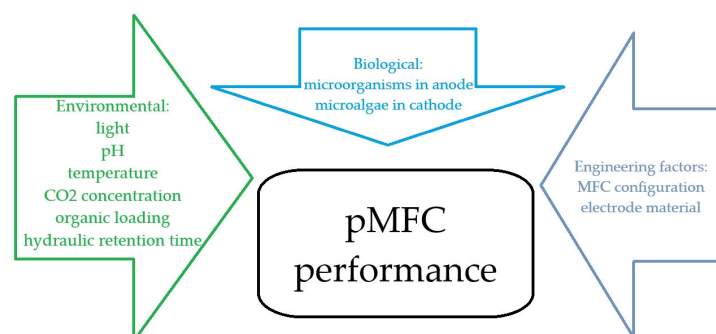


Figure 2. Factors influencing pMFC performance.

Since photosynthesis is essential for the operation of a pMFC, the influence of light and its intensity is a critical factor. The electrochemical behavior of pMFCs varied with the use of different wavelengths, suggesting that light quality and photosynthetically evolved oxygen increased pMFC voltage. This is because different microalgae species prefer different wavelengths; for example, *Scenedesmus obliquus* showed the maximum growth rate under blue light [48]. However, the generally recommended wavelength range for optimal photosynthetic activity falls between 400 and 500 nm, aligning with the absorption spectra of photosynthetic pigments.

The optimal light intensity should be selected based on the microalgae species used in the cathode chamber. For instance, *Chlorella vulgaris* achieved a maximum power density of 126 mW/m³ under a light intensity of 5000–6500 lx, whereas a continuous light intensity of 10,000 lx resulted in the highest biomass concentration of 4 g/L [69]. Therefore, the selection of light intensity should be tailored to the specific objectives of the system, whether maximizing power generation or enhancing biomass production. Moreover, the variability in light intensity preferences among different species underlines that the selection of the light source and intensity should be based on prior research before experimentation.

Furthermore, studies have demonstrated that continuous illumination increases power density by 12.7% compared to an intermittent light cycle [70]. During dark periods, power production is significantly reduced or absent [3]. However, incorporating a light/dark cycle is essential for maintaining the physiological health of the microalgae and promoting mixotrophic growth, which is beneficial for biofuel and bioproduct synthesis [69]. During the dark phase of photosynthesis, CO₂ produced within the system is converted into valuable products such as sugars and biomass (above-mentioned in the Introduction). Not only does a light regime that includes a dark phase provide an economic benefit by reducing energy consumption, but the accumulation of valuable compounds in the biomass can also offer additional advantages.

A neutral pH (6–8) is optimal for maximizing MFC performance and ensuring efficient microbial metabolism in both the anode and cathode chambers [71]. Proton generation primarily results from the electrochemical oxidation of organic substrates rather than microbial degradation within the anode chamber [72]. The accumulation of protons leads to the formation of a pH gradient, which increases internal resistance and gradually diminishes the pH gradient potential over time [73]. This, in turn, can lower current density and electrode potential [71]. Under optimal conditions, cathodic reactions facilitate the reduction of O₂ by protons and electrons, forming water. Any imbalance in electron transfer, proton availability, or oxygen concentration disrupts the pH gradient, ultimately reducing electricity generation. While the theoretical cathode potential at pH 7.0 is 0.81 V, experimental values are consistently lower due to the mixed potential effects of contaminant species.

The other key parameter influencing microalgae growth and affecting pMFC performance is CO₂ concentration. Microalgae exhibit varying responses to different carbon

dioxide (CO₂) concentrations with most species thriving at low levels and experiencing growth inhibition at elevated concentrations. In general, species inhibited by CO₂ concentrations below 2–5% are considered CO₂-sensitive, while those that can cope with up to 20% CO₂ are deemed more tolerant [74]. Some species, however, have developed mechanisms to tolerate and even flourish under higher CO₂ conditions. For instance, *Dunaliella salina* has been shown to tolerate CO₂ concentrations up to 30%, achieving a maximum biomass concentration of 1.13 g·L⁻¹ after 8 days of incubation, which is 1.85 times higher than that under atmospheric CO₂ levels [75]. Notably, certain microalgal species can withstand extremely high CO₂ levels. For example, *Cyanidium caldarium* can grow in pure CO₂ environments, and *Scenedesmus* species have been reported to tolerate CO₂ concentrations up to 80% (v/v). However, *Scenedesmus* and *Chlorella* had about the same growth rate when the CO₂ concentration was in the range 10–30% [76]. For most of the algae species, adaptation to higher CO₂ concentrations is required for the direct use of carbon dioxide from exhaust gases/anodic chamber. It was found that a gradual increase in CO₂ concentration gave a better growth rate [77]. Overall, the CO₂ tolerance of microalgae is dependent on cell density, pH, nutrients, light, and, as mentioned above, species selection. Microalgae in the cathode chamber used CO₂ produced by the degradation of organic waste in the anode. In one study, a pMFC was constructed to capture carbon by microalgae through directing the anode off-gas into the cathode chamber, promoting the growth of *Chlorella vulgaris* [78]. *Chlorella* sp. was also cultivated in the cathode chamber with the addition of flue gas (10% CO₂ v/v), resulting in high biomass production (1.34 g/L) and a CO₂ sequestration rate of 190.9 mg/(L·d) [66]. In another study, pMFC fed with hydroponic wastewater also effectively captured CO₂ from the anode chamber with an algal biomass yield of 0.01379 g/(L·d) and a CO₂ fixation rate of 0.02528 g/(L·d) [65]. In contrast, *Tetraselmis indica* exhibited a CO₂ fixation rate of approximately 0.087 g CO₂/(L·d) [72]. However, when CO₂ is solely derived from the anode chamber, it may hinder energy production due to its limited quantity [79]. The biogas with high sulfide (961 to 2800 ppm) and ammonia (1300 to 2800 ppm) concentrations might be also treated in pMFC [79]. The obtained biogas contained 85–93% CH₄ with no traces of CO₂, H₂S, or NH₃. The pMFC produced power and current densities of 17.3 mW/m² and 170 mA/m², respectively. The CO₂ captured in the form of bicarbonate resulted in total algal biomass production of 6.8 g/L. Microalgal CO₂ fixation is a complex process. Usually, most of the physicochemical and hydrodynamic process parameters have important nonlinear effects on microalgal growth [80]. However, their detailed reaction mechanisms, especially the impact on the activity of carbonic anhydrase and carbon concentration mechanism, have not yet been completely understood and still need to be explored.

Both the carbon dioxide content and the intensity of light translate into a parameter that directly affects the pMFC output, i.e., the dissolved oxygen content. By properly controlling these parameters, we influence the photosynthesis process, as a result of which a difference in potentials is created between the pMFC compartments, increasing the amount of energy produced.

A broad temperature range (15–45 °C) can be applied for the anode chamber reactions. However, the cathode chamber temperatures should be in range of the optimum for microalgae cultivation. *Chlorella* sp. is characterized by a higher spectrum of temperatures, whereas *Arthrospira platensis* requires higher temperatures for optimal growth [71].

Technological parameters such as hydraulic retention time (HRT) and organic loading rate (OLR) significantly influence electricity generation and the overall efficiency of pMFCs. These parameters directly affect the morphology of the biofilm and the growth dynamics of the microbial community. For instance, a study by Nam et al. [81] demonstrated that electricity generation increased from 1884 to 2981 mW/m³ when the substrate loading

rate was raised from 1.9 to 3.9 g/(L·d). While a higher organic loading rate can enhance power output, it is often accompanied by a reduction in Coulombic efficiency, indicating a trade-off between these performance metrics. Excessive COD loading can lead to competition between exoelectrogenic and non-exoelectrogenic bacteria, as a high COD corresponds to increased saturation of the anode surface. Electrical energy generation was higher at low OLRs than at high OLRs, as exoelectrogens were unable to effectively oxidize the entire substrate to generate electricity. As a result, excess substrate created niches for methanogens, leading to electron utilization for methane production [14]. Since excessively high COD reduced power density, it is crucial to determine the optimal OLR for the effective harvesting of maximum energy for bioelectricity generation. Furthermore, excessive OLRs can also increase the risk of membrane fouling, which may adversely affect energy generation. On the other hand, a lower OLR results in a longer retention time of wastewater in the anodic chamber. This allows the bacteria more time to assimilate the substrate and achieve higher COD removal efficiency. Additionally, nitrogen and phosphorus removal rates also could decrease when the OLR is too high.

Appropriate HRT in the anode also determines whether the anodic effluent of the pMFC can be utilized in the cathode chamber as a growth medium for microalgae. If a high COD is introduced into the cathode chamber, microalgae will perform heterotrophic metabolism, which diminishes photosynthesis and oxygen production. In a study using synthetic wastewater with a COD of 1500 mg/L in a pMFC with *Chlorella vulgaris* in the cathode chamber, after 10 days, the efficiency of COD removal was 60% and the power generation was 0.0254 kWh/kg COD. After an additional 10 days in the cathode, the efficiency of COD removal increased to 74%, and N-NH₄ removal reached 79% [82].

The performance of microalgae-assisted MFCs is influenced by a complex interplay of different factors. To optimize performance, it is crucial to carefully manage parameters such as light intensity, nutrient availability, pH, temperature, electrode materials, and system configuration and algae species.

4. Microorganisms in Photosynthetic Microbial Fuel Cell

One of the primary advantages of pMFCs is their use of microorganisms as biocatalysts. These microorganisms possess the molecular machinery necessary to transfer electrons to the electrode without the need for external mediators. Electrical current is generated through a continuous electron flow, which results from bacterial metabolism within anodic biofilms. The electron transfer rate depends on the electrochemical behavior of bacterial metabolism in these biofilms. Bioelectroactive species—such as outer membrane cytochromes, conductive pili, and extracellular exudates—play a crucial role in facilitating electron transfer between the biofilm and the anode electrode [83]. Additionally, biofilm thickness on the anode should be controlled at approximately 40 µm to ensure sufficient substrate availability for microorganisms directly attached to the electrode [84]. Several abiotic factors can also influence the performance of bioelectroactive species, including oxidation-reduction peaks observed in cyclic voltammetry of the anodic biofilm, which vary depending on pH [85]. The biofilm formed on the anode is a diverse microbial consortium with a complex spatial structure. Its thickness and species composition vary depending on the type of substrate and operational conditions [86]. Processes occurring in the cathode chamber also influence biofilm composition. For instance, in batch-fed pMFCs, microorganisms capable of producing redox mediators are selectively enriched, whereas in continuous-flow systems, frequent fluid exchange alters microbial selection pressures.

The microbial communities within the anode chamber vary significantly depending on the type of wastewater or substrate used, as they are responsible for hydrolyzing organic matter. For instance, in cellulose-based wastewater treatment, *Enterobacter cloacae* IIT

BHU M2V2 [54] and the facultative strain *Cellulomonas* Lsc-8 [55] have been successfully employed. The latter strain also demonstrated the ability to remediate Cr(VI), reducing the highly toxic Cr(VI) to the less toxic Cr(III) while simultaneously generating electricity using cellulose as a carbon source. In cafeteria wastewater treatment, *Saccharomyces cerevisiae* yeast was cultivated in the anode chamber [45], whereas pharmaceutical industry wastewater was treated using a *Bacillus cereus* bacterial culture [42]. Another study utilized *Klebsiella pneumoniae* to treat food wastewater in the anode chamber [46], and *Klebsiella* was also identified as the dominant species in a pMFC treating aquaculture wastewater [47]. However, a separate study on aquaculture wastewater treatment found that *Azospirillaceae* (*Proteobacteria*), followed by *Geobacteraceae* (*Thermodesulfobacteriota*), were predominant in biofilm formation [48]. Due to cost considerations, mixed microbial consortia are often employed. Microbial community analyses revealed that anodic biofilms predominantly consist of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, highlighting the diversity and adaptability of microbial populations in facilitating substrate degradation and electron transfer [62,87].

Microalgae are employed in the cathode chamber to enhance oxygen availability and to provide secondary treatment of effluent from the anode chamber. To maximize their remediation effectiveness, careful strain selection and the optimization of light intensity, temperature, nutrients, and cultivation conditions are essential. The most commonly used microalga in cathode chambers is *Chlorella vulgaris*, which is valued for its ability to produce lipids for biodiesel production. Genetic modification techniques may also be applied to enhance the ability of microalgae to degrade pollutants or synthesize valuable products. For example, *Chlamydomonas reinhardtii* has been engineered for carotenoid production [88]. Specific genes responsible for hydrocarbon degradation pathways can be introduced to enhance algal remediation capabilities. Various microalgae species have been utilized in this context, such as *Chlorella sorokiniana*, which was isolated from bilgewater [55]. *Arthrospira platensis* has been shown to produce sufficient oxygen to enhance electrochemical performance compared to a water-fed cathode when treating swine farm wastewater [59].

For specialized effluents such as bilgewater, some researchers have isolated microalgae directly from wastewater [56] or from natural water bodies [57,60] to enhance growth and treatment efficiency. Selecting an appropriate algal species can also improve oxygen availability in the cathode chamber. For instance, *Scenedesmus dimorphus* has been inoculated in cathode chambers, while pre-treated anaerobic sludge from dairy wastewater has been supplemented in anode chambers. Additionally, *Tetraselmis indica* has been cultured in photobioreactors for the further treatment of pharmaceutical industry wastewater following pMFC processing [72]. *Arthrospira platensis* offers an advantage over other microorganisms due to its alkaliphilic nature, allowing it to thrive in media derived from swine wastewater [63].

Experiments have also been conducted to enhance cathode chamber efficiency by integrating activated sludge with microalgae. These studies demonstrated that such hybrid systems provided higher power densities and improved nitrogen removal. The coexistence of both suspended and attached microorganisms, along with biofilm formation, facilitated nitrification and denitrification processes under periodic illumination, thereby increasing pollutant removal efficiency and system stability.

The selection of the most suitable microalgal species depends on wastewater characteristics and specific treatment objectives. Commonly used genera such as *Chlorella*, *Scenedesmus*, and *Arthrospira* are preferred due to their rapid growth, high nutrient uptake efficiency, and resilience under various environmental conditions. The optimal choice of algae requires balancing factors such as the nutrient removal capacity, growth rate, environmental adaptability, and ease of harvesting to maximize treatment efficiency and overall system performance.

5. Advantages of pMFC

pMFCs offer several additional advantages, including (i) operation across a broad temperature range under neutral pH conditions, (ii) the ability to utilize a wide variety of complex wastewaters as substrates, (iii) direct energy production from substrate conversion, (iv) value-added product generation, and (v) a moderate environmental impact [89,90]. These benefits make pMFCs highly versatile for applications such as electricity and bioenergy production as well as wastewater treatment.

A comparison between pMFCs and conventional wastewater treatment plants (WWTPs) highlights significant differences in energy consumption and efficiency. pMFCs stand out due to their remarkably lower energy demand and the added advantage of energy recovery from waste. In contrast, conventional WWTPs, particularly those relying on activated sludge processes, require substantial energy input. The activated sludge method depends on aeration to facilitate the microbial degradation of organic pollutants with aeration alone accounting for the largest portion of energy consumption. On average, conventional activated sludge processes consume approximately 0.8–1 kWh per kg of COD removed, depending on wastewater characteristics and treatment configurations. This high energy demand represents a major operational cost and contributes to the overall carbon footprint of wastewater treatment [91,92].

In comparison, pMFCs consume significantly less energy, requiring only 0.024 kW or 0.076 kWh/kg COD. This represents an approximately tenfold reduction in energy consumption compared to conventional activated sludge bioprocesses [93,94]. The energy savings primarily result from the unique nature of pMFCs, where bacteria in the anodic chamber degrade organic matter, simultaneously recovering energy, while microalgae in the cathodic chamber enhance system efficiency.

The integration of microalgae as biocatalysts and oxygen suppliers in pMFCs further improves economic and energy balance by reducing or even eliminating aeration costs. Additionally, microalgae contribute to greenhouse gas mitigation by directly consuming CO₂ produced in the anode. Beyond energy benefits, microalgae enhance the circular economy potential of wastewater treatment by enabling the recovery of valuable materials and biofuel precursors. In particular, algae biomass conversion can yield liquid biofuels, further increasing the economic viability of the system [46]. Beyond biofuels, algae biomass contains substantial amounts of proteins, carbohydrates, and other nutrients, opening opportunities for alternative high-value applications. These include the production of chemicals, the recovery of various materials, and the development of slow-release fertilizers, which can be obtained in the form of dried biomass or biochar through pyrolysis, depending on processing conditions. Biofertilizers and biostimulants have emerged as particularly promising and economically attractive sectors within algae technology [95].

Thus, the integration of pMFCs with microalgae presents a sustainable and economically viable approach to wastewater treatment. By leveraging resources embedded in liquid waste streams, reducing energy demands, and enhancing product recovery from algae biorefineries, this technology has the potential to deliver far-reaching environmental and economic benefits beyond bioelectricity production alone.

6. Conclusions

Although microalgae-based microbial fuel cells (pMFCs) offer an eco-friendly and sustainable solution, the technology still faces several challenges. These include controlling biofilm formation in the anode chamber, selecting suitable microalgae strains, and characterizing their biochemical composition to assess their potential for power generation—all of which impact net energy yield. Future research should focus on scaling up pMFC systems, enhancing electricity production, and leveraging genetic engineering to optimize

microbial and algal performance. Integrating pMFCs into existing wastewater treatment infrastructure presents a promising pathway toward energy-neutral or even energy-positive operations, driving a transformative shift in sustainable environmental management.

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