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Review article

Recent progress in microbial fuel cells using substrates from diverse sources

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ABSTRACT

Increasing untreated environmental outputs from industry and the rising human population have increased the burden of wastewater and other waste streams on the environment. The most prevalent wastewater treatment methods include the activated sludge process, which requires aeration and is, therefore, energy and cost-intensive. The current trend towards a circular economy facilitates the recovery of waste materials as a resource. Along with the amount, the complexity of wastewater is increasing day by day. Therefore, wastewater treatment processes must be transformed into cost-effective and sustainable methods. Microbial fuel cells (MFCs) use electroactive microbes to extract chemical energy from waste organic molecules to generate electricity via waste treatment. This review focuses use of MFCs as an energy converter using wastewater from various sources. The different substrate sources that are evaluated include industrial, agricultural, domestic, and pharmaceutical types. The article also highlights the effect of operational parameters such as organic load, pH, current, and concentration on the MFC output. The article also covers MFC functioning with respect to the substrate, and the associated performance parameters, such as power generation and wastewater treatment matrices, are given. The review also illustrates the success stories of various MFC configurations. We emphasize the significant measures required to fill in the gaps related to the effect of substrate type on different MFC configurations, identification of microbes for use as biocatalysts, and development of biocathodes for the further improvement of the system. Finally, we shortlisted the best performing substrates based on the maximum current and power, Coulombic efficiency, and chemical oxygen demand removal upon the treatment of substrates in MFCs. This information will guide industries that wish to use MFC technology to treat generated effluent from various processes.

1. Introduction

Industrialization and modern lifestyles have exploited the environment, creating sanitation issues for human beings and ecosystems [1,2]. Industrial expansion boosts the economy but brings with it waste disposal problems. Environmental release of untreated wastewater from both industry and household use leads to environmental contamination due to algal blooms and eutrophication [1]. Wastewater treatment is an energy-intensive and resource-intensive process [1,3,4]. This process results in the release of greenhouse gases and volatile substances into the atmosphere, and the sludge generated also creates disposal issues [5]. As untreated waste water has many sources and tracks with local economic factors [6], treatment of wastewater should be transformed from a cost to a benefit, as is being considered with other "waste-to-wealth" initiatives [7], in which biotechnology is factoring in heavily [8]. Indeed, wastewater has a high energy content and can be used as an energy source [1,5]. This review aims to bring into focus the rapidly developing technology microbial fuel cells (MFCs) as a biotechnology that can naturally convert wastewater from various sources into energy, while remediating the waste stream. Given the range of waste streams produced around the world, the specific focus is placed on recent progress in MFCs using substrates from these diverse sources.

Microbial fuel cells (MFCs) use electroactive bacteria for simultaneous power generation and wastewater treatment. The use of MFCs in the treatment of wastewater was first proposed in the late 20th century, but since then, continuous developments have enhanced the productivity

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of this technology [9,10]. MFCs solve waste treatment issues by utilizing the chemical energy of waste material to generate electricity [11,12,13]. Compared to other waste management technologies such as conventional biological sewage treatment plants, MFCs offer a range of benefits, including operation at room temperature, minimized sludge production, and environmental friendliness while simultaneously producing electricity [1,14].

As shown in Figure 1, a typical MFC consists of an anode and is separated by an ion-exchange membrane cathode (a cation exchange membrane (CEM)), or anion exchange membrane (AEM), [9,15]. Exoelectrogens form electroactive biofilms (EAB) usually reside on the anode surface, which act as biocatalysts for substrate oxidation to produce electrons and protons by coupling certain redox processes to their metabolic cycles. The electrons are transferred into the anode from the attached EAB via direct and indirect electron transfer mechanisms [16]. Due to the difference in potential between anode and cathodes, elections travel from the anode to the cathode through an external circuit where they perform work. Thus, the current and cell potential are critical parameters in determining power outputs. The current is also related to the rate of oxidation. Protons generated from substrate oxidation usually travel through a proton exchange membrane into the cathode chamber, where they combine with electrons, protons and terminal electron acceptors, which is usually oxygen through the oxygen reduction reaction, forming water. It has been considered that economically and environmentally sustainable systems will use air cathodes for oxygen reduction [17].

There are two types of electron transfer mechanisms in MFCs: mediator and mediator-less [18,19]. The mediator-type MFC requires soluble electroactive molecules to shuttle electrons to the anode surface. The use of mediators poses economic and safety constraints on the MFCs. Mediator-less MFCs exploit the ability of metal-reducing bacteria such as *Geobacter metallireducens*, *Aeromonas hydrophila*, *Shewanella putrefaciens*, *Rhodoferax*, *Klebsiella pneumonia* to generate electricity without mediators via direct electron transfer through physical contact [18,19]. On the cathode side, the reduction of an electron to water can be catalyzed by metals such as platinum or palladium in a single-chamber air cathode setup. When using well-defined substrates, (e.g., glucose or acetic acid), known anode potentials can be used in conjunction with respective cathode potentials to calculate basic thermodynamic features of the cell, such as open circuit potentials which typically range between 0.3 and 0.8 V [20]. Recently, useful methods have been demonstrated that allow researchers to measure individual anode and cathode potentials which will be helpful in benchmarking fundamental substrates and their mixtures, as discussed in this work [21].

Substrates provide food to the bacteria in MFCs and hence play a significant role. Thus, substrates play a vital role in the development of biofilms. The biofilm can be modulated by optimizing the substrate feed, and different substrates have been used in MFCs. There are several strategies to modulate microbiome of MFCs, but a more effective strategy than optimizing feed would be by adopting particular start-up and operating conditions [22]. Several factors affect the efficacy of the MFCs, such as the type of substrates used (simple or complex), the electrode material, and the microorganisms used to oxidize substrate molecules [23,24]. MFCs can use substrates from diverse wastewater sources ranging from domestic [25,26,27] to agricultural [1,28,29], industrial [14,30], pharmaceutical [31], and animal [32,33], among others. It is easier for the EAB to metabolize liquid waste; solid waste is difficult to metabolize due to low hydrolysis, and heterogeneity could add to slow mass transport [34].

This review focuses on the recent expansion of MFC technology for treatment and electricity generation from an ever-expanding range of wastewater sources. For example, complex substrates are a mixture of different chemical compounds and often contain microbial communities [35]. These complex substrates primarily include domestic wastewater from municipal [36,37], kitchen, and food waste [38,39] sources. Industrial wastewater typically originates from distilleries [25,40], palm oil factories [41], dark fermentation systems [42], landfill leachate [43], petroleum industry [44], textile factories [45,46] and the chocolate industry [47]. Agricultural sources include waste from cellulose [48,49], soybean [50], molasses [51,52], lignocellulosic biomass [53,54] and tofu [3]. Animal waste includes waste from fish markets [55], swine [33,56], cattle [57], seafood processing [58], slaughterhouses [59,60], biogas slurry [61] and poultry. Fruit waste includes the peels of fruits [28] and juices [29]. Chemical waste comes from sources such as azo dye [45,62], ethanolamine [63,64], sulfide [65], nitrate [66], isopropanol [67], and pharmaceutical components [31]. Dairy waste [68] consists of cheese whey [69] and yogurt waste [70]. Also, applications of MFCs for greywater treatment in view of non-potable reuse has been reported [71] as have been options for groundwater remediation. It should be noted that many of these sources are based on specific processes or input parameters

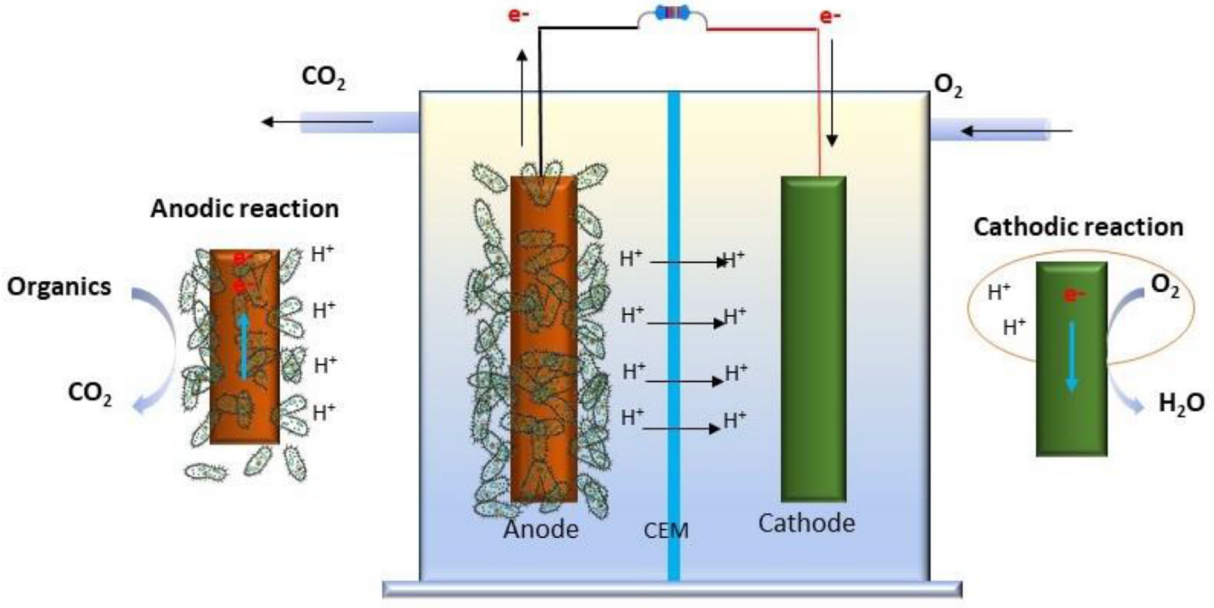

Figure 1. Schematic and working principle of the typical microbial fuel cell.

that may vary. This is especially the case for complex substrates with often unpredictable compositions (example kitchen waste and wastewater). All of these materials have been examined as substrate sources for MFCs in recent years and are reviewed in detail in the next section. This includes operating pH, and chemical composition of the substrate molecules, which directly influence the MFC performance.

The potential to commercialize MFC technology can be dictated by a series of performance indicators, each of which are significantly influenced by substrate type. Here we consider the most important metrics to be (i) current density (CD), which is a direct indicator of the reaction kinetics; (ii) power density (PD), which is the most important indicator of the potential of the MFC to be used as a power source; (iii) Coulombic efficiency (CE), which describes the efficiency in converting redox molecules to electrons; and (iv) carbon oxygen demand (COD) removal efficiency (ΔCOD), which describes the ability to remove organic materials from waste streams, or roughly speaking, to clean the waste stream. While power production is an enticing direction for MFC, its role in future energy markets is still undefined. On the other hand, the potential to remediate waste streams without external energy requirements (nor their associated CO_2 emissions) stands out as an important capability for near-term MFC implementation. Therefore, with the goal of strategically incorporating MFC technology, COD removal efficiency may be the most important figures of merit in the near-term. As CD is a marker of the rate of COD removal, this should be considered closely as well. The literature shows that COD removal can be efficient down to concentrations as low as 100 mg L^{-1}, and that current densities become reduced and stop outright at 50 mg L^{-1} [72,73], but recent studies have demonstrated methods to reduce this limit by 50 times [74].

We also note that MFCs can be further improved using highly efficient electrodes with nanoactive materials, and ions selective ceramic-based membranes are to be developed and investigated for cost-effective scale-up of MFCs. Further, to reduce the cathode cost and improve cathodic performance, photosynthetic bacteria could be used to make self-sustained cathode. Upon these modifications, MFCs can be used for potential applications in environmental sensors using 3D-printed biofilms for large-scale power generation and deployment in the wastewater treatment process.

2. Defined substrates

2.1. Acetate

Acetate is a widely acceptable substrate of MFCs, and a simple substrate used by electroactive microbes as the primary carbon source. Acetate is a preferred substrate in MFCs because it is inert for microbial conversions such as fermentations or methanogenesis at room temperature [75]. Electrogenic bacteria prefer acetate as their substrate [76]. The production of current increases rapidly in MFCs with acetate as a substrate. Moreover, the biofilm at the anode comprises a large amount of electrogenic bacteria, leading to higher *CE* and cell voltage compared to xylose as substrates. Acetate is popularly considered a substrate for the production of electricity. It is used as a substrate to scale reactor designs, constituents of MFC, and operational designs because it is inert towards other conversions like methanogenesis and fermentations at room temperature. Acetate is also the end product for other carbon sources with numerous metabolic routes [77].

An air-cathode single-chamber mediator-less MFC was constructed to use acetate as a feed for assessment of the MFC efficiency for electricity generation and wastewater treatment [75]. A study based on the utilization of wastewater as a substrate pointed out that the use of acetate in pretreatment of wastewater through the process of anaerobic acidogenesis increases the efficiency of the MFC [78].

An investigation compared four different substrates, namely, acetate, xylose, a 1:1 mixture of acetate and xylose, and bioethanol effluent (BE), to investigate the electricity generated by MFCs. Each substrate was sequentially used as the initiator, followed by the addition of another substrate [79]. The results show that when the MFCs were initiated by acetate, the initiation time was one day, shorter than other carbon sources, and the cell *CE* was 31.5 ± 0.5 %. The initiation time was shorter, and the cell voltage and *CE* values were higher than in MFCs initiated by a combination of acetate, xylose, or only xylose. Also, more electrogenic bacteria such as *Geobacter sulfurreducens* and *Desulfuromonas acetexigen* were present in acetate-initiated MFCs. Furthermore, when the substrate in acetate-initiated MFCs was switched to BE, the *CE* values were 25 ± 0.5%, higher than with use of BE as the sole substrate or in combination with other substrates.

2.2. Synthetic wastewater

Synthetic wastewater, also known as artificial wastewater, has been popularly used as a defined substrate for MFCs to estimate efficiency in the presence of realistic yet repeatable substrate conditions. The composition generally mimics one or another form of wastewater. Synthetic wastewater is usually rich in one of three carbohydrate molecules: glucose, soluble starch, and sucrose. Additionally, proteobacteria are found abundantly in synthetic wastewater. Chemical wastewater or synthetic wastewater is widely used in research studies due to the ease of modulating the parameters such as conductivity, loading strength, and pH. Reduced sulphur is present in high-strength wastewater and acts as an abiotic electron donor, which enhances the short-term generation of current [80]. The short-term generation of current masks the actual activity of the system, which can be averted by adding a small amount of acetate or glucose to the wastewater medium.

In one study, those formulations were used in inoculations for anaerobic sludge and a microbial mixture to evaluate the performance of single-chamber membrane-less MFC [24]. In that study, the MFC fed with glucose-based synthetic wastewater showed the highest efficiency parameters, specifically, when using a microbial solution as inoculum. The power density and *COD* removal rates were 218 mW m^{-2} and 98.8%, respectively; in the case of an anaerobic sludge used as inoculum, these values were 456.8 mW m^{-2} and 94.3%, respectively. The results indicate that the MFC efficiency was progressively impeded with the increasing complexity of the substrate utilized. Further, it was observed that waste removal efficiency was affected by the microbe species used in the inoculum of microbes.

Another study using synthetic wastewater assessed the efficacy of four different plant species (*Typha angustifolia L.* (Typhaceae)), *Typha latifolia L.*, *Juncus gerardii Loisel.* subsp. *gerardii* (Juncaceae), and *Carex divisa HUDSON* (Cyperaceae) for wastewater treatment. The electricity generation using MFCs revealed that the species *Typha angustifolia* efficiently treated synthetic wastewater and generated electricity with PD_{max} of 7.47 ± 13.7 mW m^{-2} and *CE* of 8.28 ± 10.4% [81].

Nitrobenzene is a toxic pollutant mostly used in dyes, explosives, rubber, etc., and can accumulate in groundwater. The treatment of nitrobenzene-containing wastewater is a major environmental concern. Xie et al. 2018 studied the efficacy of a single-chamber membrane-less MFC coupled with constructed wetland (MFC-CW) in the degradation of nitrobenzene-incorporated wastewater [82]. The study results showed that the MFC-CW produced a PD_{max} of 1.53 mW m^{-2}, which was an improvement over a plain MFC with a PD_{max} of 0.59 mW m^{-2}. The nitrobenzene removal rate in the MFC-CW was 92.28%, and the *COD* removal efficiency was between 67.92% and 78.30%. The study shows that the MFC, when coupled with wetlands, can be a possible solution for treating nitrobenzene wastewater and electricity generation.

In another study, synthetic wastewater with xylose was used to assess the power generated by a two-chamber up-flow MFCs under the fed-batch condition. Synthetic wastewater with xylose was made to mimic the lignocellulosic material, and the bacterial composition was also assessed using molecular detection techniques. Different recirculation rates were used to check the mass transfer and yield of electricity [83]. The results showed a recirculation rate of 4.8RV h^{-1}, PD_{max} of 356 ± 24 mW m^{-2}, and a *CE* of 21.3 ± 1.0%.

One of the studies compared the microbial composition in two-chambered MFCs with either synthetic wastewater or a pig slurry as the substrate source [84]. To reduce the competition between exoelectrogenic bacteria and methanogens, both of the MFCs were also fed with the methanogenesis inhibitor 2-bromoethanesulfonate (BES-Inh). The inoculum for the MFC fed with pig slurry was the biofilm from the anode of the MFC with synthetic wastewater taken 45 weeks after the MFC operation. The study shows that the PD_{max} in synthetic wastewater was 2.138 W m^{-3}, which was less than that of the pig slurry-fed MFC with a PD_{max} of 5.623 W m^{-3}. Also, the addition of BES-Inh to the synthetic wastewater MFC didn't result in any change in the PD_{max} values. Furthermore, the study results reveal that the microbial community depended on the substrate type, with different species dominating with the use of each substrate. In addition, the microbial composition was affected by 2-bromoethanesulfonate (BES-Inh). The study identified the presence of exoelectrogenic bacteria and their efficiency using synthetic wastewater.

2.3. Glycerol

Glycerol is a major residual by product of biodiesel production and is an essential ingredient for the pharmaceutical, tobacco, food, and beverage industries. Like any other commodity, the price of glycerol is influenced by the demand and supply chain but is mainly dependent on biodiesel production. To make the production process of glycerol economical, researchers decided to use it as an energy source for generating bioelectrochemical energy [85]. Glycerol is highly available and low in cost, with a reduced chemical structure, making it a suitable feed stock.

Additionally, glycerol can be transformed into multiple platform products and intermediates, and microorganisms can metabolize glycerol naturally. Glycerol fermentation produces different types of alcohols such as ethanol, n-butanol, 2,3-butanediol, and 1,2-propanediol. Thus, the conversion of glycerol via modified technology is still in high demand.

A two-chamber MFC was used in the study with a variable concentration of glycerol, i.e., 0.5–5.2 g *COD* L^{-1}, and the results show that at a concentration of 3.2 g *COD* L^{-1} the efficiency of MFC was the highest with CE_{max} of 34.1%, *COD* removal efficiency of 99% and PD_{max} of 65.4 mW m^{-2}. A study was conducted to evaluate the efficacy of glycerol as a substrate in a MFC by applying concentration pulses and identifying the reaction intermediates [86]. The study aimed to improve the understanding of reaction dynamics, which can enhance electricity production through glycerol. However, glycerol is a defined substrate for MFCs but is not widely used due to its refractory properties and a lack of exo-electrogenic bacteria that use it as a substrate. A study conducted to enhance glycerol-based MFC efficiency revealed that using a co-culture of *Shewanella oneidensis* strain MR-1 and *Klebsiella pneumonia* strain J2B showed a PD_{max} of 2.15 mW m^{-2} [87]. The study results show that combining these two bacterial species improved energy recovery from the fuel cells.

Table 1 provides an account of performance of defined substrates in MFCs.

3. Complex substrates

3.1. Domestic wastewater

Domestic wastewater, which is a part of municipal wastewater, is categorized as a weak, medium, and strong based on the concentration of dissolved substances. Concentrations of dissolved organic materials of 250 mL^{-1}, 500 mL^{-1}, and 1000 mL^{-1} denote weak, medium, and strong wastewater, respectively [90]. Domestic wastewater is generated from households by day-to-day activities like washing, bathing, flushing, cleaning, etc. It is a major nuisance to the cleanliness of society. Despite a low *COD* relative to other wastewaters, domestic wastewater still needs to be treated before being discharged into environmental waterbodies. Earlier studies showed that a sudden substrate change from acetate to domestic wastewater reduced the MFC efficiency. Therefore, a study was performed to assess whether this negative effect can be reduced by gradually exchanging acetate for domestic wastewater. A flat-panel air-cathode MFC (FA-MFCs) was used to test the conductivity and biodegradability of wastewater [91]. A substrate was added with an increasing percentage of domestic wastewater, i.e., only artificial medium, 25%, 50%, 75%, and 100% wastewater mixed with acetate and raw domestic wastewater. The results showed that increasing the domestic wastewater concentration reduced the PD_{max} from 187 to 60 W m^{-3} and the organic removal efficiency from 51.5 to 37.4%. Further, no change in *CE* was observed, which remained around 18–19%. This study concludes that FA-MFCs provide a practical solution for treating domestic wastewater with low conductivity and low biodegradability. In another study, the performance of an air-cathode MFC was evaluated using three different types of substrate pre-acclimation strategies [92]. In the first case, serial pre-acclimation was done using acetate and glucose before adding domestic wastewater. In the second case, only acetate was used for pre-acclimation, followed by the addition of domestic wastewater, and in the third case, no pre-acclimation was done. Each strategy gave rise to a diverse microbiome with (i) growth of EAB in the first case, (ii) inhibition of fermentative bacterial growth in the second, and (iii) uncontrolled and spontaneous growth of bacteria in the third case. The results show that the first strategy resulted in a high current generation with a value of 1.4 mA and *CE* of 33.5%, while the second and third strategies showed 0.7 mA and 9.4% and 0.9 mA and 10.3% for values of current and *CE*, respectively [92]. The *COD* removal for all three strategies was 69%. The bacteria (*Proteobacteria*, *Actinobacteria*, *Geobacter*) were identified using pyrosequencing, and it was observed that pre-acclimation positively influenced the growth of bacteria capable of generating electricity. Thus, pre-acclimation can be a useful method to enhance electricity generation and treatment of domestic wastewaters.

Although MFCs can treat domestic wastewater, they are unsuccessful in removing the major ionic pollutants from the water found in their effluent. A novel approach toward electricity generation and treatment using domestic wastewater effluent was developed using a combination of MFC and capacitive deionization (CDI) [93]. The results show that MFCs could reduce 90% of *COD* and ammonium during the secondary treatment of water and that CDI can further purify the effluent of secondary treatment by removing ionic pollutants. In a separate study, 40 individual air-cathode MFC units were used individually, connected in parallel and connected in series. The efficacy of the three different connection systems was assessed for efficacy in domestic wastewater treatment [94]. The connection system parameters tested in all three cases were the variations in type of bacteria deposited at the anode surface and changing the connection from individual to either series or parallel configurations; all affected the performance of the MFC. In the case of a stacked MFC in series connection, the electricity produced was much higher, with a PD_{max} of 2500 mW m^{-2} and maximum current density (CD_{max}) of 500 mA m^{-2}, along with high biodegradation with *COD* removal of 84% and ammonia removal of 80%. To improve the efficacy of MFC in treating real domestic wastewater, the MFC was coupled with constructed wetlands and different external resistance, and the anode material was tested for improving the electricity generated [95]. The study concluded that an external resistance of 220 Ω was optimal for increasing the efficiency of the MFC. Gravel or graphite base anodes coupled with stainless steel mesh as the electron collector is also a viable alternative. Decentralized sanitation and water reuse with hybrid nature-based systems that combine CWs and MFCs were reported [96]. Tubular photo-MFC combination reactors were tested as a wastewater polishing treatment step with simultaneous electricity production [97, 98]. Overall, the study concludes that a combination of wetlands with MFC is a promising approach.

MFCs with floating carbon-cloth air cathodes were modified using oxygen reduction reaction redox catalysts from platinum nanoparticles or

Table 1. An account of defined substrates explored in MFCs.

Substrate type	Inoculum	Type of MFC	Working volume (mL)	Anode	Cathode	CD_{max} (mA m^{-2})	PD_{max} (mW m^{-2})	OCV_{max} (mV)	*CE* (%)	*COD* removal (%)	Reference
Glucose based synthetic wastewater	Microbial solution	Single-chamber membrane-less	500	Graphite	Graphite	NA	218	351	26.20	98.80	[24]
	Anaerobic sludge					NA	456	508	55.40	94.30	
Glycerol	Anaerobic sludge	Two-chamber (H-type)	250	Carbon paper	Carbon cloth	225	65.4	55	34.10	99	[85]
Glycerol	*Shewanella oneidensis MR-1* and *Klebsiella pneumonia*	Two-chambered	301	Carbon paper	Carbon paper	10	2.15	22.6	NA	NA	[87]
Synthetic wastewater with xylose	Exoelectrogenic culture	Two-chamber up-flow	500–anode 400–cathode	Graphite	Graphite	356	58 ± 8	NA	21.3 ± 1.0	NA	[83]
Synthetic wastewater	Aerobic activated sludge (mixed)	Air-cathode single-chamber mediator-less	50	Carbon paper (Laydel)	Carbon Vulcan XC-72R dispersed in PTFE and loaded with Pt	275	52	0.63	55	94.40[a]	[88]
Synthetic wastewater	Biomass from anaerobic digester	Two-chambered	165	Granular graphite and carbon felt	Stainless steel mesh	47	2138[b]	NA	82	70	[84]
Modified synthetic wastewater	Differed vegetation	Microcosm-constructed wetland modules combined with MFC device	NA	Graphite	Magnesium	22.9 ± 19.4	7.47 ± 13.7	1001 ± 0.1	8.3 ± 10.4	85–88	[81]
Acetate substrate	Sludge of domestic wastewater treatment	H-type	335	Graphite foil (0.4 mm thick, Alfa Aesar)	Graphite felt (11.2 mm thick, Alfa Aesar)	NA	112	0.41	20	44 g DOC m^{-2} h^{-1}	[89]
Peptone substrate						NA	114	0.41	19	52 g DOC m^{-2} h^{-1}	
Acetate synthetic media	Activated sludge	Air–cathode single-chamber mediator-less MFC	50	Carbon paper (Laydel)	Carbon paper (Laydel)	354	86.1 at 500 Ω 80.5 at 1000Ω	243 at 500 Ω 317 at 1000 Ω	65	96	[75]

DOC - Dissolved organic carbon.

[a] Degradation rate in %.

[b] Volumetric power density (mW m^{-3}).

manganese oxide (MnO_x), and their performance was evaluated for the treatment of domestic wastewater from a primary settling tank [99]. MnO_x showed a higher *CD*, but after 55 days, platinum MFCs had a higher PD_{max} of 65.4 ± 4.6 mW m^{-2}. Thus, the study shows the possibility of MnO_x as a low-cost catalyst, an alternative to expensive platinum catalysts. Other studies assessed the performance of a single-chamber single-electrode MFC for generating electricity from sewage sludge, cattle dung, and kitchen waste. In the case of sewage sludge, PD_{max} of 988.32 mW m^{-2} was obtained on day 5 [100,101].

Wastewater from a student hostel in Nigeria was used as a substrate for two different MFCs, one using both carbon and the other using copper as the electrodes [9]. The copper fuel cell displayed better performance than a carbon fuel cell. Further, the combination of both cells in series provided maximum output with a combined voltage of 138 mV. The study also analyzed the microbial species present in the biofilm and observed both aerobic and anaerobic bacteria (*Bacillus spp., Corynebacterium spp., Staphylococcus spp., Enterococcus spp., and Micrococcus spp.*), which shows their capacity to generate electricity using wastewater.

3.1.1. Municipal wastewater

The concentrations of dissolved inorganic, organic, and suspended solids are low in municipal wastewater. The most common treatment for municipal wastewater is the conventional activated sludge system. This process is effective for the elimination of pollutants, but its economic deficiency and energy have become a cause of concern [102]. The energy used in the conventional activated sludge system is 0.3–0.6 kWh m^{-3}, thereby consuming 4% of the total generated energy across the world [103]. In Europe and USA power required to treat wastewater is 0.3–2.1 kWh m^{-3} and 0.41 to 0.87 kWh m^{-3}, respectively. Thus, these wastewater types have the potential to be used as substrates in MFCs to produce electricity [4].

One of the studies evaluated the efficiency of a double-chamber MFC in recovery of nutrients using municipal wastewater [104]. By recovering nutrients from the MFC, pH adjustments can be reduced, making these MFCs energetically efficient. The study evaluated the concentration of ammonium and phosphate ions and the *COD* levels using three different types of separating membranes, i.e., CEM, forward osmosis (FO), or nonwoven. The results show that the average *COD* removal efficiency in all three cases exceeded 80%. The double-chamber MFC with CEM as a separating membrane could recover a higher percentage of ammonium and phosphate ions, with values of >97.58% and >94.9%, respectively. The study suggests that MFCs can be an emerging technology for wastewater treatment and precious materials recovery.

MFCs with multi-panel stainless steel/activated carbon air cathodes operating under submergible conditions were evaluated using municipal wastewater [105]. At a flow rate of 144 L d^{-1}, the device produced PD_{max} values of 78 mW m^{-2} $_{Cat}$ (normalized by cathode surface areas) and 317 mW m^{-3} (normalized by volume), *COD* of 41 ± 16%, and *CE* of 29.5 ± 14%. In the initial four days, the device showed excellent performance, but electricity generation decreased due to severe inorganic cathode fouling. This fouling was attributed to the high salt content in the wastewater, which damaged the cathode.

Most of the MFCs that are tested on artificial wastewater lose their efficiency when treating actual wastewater because real wastewater has low biodegradability, low buffering ability, and low conductivity, all of which reduce the ability of microbes to degrade molecules. Furthermore, the reactor scale-up process often reduces MFC efficiency. A 1000 L MFC system was constructed in China to treat actual municipal wastewater using 50 separate modules [106]. The system was provided with two different types of wastewaters, with low (average 80 mg L^{-1}) and high initial (average 250 mg L^{-1}) *COD* concentrations. The results show that the *COD* removal rate was 70–90%. At the same time, the PD_{max} in the case of the MFC fed with artificial wastewater was 125 Wm^{-3} (7.58 Wm^{-2}), and that fed with municipal wastewater was 7–60 W m^{-3} (0.42–3.64 Wm^{-2}). The recent report demonstrates that the MFC generated electricity during one year of operation, and the modularized system was cost intensive.

Anaerobic sludge samples from a sugar factory and a municipal wastewater treatment plant were used as inoculum to check the MFC efficiency in treatment of municipal liquid waste as the substrate source [107]. The study results showed that MFC that were inoculated with sludge from the wastewater treatment plant improved energy yields by more than 65% from the sugar factory. Thus, significant observations were that MFC performance was greatly influenced by the type of inoculum used and that bacteria belonging to *Firmicutes*, *Proteobacteria*, and *Actinobacteria* are preferable.

Another study reports on *Pseudomonas aeruginosa* isolated from municipal wastewater and used as inoculum to test the MFC efficiency in treating synthetic waste streams consisting of different concentrations of simple carbon substrates (glucose, fructose, and sucrose) [108]. The results show that *P. aeruginosa* could efficiently utilize hexose and pentose sugars. Further, using glucose as a substrate produced the highest PD_{max} and *COD* removal rates, with respective values of 136 ± 87 mW m^{-2} and 88.5% ± 4.3%. Fructose substrates only produced PD_{max} of 3.6 ± 1.6 mW m^{-2} but had a high *COD* removal of 67.5% ± 2.6%. Sucrose produced a moderately higher power of 8.606 ± mW m^{-2} and *COD* removal of 54.2% ± 1.9%. This led to the conclusion that the type of electron donor controls the growth of anode-respiring bacteria (ARB).

To reduce the cost of MFCs operating in real conditions, a four-air cathode single-chamber MFC was designed using a carbon granule bed as the anode. The system used GORE-TEX as a separator instead of the expensive polymer electrolyte membrane and MnO_2 as a catalyst in place of the expensive noble metal catalyst [15]. The system was run in anaerobic conditions, further reducing the treatment costs of synthetic and municipal wastewaters. The study results showed that synthetic wastewater as a substrate yielded *COD* removal of 85% and *CE* of 21%, compared to municipal wastewater with *COD* removal of 45% and *CE* value of 7.8%. However, the performance with the use of additional salt added to the municipal wastewater increased the *CE* to 22.3%. Further, it was observed that catalyst degradation and deposition of non-exoelectrogenic bacteria eventually occurred, thereby reducing the efficiency of the system in the long term. The study shows potential for cost-effective MFCs that can provide electricity and treat wastewater simultaneously, but more work is needed to address limitations during a continuous performance.

Activated sludge from municipal wastewater was used as the inoculum for a mediator-less single-chamber MFC applied to synthetic wastewater [88]. The ability of the MFC to utilize glucose as a substrate was assessed, and the microbial composition of the anode was also evaluated. Increasing the glucose concentration in the synthetic wastewater to 5.0 g L^{-1} resulted in increases in outputs, which reached PD_{max} of 52 mW m^{-2} with *COD* removal of 94.4%. Further increasing *COD* resulted in increased current and reduced internal resistance.

Researchers have developed high-energy harvesting systems (EHS) to enhance single MFC efficiency with low input voltage. The power output efficiency and the output voltage is enhanced in MFCs by introducing a charge pump, capacitor, and boost converter [109]. A suitable tactic incorporates maximum power point tracking (MPPT) for optimization of the power output by identifying the maximum power voltage (MPV) of the MFC. A study was conducted with 48 MFC modules with a total 200 L capacity connected in parallel or series, using a commercially available EHS BQ25504 to convert voltage ranging from 0.8-2.4 V to 5V [110]. The energy produced by the MFC was extracted by BQ25504 using four different connections for charging the ultra-capacitors. The process of charging was boosted by increasing the number of MFC modules. The MFC functionality was tested using municipal wastewater. The results showed that the MFCs arranged in three rows had a conversion efficiency of 80%, and the power output was 114 mW. These values were the highest, and poor performance in any single row of the MFC severely affects the overall MFC performance. The study reports that this configuration of MFC was able to treat actual municipal wastewater.

3.1.2. Sewage sludge

Sewage has been increasing exorbitantly due to an increase in population, an increase in construction, and improvement of sewer treatment facilities. The biological treatment of domestic sewage water produces substantial amounts of active sludge in the plants that treat sewage. Active sludge is disposed of in ponds as wet cakes, which pollute the groundwater, soil, and air. Thus, active sludge can be remediated by using them as substrates in MFCs and producing electricity [111].

A laboratory-scale MFC was fed with sludge and wastewater from the primary settling tank of a wastewater treatment plant [112]. The investigation showed that the average current generation from domestic wastewater used as a substrate was 640 mA, and the degradation rate was 63%. These values were higher than that of an on-sludge-fed MFC, leading the authors to conclude that domestic wastewater shows better efficiency than sludge, possibly due to increased resistance in the sludge. Biomethane can be produced from organic waste such as food waste or sewage sludge and provides a greener alternative to non-renewable fuels used in households. Using a double-chamber MFC, a study was conducted on electricity generation and methane production with food waste and sewage sludge as the inoculum [113]. Food waste and sewage sludge were added as fuel in three different ratios. An OCV_{max} of 600 mV was obtained after 17 days of operation when both wastes were combined in equal proportions [113]. Methanogens enhanced the methane yield in the anode chamber with a maximum yield of 168 mL in ratio 2. In general, methanogens are antagonistic to electroactive biofilms which results lowers electricity production. However, from this study, it was concluded that an equal ratio of food waste and sewage sludge was the best combination for enhanced electricity generation and methane production.

Fermented industrial food waste can also be used in MFCs. The power generation and *COD* removal rates of an MFC were evaluated using different fermented sludge concentrations, pH values, and aeration rates [114]. *Saccharomyces cerevisiae* was used as the biocatalyst. The results show that the maximum current density (CD_{max}) of 994 ± 41 mA m^{-2} was found when the substrate concentration was 60% at an aeration rate of 160 mL min^{-1} and pH 6.

Sewage sludge with a high organic content can be a promising source of substrate molecules for sediment MFCs, which generally have limited applicability due to low organic content in the sediment. A sediment MFC was tested for efficiency using sewage sludge as the substrate for the anode [115]. The sewage-supplied sediment MFC shows improved performance with PD_{max} of 187 mW m^{-2} and charge transfer resistance of 84.7 Ω. Further, microbiome analysis revealed the presence of electrogenic bacteria in the anode biofilm, also supplementing the power generation capacity of the MFC. The study offers a unique practical application. Oscillating temperatures applied to sediment inert MFC have been shown to increase the power outputs by up to 400% [13]. This strategy may further improvement the performance of sewage-supplied sediment MFCs.

Sewage sludge from the primary settling tanks of the domestic wastewater of Irkutsk, Russia, was taken as a substrate source for a double-chamber MFC. A preparation of spores from four soil organisms of *Bacillus sp* named "Doctor Robik 109" was used as inoculum [116]. The study shows that after six days of incubation with the bacterial culture and an external resistance of 1 kΩ, a stable *OCV* of 300 ± 16.1 mV was achieved, and the current was 212.0 ± 15.2 μA. The addition of peptone to the sludge increased the *OCV* up to 568 mV and the current to 300 μA, whereas the addition of sodium acetate did not result in much increase. Thus, the study shows that even in the absence of bacterial inoculum, sewage sludge fed MFC can simultaneously produce electricity and treat wastewater.

In one of the studies, the performance of a two-chambered MFC with a platinum catalyst and Nafion membrane was evaluated for sewage wastewater treatment [117]. The results show that changing the pH from 7.65 ± 0.6 to 7. 31 ± 0.5 reduced the biological oxygen demand (*BOD*) from 290 ± 30 mg L^{-1} to 175 ± 10 mg L^{-1}. The CD_{max} was 0.54 mA m^{-2}, PD_{max} was 810 ± 10 mW $m^{-2,}$ and *COD* removal was 78%.

A single-chamber single-electrode MFC was constructed, and 6 single-chamber MFCs equipped with graphite electrodes (acting as anode and cathode) were connected externally in series. In this design, 30% of the electrodes were exposed to air (cathode), and 70% of the submerged electrode behaved as the anode. The substrate layer divides the anode and cathode region, where the submerged part of the electrode can be compared to the anaerobic chamber acting as an anode. The system generates electricity from sewage sludge by operating the MFC at two different temperatures, i.e., 25 ± 4 °C and 32 ± 4 °C, under aerobic conditions [118]. In contrast, the exposed part in the aerobic chamber serves as a cathode. At a temperature of 25 ± 4 °C, OCV_{max} and surface PD_{max} were 2890 mV and 1108.29 mW $m^{-2,}$ respectively, whereas, at a temperature of 32 ± 4 °C, these parameters were 1652 mV and 865.57 mW m^{-2}. The study shows that compared to other single-chambered MFC, the tested MFC generated more electricity and was also cost effective due to the lack of expensive platinum electrodes. However, the current MFC configuration did not successfully remove *COD* effectively and thus requires more research.

Seawater-based domestic wastewater sewage sludge (SWS) is unique to a few cities and can be considered a possible substrate for MFC-based electricity generation. The ionic conductivity value of SWS is ambiguous because it increased the MFC power density in one study [119] while reducing the same in another study [120]. Thus, the ionic conductivity of the wastewater was reduced by mixing it with freshwater-based domestic sludge (FWS) or deionized water [121]. To assess the effect of ionic conductivity on the utilization of SWS as a substrate source, four different combinations of feed solutions were tested, i.e., only SWS, only FWS, SWS mixed with 50% deionized water, and an equal mixture of both SWS and FWS. The results show that the same ratio of SWS and FWS resulted in CE_{max} of 28.6 ± 0.5% and *COD* removal of 59 ± 3%. The authors concluded that if the ionic conductivity was maintained at 12 mS cm^{-1}, the SWS can itself be used as a substrate for wastewater treatment and electricity generation via MFCs.

MFCs can naturally catalyze the oxidation of certain organic molecules and inorganic ones (e.g., ammonia), but generally they cannot reduce nitrates, due to lack of a suitable catalytic pathway. Autotrophic denitrification uses inorganic carbon as carbon source (e.g., bicarbonates), and an electron source required to activate their metabolism [122]. A membrane-less MFC study was conducted to assess its efficacy in biodegradation, electricity generation, and nitrogen removal [123]. The cathode chamber of the MFC was intermittently supplied with air, and the performance was assessed in both open- and closed-circuit operations. In the closed circuit, the PD_{max} was 2.05 W m^{-3}, CD_{max} was 6.05 A m^{-3}, *COD* was 91.7 ± 0.3%, and ammonia-nitrogen (NH_3–N) removal was 98.2 ± 0.3%. In *OCV*, *COD* removal dropped to 81.1 ± 0.6%, and ammonia-nitrogen removal dropped to 80.4 ± 0.9%. Thus, the study concluded that this MFC could generate electricity, degrade organic waste in domestic sewage, and simultaneously remove ammonia and nitrogen.

The use of expensive metals for oxygen reduction catalysis on the cathode can be replaced with microorganisms to produce a biocathode [124]. The bacteria behave as biocatalysts for accepting electrons from the cathodes. The sustainability of the MFC is enhanced because metal poisoning can be discarded. A study was conducted using a biocathode from *Saccharomyces cerevisiae sp*. to treat sewage sludge. The maximum voltage generated was 2.5 V [125]. The study assessed the MFC performance at different substrate concentrations, pH values, and oxygen flow rates and concluded that minimizing the internal resistance in the MFC enhances power generation.

A two-chamber MFC built using platinum-coated carbon cloth electrodes was used to treat sewage wastewater [126]. The *COD* removal efficiency was 78%, OCV_{max} was 800 mV, and PD_{max} was 204 ± 0.38 mW m^{-2}. The high performance was attributed to the use of a porous membrane (Nafion-117), which has high conductivity, high porosity, and non-fouling capability.

3.1.3. Kitchen waste

Kitchen waste contains a large amount of organic compounds such as glucose sucrose that are favourable as substrates for MFC, as they are a rich source of carbohydrates and favour bacterial growth. A biocathode-based MFC was used to treat kitchen wastewater. Two photosynthetic microorganisms, i.e., *Synechococcus sp. and Chlorococcum sp.,* were used as cathode catalysts [127]. The results show that in the case of *Synechococcus sp.*, PD_{max} was 41.5 ± 1.2 mW m^{-2}, *CE* was 16.5%, and *COD* removal was 73.5%, whereas, in the case of *Chlorococcum sp.*, PD_{max} was 30.2 ± 0.8 mW m^{-2}, *CE* was 11.4%, and *COD* removal was 69.4%. The study reveals the possibility of using these two species as a biocathode for enhanced MFC efficiency and a greener alternative to expensive metal cathodes; additionally, an increase in light intensity enhanced the power density [127].

A novel PVC hydrophobic matrix membrane using hydrophilic zeolite 4A was constructed to treat kitchen wastewater via MFC [128]. The performance of an MFC using this novel membrane was assessed using plain PVC and Nafion membranes. The PVC/4A membrane showed an optimum PD_{max} of 250 ± 5 mW m^{-2} and *COD* removal of 89%, whereas the plain PVC and Nafion membrane yielded a PD_{max} of 92 ± 5 mW m^{-2} and 125 ± 5 mW m^{-2}, respectively. The membrane cost was less compared to other standard membranes. The study concludes that the novel membrane improved the efficacy of MFC and was also cost effective.

One *Exioguobacterium sp* SU-5 was used in a mediator-less MFC to generate electricity from carboxymethyl cellulose (CMC) and kitchen waste [12]. The MFC was fitted with a Nafion membrane or salt bridge. The two substrates were different because CMC is a single substrate, whereas kitchen waste consists of multiple microbes. The results of the study show better performance of the MFC with the Nafion fitted membrane. Hence, the MFC was inoculated and fed by food waste.

One approach to enhancing MFC efficiency includes the use of exogenous compounds known as redox mediator molecules. These molecules include dyes and metal organic molecules. A study assessed the effect of five such mediators, i.e., ethylenediaminetetraacetic acid (EDTA), methylene blue (MB), potassium ferricyanide, neutral red (NR), and potassium permanganate, on electricity generation by MFC [130]. In the control MFC with no mediators, the PD_{max} was 84.58 mW m^{-2}, whereas in the case of mediators, the highest *PD* and energy contribution values were found in the following order: (i) potassium ferricyanide: PD_{max} was 924.79 mW m^{-2} (energy increase of 993.39%); (ii) EDTA: PD_{max} was 803.71 mW m^{-2} (energy increase of 850.24%); (iii) methylene blue: PD_{max} was 340.45 mW m^{-2} (energy increase of 302.52%); and iv) potassium permanganate: PD_{max} was 192.14 mW m^{-2} (energy increase of 121.17%). The *COD* removal percentage was highest for potassium permanganate at 21.89%, followed by 19.16% for ferricyanide, 11.67% for neutral red, 6.13% for EDTA, and 4.96% for methylene blue [130]. The research also shows some toxic effects of these mediators for the survival of electrogens, thus requiring further research to improve the practical application of the MFC.

Hou et al., 2017 reported the use of *Golenkinia sp.* SDEC-16 for bioelectricity generation; however, the effects of wastewater concentration on the growth of the microbial species and its efficacy were explored earlier [134]. Thus, the investigators used different dilutions of anaerobically digested effluent from kitchen waste (ADE-KW) in a dual-chamber MFC for electricity generation and degradation. The results showed that dilution by four times improved the *COD* removal from 48.2% to 76%. The total nitrogen (TN) removal at the anode was more than 80%, while TN and TP removal at the cathode was 90%. Without dilution, the OCV_{max} was 400 mV, PD_{max} was 400mW, and a total lipid content of *Golenkinia sp.* SDEC-16 of 55.85% was reported.

3.2. Food waste leachate

Food waste degradation to produce hydrogen through biohydrogen fermentation releases promising substrates for MFCs such as acetate and butyrate. Food waste leachates consist of polluted effluents of landfills with four different types of pollutants, mainly xenobiotic organic compounds and dissolved organic materials. MFCs have been used to simultaneously produce electricity and degrade the leachate.

A study evaluated the MFC performance using food waste leachate used for biohydrogen fermentation [34]. The results show that in batch mode, efficiency parameters such as CE_{max} were 88.8%, energy efficiency (EE) was 18.8%, PD_{max} was 1540 mW m^{-2}, and *OCV* was 0.56 V. The study shows that products of biohydrogen fermentation can be effective substrates for MFC and that combination of MFC with biohydrogen production can be an energy-efficient option.

Table 2 provides an account of MFC performance with different domestic wastewaters as substrate source.

3.3. Industrial wastewater

Wastewater from the leather, palm oil, dairy, distillery, and petroleum industries contain significant pollutants that can be treated by MFCs to reduce costs and enhance practical application. Organic biopolymers, chitin, and cellulose are primarily present in industrial wastewater sources [143]. These polymers are good substrates for the generation of electricity.

A single-chamber MFC was used to treat real cooking wastewater, removing carbon and nitrogen and maintaining alkaline consumption. The MFC was run in both batch-fed and continuous-flow mode [144]. A *COD* removal efficiency of 83.8 ± 3.6% and total nitrogen removal of 97.9 ± 2.1% were observed, which were better in the MFC than in a traditional aerobic biological reactor (ARB), which achieved only 73.8 ± 2.9% *COD* removal and total nitrogen removal of 50.2 ± 5.0%. Furthermore, the MFC also showed better performance in the degradation of phenolic and nitrogenous heterocyclic compounds and showed a higher abundance of heterotrophs, nitrifiers, and denitrifiers [144].

To reduce cost, an economically fabricated MFC was used to treat industrial wastewater (leather and dairy sources). The experiment was performed in two different phases, one in which only the wastewater was used and a second in which a ferroin mediator was added to the wastewater [145]. The *COD* and *BOD* removal percentages were calculated based on effluent treatment using *Saccharomyces cerevisiae*. With no mediators, the *COD* removal, *BOD* removal, and power generation for leather effluent were 80.4%, 59.3%, and 385.25 μW, respectively, while those of dairy effluent was 83.4%, 64.3%, and 304.5 μW, respectively. By adding a mediator, the maximum power generation reached 1.98 mW for dairy leather. In both cases, the degradation and current was reported to be higher in dairy wastewater [145]. More power was generated in the second phase, with the mediator addition indicating a positive effect.

The use of highly porous activated carbon (AC) as an anode has been proposed as an alternative to expensive carbon cloth [146]. A MFC based on AC as the anode was constructed for electricity generation and wastewater treatment from real industrial wastewater without any prior treatment or addition of microbial mediators. The activated carbon cathode performance was compared to that of other anodes such as carbon paper or cloth, with or without Teflon. The results show that the highest *CD*, *COD* removal efficiency, and *CE* values were 1792 mA m^{-2}, 60%, and 71%, respectively, compared to other MFC with different anodes. The study shows the possibility of a cost-effective MFC that can be used to treat industrial wastewaters.

A laboratory-scale experiment was conducted using a dual-chamber MFC using wastewater from glass and marble, vegetable oil, chemical, metal, and combined industrial effluents [147]. For efficiency parameters, *COD* was 85–90% and OCV_{max} was 890 mV, and a CE_{max} value of *CE* 5184.7C was obtained for the vegetable oil industry [147]. The study provides a comparative insight into the efficiency of different industrial wastewaters using the same MFC.

Table 2. An account of domestic wastewater explored in MFCs.

Substrate type	Inoculum	Type of MFC	Working volume (mL)	Anode	Cathode	CD_{max} (mA m^{-2})	PD_{max} (mW m^{-2})	OCV_{max} (mV)	*CE* (%)	*COD* removal (%)	Reference
Hostel wastewater	NA	Dual-chamber MFC	1000	Graphite	Copper	NA	NA	833	NA	NA	[9]
Municipal solid waste	Mesophilic anaerobic sludge	dual-chamber MFCs	60	Carbon cloth fixed on a graphite rod	Carbon cloth fixed on a graphite rod	218	11.9	54.6	1.95	87.3	[129]
Municipal wastewater	NA	Submergible prototype	255000	Graphite fiber brush (GFB) (MillRose Company, USA)	Stainless steel/ Activated carbon (Vito NV, BEL)	NA	78	NA	29.5 ± 14	41 ± 16	[105]
Municipal wastewater	Anaerobic sludge	Four-air cathode single-chamber microbial fuel	938	Graphite granules	GORE-TEX® cloth with MnO_2 catalyst	NA	136000[c]	570	22.30	45	[15]
Domestic sewage	NA	Anoxic/oxic	1050-Anode 2460-cathode	Graphite granule	Carbon fiber brushes	6050[a]	2050[c]	NA	NA	91.7 ± 0.3	[123]
Seawater-based domestic wastewater sewage sludge	NA	Two-chambered MFCs	75.6	Titanium wire inserted into carbon felt	Titanium wire inserted into carbon felt	109[a]	41000[c]	NA	28.6 ± 0.5	59 ± 3	[121]
kitchen wastewater	Mixed bacterial culture	Two-chambered MFCs	600	Graphite	Graphite	300	710 ± 3	920 ± 3	20.1	89	[128]
Kitchen wastewater	NA	Single-chamber air cathode	28	Graphite	Graphite	1.724[b]	924.79	810	NA	25.4	[130]
Food waste leachate	NA	Two-chamber	1350 anode 1000 cathode	Carbon rods	Carbon rods	150.30	29.32	560.2	14.22	72.27	[131]
Domestic wastewater	Acetate fed	Flat-panel air-cathode	150	Graphite felt	Wet-proof carbon cloth	NA	187000[c]	700	18–19	51.5	[91]
Domestic wastewater	Activated sludge	Cubic type air-cathode	260	Graphite felt	Wet-proof carbon cloth	1.4[b]	NA	NA	33.5	69	[92]
Domestic wastewater	*Scenedesmus quadricauda* (SDEC-8)	Algae biofilm	1600	Titanium fixed carbon cloth	Titanium fixed carbon cloth	NA	62.93	580	17.01	80.20	[132]
Domestic wastewater	Activated sludge	Single-chamber	650	Graphite felt	Stainless steel mesh with multi-wall carbon nanotube	335	2190[c]	634	NA	35.3	[133]

[a] Volumetric current density (mA m^{-3}).
[b] Total current (mA).
[c] Volumetric power density (mW m^{-3}).

3.3.1. Distillery/brewery/winery wastewater

Wastewater from wineries is rich in organic load and poor in inorganic nutrients such as nitrogen and phosphorus [148]. Therefore, winery wastewater treatment and electricity generation using MFCs is a sustainable method for this industry. Wastewater from breweries is popularly used by scientists as a substrate in MFCs because of its low strength. It is appropriate for electricity production in MFCs because it is rich in organic matter and has a low concentration of inhibitory substances like ammonia. The concentration of brewery wastewater is within the range of 3000–5000 mg of *COD* L^{-1}, which is 10 times more concentrated than domestic wastewater [149].

A study was conducted to assess the effect of unfavourable *COD/N* and *COD/*P ratios on MFC performance using a dual-chamber model. The research shows that by increasing the nutrient concentration and daily removals to 1000 mg L^{-1} d^{-1}, an MFC could only reach a *COD* removal value of around 17%. An increase in the concentration of nutrients (nitrogen, phosphorus) positively influenced electricity generation by increasing the *CE*% between 2% to 15% and PD_{max} from 105 to 465 mW m^{-2} [148]. The study concluded that MFCs could efficiently generate electricity using winery wastewater; however, the treatment efficiency is currently too low for industrial application.

To optimize the MFC condition for effective scale up, a 20 L dual-chamber MFC was constructed, and electricity generation was assessed using brewery wastewater [150]. A flow rate of 1 mL min^{-1} (HRT ¼ 313 h) resulted in the highest *COD* removal efficiency of 94.6 ± 1.0%. The system showed an effective option for a cost-effective MFC without the use of any catalyst or expensive membranes.

The effect of sludge age on the efficiency of MFC treatment and electricity generation from winery wastewater was studied using six solid retention times (SRT) [151]. The results showed that decreasing the SRT was associated with higher electricity generation; it enhanced *CE* (from 3.4% to almost 42.2%) and PD_{max} (from 58 to 890 mW m^{-2}) but did not change the *COD* removal percentage.

The effect of fermentable or non-fermentable substrates in single pure or mixed sources on the MFC performance was studied using a single-chamber MFC fed with single pure substrates (glucose, butyrate, propionate, acetate) and a mixed substrate with brewery wastewater [152]. Glucose-fed MFCs (single substrate) showed the highest PD_{max} of 1.5 mW m^{-2}. With brewery wastewater, PD_{max} was 0.552 mW m^{-2}, more than that with acetate, butyrate, or propionate-fed MFCs. Also, when the substrate was switched to brewery wastewater, a reduction in power generation was observed in all cases. The study further concludes that fermentable substrates enhanced electricity production compared to real wastewater [152].

A single-cell MFC with an air cathode was used to study electricity generation and wastewater treatment from brewery and pig slurry wastewater. The mixed-fuel MFC (brewery and pig-farm liquid manure combination) produced OCV_{max} of 199.8mV, PD_{max} of 340 mW m^{-3}, and a *CE* value that was 11% higher compared to values from pure brewery samples [14]. However, the *COD* removal efficiency of the combination was 53%, less than that with a pure brewery wastewater fed MFC (where a *COD* removal of 93% was obtained).

A dual-chamber MFC with a tin-coated copper mesh anode was used to assess electricity production from brewery wastewater by varying the hydraulic retention time [153]. MFC-1 was incorporated with brewery wastewater from the inlet of an anaerobic digester, and MFC-2 was incorporated with the outlet water of the digester. The results show that at a hydraulic retention time of 0.5 d, the MFCs show the best performance with PD_{max} values of 80.01 and 18.43 mW m^{-2} from the MFC-1 and MFC-2 reactors respectively. The study further analyzed the bacterial species present, and the fouling effect shown by the Nafion membrane to optimize the practical use of the MFC. The MFC-1 was referred to as inflow, and the feed solution was wastewater acquired from the inlet. The MFC-2 was referred to as outflow because the feed water was obtained from the outlet.

A 90 L stackable MFC was constructed to treat brewery wastewater in a self-sufficient manner [154]. The MFC was operated in two different stages: stage 1 with diluted wastewater and stage 2 with wastewater. In the case of diluted wastewater, the efficiency parameters such as *COD,* SS removal, energy produced, and net electrical energy were 84.7%, 81.7%, 0.056 kWh m^{-3}, and 0.021 kWh m^{-3}, respectively; in case of industrial wastewater, these parameters were 87.6%, 86.3%, 0.097kWh m^{-3} and 0.034 kWh m^{-3} [154]. The study proved to be a milestone for using self-sufficient energy generated by MFC treatment of real wastewater.

3.3.2. Palm oil mill effluent sludge

The processing of one ton of fresh fruit to produce palm oil requires 1.5 m^3 of water, which generates a large amount of palm oil mill effluents containing 0.7% oil, 96% water, and 5% total solids. These colloidal effluents are not toxic but are acidic with a pH of 4–5 and a discharge temperature of 85 °C [155]. This effluent has been considered the largest pollutant in Malaysian rivers. Although the ponding system is used for effluent treatment, the final discharged water still contains a considerable amount of suspended solids and *COD* due to cellulose mixed with oil, grease, and fat. Thus, the MFC has been considered an alternative process for the disintegration of these effluents.

Palm oil mill effluent (POME) is organic waste material and has been considered as a substrate source for MFCs in biodegradation and electricity generation [156]. A study was conducted using both single- and double-chamber MFCs, with EAB from *Pseudomonas aeruginosa* (ATCC – 27,853) as the inoculum. The double-chamber MFC showed a better PD_{max} of 4.2 W m^{-3} and *COD* removal of 54% compared to the single-chambered MFC with 1.7 W m^{-3} PD_{max} and 41% *COD* removal [156].

POME water with a *COD* of 68,360 mg L^{-1} was ultrasonicated. The effect of ultrasonically pre-treated and untreated substrate on electricity generation by an MFC was studied using the fermentative facultative anaerobe *K. variicola* as a biocatalyst [157]. The study results show that the pre-treated MFC had a PD_{max} of 1.6 W m^{-3}, while in the case of the untreated MFC, the PD_{max} was 1.2 W $m^{-3,}$ and the *COD* removal efficiencies were 74% and 48%, respectively. The study highlights the positive effect of pretreatment on the efficacy of MFC [157].

A study was performed to evaluate the application of natural microbiota and pure culture bacteria (isolated from anaerobic POME sludge) for generation of electricity using a double-chamber MFC [158]. The substrate source for the MFC was sterilized POME without any nutrients. In the case of natural microflora, the efficacy parameters, i.e., PD_{max} and CD_{max}, were 0.085 W m^{-2} and 91.12 mA m^{-2}, respectively, and in the case of pure culture (*Pseudomonas aeruginosa* strain ZH1), these values were 0.45 W m^{-2} and 654.90 mA $m^{-2,}$ respectively. The results show the increased efficiency of MFCs operating with pure culture as inoculum [158].

3.3.3. Dark fermentation effluent

Anaerobic digestion of biowaste, known as dark fermentation, releases a high amount of volatile pollutants such as acetic acid. Dark fermentation effluents are high in carbon sources, with the presence of organic acids such as propionate, acetate, lactate, butyrate, caproate, and valerate. Thus, it is crucial to treat the effluent discharged from this process. Volatile fatty acids are the main constituents of dark fermentation effluents. These effluents can be treated by integrating MFCs for the production of electricity.

A dual gas diffusion cathode design was used in three different MFCs operating under batch, semi-continuous (fed-batch flow mode), and continuous flow modes using a dark fermentation effluent as the substrate source [42]. The results show that MFCs under continuous flow mode show better performance out of all three with a PD_{max} of 15.53 ± 2.51 mW m^{-2} and *CE* of 9.85 ± 1.02%. Furthermore, stack MFCs were placed in a series of connections to enhance the continuous flow mode treatment efficiency output. The stack MFC at an external resistance of

2KΩ shows a volumetric PD_{max} of 3.16 W m^{-3} (0.19 W m^{-2}), and treatment efficiency of 80 ± 2% [42].

3.3.4. Petroleum wastewater

Petrochemical plants and petroleum refineries generate a large amount of wastewater that contains both inorganic and organic pollutants such as heavy metals, hydrocarbons, BTEX, sulfides, and phenols [159]. Petroleum wastewater is not commonly used in MFCs because it is complex to degrade. This wastewater includes aromatic, aliphatic, and phenolic compounds. Additionally, interaction of the microbes with these compounds is difficult, thereby showing poor performance in MFCs when used as substrates. Anaerobic microorganisms have to be externally added to degrade this wastewater and produce electricity.

Similar to other wastewaters, it is crucial to treat petroleum wastewater with a high *COD* in an energy-efficient manner [160,161]. The efficacy of anaerobic sludge as the MFC inoculum for petroleum wastewater from an acrylic acid plant was evaluated in a double-chamber MFC [162]. The MFC shows a PD_{max} of 0.75 W m^{-2}, CD_{max} of 412 mA m^{-2} (after seven days), maximum output voltage of 0.45V, *CE* efficiency of 13.11% (after ten days), and *COD* removal of 40%. The study demonstrates the use of anaerobic sludge-based MFC to treat petroleum wastewater [162]. In another study, petroleum refinery wastewater was used to generate electricity. The electricity generated was then used for seawater desalination using an osmotic MFC, which was connected to an up-flow microbial desalination cell [161]. Anaerobic mixed sludge was used in the anodic chamber in both cells, and petroleum wastewater flowed from the osmotic MFC with 1000 Ω external resistance to the desalination cell with 100 Ω external resistance. Overall, 93% *COD* removal and 48% salt removal were observed using the combined system at a feed flow rate of 100 mL h^{-1} [161]. The two systems worked in synchrony to enhance the final performance with petroleum refinery wastewater conductivity. The dilution of saltwater in the osmotic microbial fuel cell further helped to lower the salt concentration for the up-flow desalination cell. A study assessed the efficacy of purified terephthalic acid wastewater as a substrate for a single-chambered membrane-less MFC due to its high organic content [133]. The concentration and pH of the petroleum wastewater were varied. The results showed PD_{max} values of 10.5, 43.3, 55.5, and 65.6 mW m^{-2} for ten times dilution, four times dilution, two times dilution, and raw wastewater, respectively. In terms of pH, the power generation reached a maximum at pH 8.5 because this alkaline pH supports the growth of electrogenic bacteria [133].

3.3.5. Chocolate industry wastewater

A large amount of wastewater is generated from the chocolate manufacturing industries. This wastewater consists of total solids, color, *COD*, and *BOD* [163]. The wastewater obtained from chocolate manufacturing industries is not toxic because of the absence of any harmful compounds. As a microorganism source, activated sludge should be added to these wastewater sources.

This wastewater was used to optimize the electrode spacing, material, and surface area and enhance the efficacy of an annular single-chamber microbial fuel cell (ASCMFC) [140]. The results show that reducing the electrode spacing to 0.7 cm and using a spiral anode decreased the internal resistance to 50 Ω, increasing the PD_{max} and current to 22.898 W m^{-3} and 6.42 mA, respectively. A study was conducted, with the objective of simultaneous generation of power and treatment of waste, using an up-flow anaerobic MFC operated under variable organic load. The study results show that at an HRT of 15 h, PD_{max} of 98 mW m^{-2} and *COD* removal of 70% were obtained. Also, a substrate concentration of 0.8 g L^{-1} was optimal for achieving a PD_{max} of 104.9 mW m^{-2}. Analysis of the anode microbes revealed the presence of four prominent bacterial strains that enhance the power generation by MFC [164].

3.3.6. Landfill leachate

Landfilled waste transforms physiochemically and biologically to generate highly polluted wastewater known as leachate. Landfill leachate consists of *BOD*, *COD*, inorganic materials, organic contaminants, toxic materials, ammonia, and heavy metals. Additionally, it consists of refractory compounds like humic substances [165]. Landfill leachate is a major toxic pollutant with extremely high concentrations of inorganic minerals such as ammonia, nitrogen, phosphorus, and heavy metals needs to be treated before dispersal.

Hydraulic retention times were varied in an algae-cathode MFC using diluted landfill leachate (15% v/v) [166]. The efficacy of diluted landfill leachate was given by the OCV_{max} of 303 mV for 20-hour HRT, whereas at 60-hour HRT, the *COD* removal was 26%, the *BOD* concentration was 5.3 mg L^{-1}, ammonia removal was 76.4%, and phosphorus removal was 86.3% [167]. These studies show the algae-based MFC's success in treating landfill leachate and electricity generation. In one of the studies, a direct air-breathing cathode-based single-chamber MFC was used to evaluate the maximum output voltage using landfill leachate [141]. The highest ever reported *OCV* of 1.29 V using landfill leachate was observed in the study with three different MFCs, each with different cathode areas. In another study, the effect of young and old landfill leachate on the efficacy of MFC was reported [168]. The results show that an MFC running in batch mode with 60% young leachate shows a PD_{max} of 96.8 mW m^{-2} and *COD* removal of 90%. In the continuous mode, using 100% young leachate resulted in a reduced PD_{max} of 75 mW m^{-2} and reduced *COD* removal of 55.5%. The study also shows that ammonium can be a possible fuel for the MFC [168]. A combination of a self-sustained single-chamber air-cathode MFC-MEC system was constructed to treat landfill leachate and generate electricity [169]. The results show a high *COD* value of 38.9% and 90% ammonia removal rates. Also, the OCV_{max} and PD_{max} were 421mV and 1330.7 mW $m^{-3,}$ respectively, which were higher than those of an independent MFC [169].

3.3.7. Surgical cotton industry wastewater

An increasing number of hospitals, dispensaries, and health centers have increased their surgical cotton requirements. The effluent released from the industries that produce this cotton are alkaline, and the wastewater can be used as a substrate source for MFC during the manufacturing process. This type of wastewater mainly consists of cellulose, and cellulose is used as a substrate in MFC to produce electricity. Cellulose must be anaerobically hydrolyzed by electrochemically active microorganisms via oxidization of the metabolite of cellulose hydrolysis.

In a pilot study using surgical cotton industry wastewater, the efficiency of an up-flow anaerobic MFC operated at different organic loads under continuous flow was assessed [142]. At an organic load of 1.9 g *COD* L^{-1} d^{-1}, the highest total *COD* removal of 78.85, surgical *COD* removal of 69%, total suspended solids removal of 62%, PD_{max} of 0.11 W m^{-2}; 2.2 W m^{-3} (volumetric)' and *CE* value of 17.8% were observed.

3.3.8. Refractory organic pesticide

Hexachlorobenzene, a common refractory organic pesticide, is detrimental to both the environment and to humans. The different remedial methods for eliminating this compound are land farming, soil washing, ion exchange, soil vapor extraction, phytoremediation, soil flushing, and ecological cleaning [170]. These conventional methods are costly and lead to loss of soil fertility. Thus, these substrates are used in MFCs for degradation and finally for the production of electricity.

Hexachlorobenzene (HCB) is a persistent organic pollutant that accumulates in the topsoil due to the use of pesticides. It is crucial to treat this pesticide to reduce soil pollution. In an attempt to achieve the goal, soil MFCs were constructed based on sandy soil as a substrate [171]. The results show that soil with HCB amounts of 40, 80, and 200 mg kg^{-1} showed removal values of 71.14%, 62.15%, and 50.06%, respectively, and PD_{max} of 70.8 mW m^{-2} was observed. Surfactants such as sodium dodecyl sulfate enhanced the MFC performance in terms of HCB removal [171].

3.3.9. Textile wastewater

The effluents from the dyed textile industries are colored and consist of *COD*, *BOD*, metals, salts, and suspended solids [172]. Approximately

200 L of water is required to generate 1 kg of textiles. Thus potable water is converted to wastewater in the textile industries and is subsequently discharged. This wastewater contains either natural fibers or synthetic fibers, depending on the type of textile industry. Moreover, some wastewater is colored, and some is colorless. The printing and dyeing units of the textile industries produce wastewater that contains dyes, chemicals, and complex organic compounds. These constituents pose a problem for treating this type of wastewater.

Microalgae-based biocathodes were used in an air-exposed single-chamber MFC to treat dyed textile wastewater and generate electricity [144]. Compared to the control, the maximum output voltage was 18–43% higher, resulting in PD_{max} of 123.2 ± 27.5 mW m^{-3}, *COD* removal of 92–98%, and Zn removal of 98%. Image processing analysis revealed that 42% of the cathode surface area was covered by the algae cells [144]. This MFC was able to efficiently treat textile wastewater; however, electricity generation was low but can be further optimized. In another study, a two-chamber H-type MFC was used to treat textile industry effluent, and the various parameters for enhancing performance were assessed [173]. The study showed a maximum current generated of 0.64 mA, which increased to 0.768 mA with use of 0.5% molasses as a substrate. The *BOD* removal efficiency was 76.4%, and 40 mM of potassium ferricyanide was used as an optional catholyte.

3.3.10. Azo-dye and dye-processing wastewater

The textile industries generate complex organic toxic wastes, including sulfides and azo dyes, which are major environmental pollutants. The most widely used synthetic dye is an azo dye that is present in large concentrations in effluents from the textile industries and dye-manufacturing industries. The strong color of the dyes causes environmental problems such as transferring oxygen to water and blocking light. These factors affect aquatic life [174]. Additionally, these dyes are highly toxic; hence these dyes have been used as substrates in MFCs to remove the colour and generate electricity.

Several studies have been performed to treat azo dye and simultaneously generate electricity [175,176,177,178]. One study used a single-chamber air-cathode MFC for electrical generation from the degradation of azo dyes [179]. The results showed that production of biogenic sulfide by the sulfate-reducing bacteria enhances the chemical degradation of azo dye. At an initial sulfide/dye ratio of 9 to 1, PD_{max} was 23.50 W m^{-2}, whereas the removal efficiencies of sulfide and azo dye were 98% and 88%, respectively. An analysis of the gene sequences from 16s RNA analysis of the microbes at the anode revealed the dominance of *Dokdonella* over other strains for transfer of electrons. In another study, a coupled system with a biofilm electrode and a MFC was used to assess the degradation of azo-dye as a co-substrate. The results show a 28.5% increase in degradation compared to the control system. The coupled system shows a PD_{max} of 1.052 W m^{-3} at an internal resistance of 220.69 Ω [180]. In a similar attempt, a three-dimensional, electro--Fenton-technique-based MFC was used to degrade azo dye and generate electricity [181]. The process enhanced the dye color removal efficiency, *COD* removal, and electricity generation and suggested the benefits of MFCs in biodegradation and bioelectricity generation. MFCs coupled with wetlands were also used for azo degradation [177,182,183,184]. In one study, at a cathode diameter of 25 cm, the highest decolourization volume of 397.64 mg L^{-1} was observed, whereas at a 27.5 cm diameter, a CD_{max} of 0.539 A m^{-2} was recorded. Further, the anode layer shows more electrogenic bacteria, whereas the cathode shows a greater proportion of anaerobes and amphimicrobes [184]. Decolourization of acid orange 7 (AO7) and simultaneous electricity generation was successfully performed using a single-chambered up-flow membrane-less MFC [185]. A study evaluated the efficiency of the degradation and electricity generation of monoazo and diazo dyes in an MFC [186]. Four dyes, namely, New coccine (NC), AO7, Reactive red 120 (RR120), and Reactive green 19 (RG19) were used in the cathode of the MFC, and the results show that dye color removal and power generation occurred in the order RG19 < RR120 < AO7 < NC. Also, the maximum *COD* removal of 73 ± 3%, dye decolourization of 95.1 ± 1.1%, PD_{max} of 20.64 mW m^{-2}, and CD_{max} of 120.24 mA m^{-2} were observed when NC was used at the cathode. Furthermore, the study shows that monoazo dyes show a 50% greater decolourization rate than diazo dyes [186]. Different combinations of stacked MFCs in individual, series, and parallel connections were assessed for bioelectricity generation and biodegradation of dye processing wastewater from the textile industries [187]. The results show that the highest power output and the maximum dye degradation rate occurred with the parallel connection (twice as high as the series and 14 times higher than the individual). PD_{max} increased to 38.6 mW m^{-2}, *COD* removal was 82.14%, TDS reduction was 68%, and dye decolourization was 74.8% upon addition of 0.5 g of corn cob biochar. The study shows the success of parallel stacking and biochar addition in enhancing MFC performance [187].

Table 3 provides an account of MFC performance with different industrial wastewater as their substrate source.

3.4. Agricultural waste

3.4.1. Lignocellulosic biomass

The resources obtained from agricultural residues and forestry are known as lignocellulose, the most available biomass in nature. These materials are currently in demand as energy storage materials and energy generation substrates based on their ease of availability, renewability, and decreased carbon dioxide emissions. The main constituents of lignocellulosic biomass are hemicellulose, cellulose, and lignin. Lignocellulose biomass is rich in organic polymers, and its treatment releases polyphenols, which can be a possible substrate for exoelectrogenic bacteria. Pinewood flour was treated with sulfuric acid, electricity generation was investigated using an MFC, and hazelnut leaves were treated with sulphuric acid for electricity generation using a microbial electrolysis cell (MEC) [188]. These complex substrates were compared with simple sucrose as the substrate. The results show a PD_{max}, *CE* %, and *COD* removal of 1995 mW m^{-2}, 32 ± 1%, and 88%, respectively, using sucrose as the carbon source. Hazelnut leaves are a lignocellulosic mass that generated electricity of 0.43 V and were efficient in hydrogen production [188]. The study shows the possibility of using lignocellulose hydrolysates containing sucrose as an effective substrate for MFC and MEC. A three-chamber MFC was used to degrade and generate simultaneous electricity from lignocellulosic biomass with sugarcane bagasse and corn cob as the substrate source [189]. A freshwater cyanobacterium, *Oscillatoria annae,* was used for cellulose hydrolysis, whereas the co-culture of *Acetobacter aceti* and *Gluconobacter roseus* was used for glucose oxidation. The results show that a PD_{max} output of 8.78 W m^{-3} at 20.95 A m^{-3} was produced with sugarcane bagasse as a substrate source, and PD_{max} of 6.73 W m^{-3} at 17.28 A m^{-3} was produced with corn cob as substrate [189]. In another study, a combination of thermophilic dark fermentation with an MFC was used to recover energy from the cellulosic substrate [190]. The combined process recovered 30.49 % of the energy. The study suggests the optimization of MFC parameters for enhancing power generation.

3.4.2. Cellulose

Cellulose, a constituent of lignocellulosic biomass, contains a long chain homopolymer of glucose. The repeating polymer unit is cellobiose. A single electroactive bacterium fails to hydrolyze cellulose directly, and thus, cellulose has to be either fermented or hydrolyzed to generate compounds for donating electrons [191]. Because a single microbe cannot reduce soil extracellular acceptors of electrons and also hydrolyze cellulose, a synergistic assembly of electrochemically active microorganisms is required that can degrade the polymers and aid in fermentation. This process will enable generation of electricity from cellulosic biomass as well as degeneration.

A large amount of biofuel is obtained from cellulose, and the functional components from cellulose have varied applications [192]. Energy generation from cellulose is dependent on obligate anaerobic bacteria and thus requires two-chambered MFCs. To better understand the

Table 3. An account of industrial wastewater explored in MFCs.

Substrate type	Inoculum	Type of MFC	Working volume (mL)	Anode	Cathode	CD_{max} (mA cm^{-2})	PD_{max} (mW cm^{-2})	OCV_{max} (mV)	*CE*% (%)	*COD* removal (%)	Reference
Distillery Wastewater	NA	Dual-chamber	250	Plain graphite plate	Plain graphite plate	580	168	725	13.5	68.2%	[135]
Distillery Wastewater	Mixed anaerobic sludge	Dual-chambered MFC	19	Carbon felt (Panex® 35, Zoltek Corporation)	Nickel foam with the platinum catalyst	1.36	4.3[a]	NA	47.4 ± 1.7	54.5 to 64.25	[136]
Palm oil mill effluent	Anaerobic sludge	Two cylindrical compartments	450	PACF	PACF	NA	22	NA	24%	70%	[137]
Dark fermentation effluent	Farm manure	Double chambered	550	Carbon cloth (Mast Carbon™, Basingstoke, UK)	VITO-CoRE™ cold-rolled gas diffusion electrodes	[c]MFC-BM-7.36 [c]MFC-SCM-5.29 [c]MFC-CM- 4.69	[c]MFC-BM- 1.31, [c]MFC-SCM- 19.06, [c]MFC-CM- 15.53	1730	MFC-BM- 0.97% MFC-SCM- 5.25 % MFC-CM- 9.23%	MFC-BM- 92.68, MFC-SCM- 71.25, MFC-CM-60.50	[42]
Petroleum refinery wastewater	Activated sludge	Double-chambered	400	Carbon rod	Graphite flake	NA	330.4[a]	255 ± 5	NA	64 ± 4	[138]
Petroleum refinery wastewater	Pre-enriched electrogenic mixed culture	Single-chamber mediator-less MFC	250	Carbon cloth with carbon coating	Carbon cloth with platinum coating	544	225 ± 1.4	648	2 ± 0.8%	84.4 ± 0.8%	[139]
Chocolate industry wastewater	Anaerobic sludge	Annular single-chamber MFC	90	Graphite coated stainless steel mesh	Carbon cloth platinum coating	6.42[b]	23[a]	742	NA	90	[140]
Landfill leachate (treatment facility)	NA	Single chambered	343	Flexible graphite sheet	Carbon paper platinum coating	NA	1513	1239	NA	NA	[141]
Surgical cotton industry wastewater	Waste activated sludge	Upflow anaerobic MFC	500	Activated carbon fibre felt	Activated carbon fibre felt	196.7	116.03	590	17.8	78.8	[142]

[a] Volumetric power density (mW m^{-3}).
[b] Total current (mA).
[c] Batch (MFC-BM), semi-continuous (MFC-SCM) and continuous (MFC-CM).

microbiota of a cellulose-fed air-cathode MFC, the bacterial consortium of the MFC was analyzed before and after the operation [193]. The results show the dominance of Firmicutes, a gram-positive bacteria, as the electrons generators and cellulose decomposing Bacteriodetes. The study concludes that bacterial communities are different for differently operated MFCs with the same substrate [193].

3.4.3. Retting wastewater

The enzymatic degradation of the inner layer of flax stalks in pools, ponds, or rivers is called retting. This is a natural process of degumming, and it produces hydrogen sulfide, butyric acid, and methane and generates a pungent rotten smell. These water pollutants are difficult to treat due to the presence of refractory organic compounds and lignin. Retting water is one of the major pollutants of the flax manufacturing process and is rich in organic material and lignin. A large amount of phenolic compounds are liberated into water, thereby decreasing the transparency and level of dissolved oxygen. Conventional treatment cannot eliminate toxic phenolic compounds. Therefore, this wastewater can be a good source of bioelectricity generation.

A continuous up-flow MFC was used to assess the electricity generation and biodegradation with retting wastewater as the substrate and different organic loads [194]. The investigation shows that PD_{max} increased with an increased loading rate from 0.45 to 2.69 g *COD* L^{-1} and showed a maximum value of 254 mW m^{-2}. At a loading rate of 0.45 g *COD* L^{-1} d^{-1}, *COD* removal of 70% was achieved, while at a loading rate of 0.28 g phenol L^{-1} d^{-1} in a reactor, 95% phenol removal was observed [194]. The study shows the possibility of upscaling the current configuration for large-scale treatment of retting wastewater.

3.4.4. Soybean

The commonly used edible soybean oil generates a high amount of organic wastewater, especially during the crude soybean oil refining process. The unrequired constituents are removed by processes such as deacidification, degumming, bleaching, neutralization, and decolourization from the soybean oil. The wastewater produced during the refining of soybean oil consists of a high concentration of *COD*, sodium salts from grease, oil, phosphates, and sulfates [195]. A dual-chamber MFC was used to treat soybean wastewater and assess electricity generation using EM4 bacteria [50]. The efficiency parameters were OCV_{max} of 441 mV at a substrate OD of 0.175, an electric current of 170 μA, and PD_{max} of 51.35 mW m^{-2}.

3.4.5. Mustard tuber wastewater

A popular pickle, the Fuling mustard tuber (FMT), is obtained from *Brassica juncea*. The production of pickles uses dehydration, salt pickling, and elutriation and generates a large amount of mustard tuber wastewater. This wastewater is highly saline with high strength, high organic load, high nitrogen, and good biodegradability. The effluents are treated anaerobically, followed by aerobic treatment of mustard tuber wastewater [196]. Energy consumption of 500–600 Wh m^{-3} is associated with aerobic treatment with 50% operational cost. Additionally, this process generated 0.4 kg of additional sludge per kg of *COD* oxidized during the treatment [197].

Mustard tuber wastewater is a by product of the mustard tuber production process and is highly alkaline. It was used for treatment and electricity generation using both anode and cathode substrate in a biocathode [198,199]. The study results show a *COD* removal of more than 90% in both the anode and cathode. Nutrient removal was limited to the cathode with total phosphorus (TP) removal of 80.8 ± 1.0%, and both nitrification and denitrification occurred at the cathode with specific bacteria for each [198]. The electrolyte was self-buffered, and this study provides a novel method for the treatment of mustard tuber wastewater.

3.4.6. Sugar mill and sugarcane molasses

A substantial amount of waste such as filter mud cake, effluent, bagasse, molasses, vinasse, and bagasse ash is generated from the sugar industries. These byproducts are rich in organic materials and other elements such as potassium, and nitrogen. Thus, this wastewater is a key source of nutrients [200]. The residual sugarcane stalks obtained after extraction of juice are known as sugarcane bagasse. Sugarcane bagasse consists of 63% of hemicellulose and cellulose, which are used as substrates for the production of electricity using MFC in the presence of microorganisms as biocatalysts [201].

A double-chamber MFC was operated using sugar mill effluent (SME) as the anode substrate, and at 50% concentration of SME, PD_{max} of 140 mW m^{-2} and *COD* removal of 56% were observed [202]. The results show the success of SME for bioelectricity generation and SME treatment using MFCs. In a similar attempt, an MFC was used to treat wastewater from sugarbeet processing and generate bioelectricity [203]. The MFC shows a PD_{max} of 14.9 mW m^{-2}, *CE* values of 0.73–6.21%, *COD* removal of more than 97%, and total suspended solids removal of 100%. A bacterial strain was isolated from molasses and identified as *Brevibacillus borstelensis* STRI1 based on 16s rRNA gene analysis [52]. The ability of sugarcane molasses to act as a substrate was assessed with the above-mentioned bacterial strain as a biocatalyst to generate bioelectricity in an MFC. The OCV_{max} was 990 ± 5 mV, and in the closed circuit, it was 453 ± 6 mV; additionally, PD_{max} was 188.5 mW m^{-2}, the maximum *CE* was 59.8%, and *COD* removal was 81.7% [52]. The study shows that use of the bacterial strain as a biocatalyst for bioelectricity generation with simultaneous treatment of sugarcane molasses was a feasible option. A double-chamber air cathodic MFC was used for treating pollutants in sugar industry wastewater (SIW) and concomitant electricity generation [204]. The MFC was used with a Nafion membrane without chemical mediators and operated in fed-batch mode. The OCV_{max} was 890 mV (340 mV in the closed circuit), and PD_{max} of 160.16 mW m^{-2} and CD_{max} of 320.9 mA m^{-2} were observed. The *CE*% was 46, and *COD* removal was 85.4% [204].

3.4.7. Molasses

A byproduct formed during the formation of sugar is molasses, which is used as raw material for the production of yeast and ethanol in the fermentation industries. The fermentation industries produce a large amount of molasses wastewater containing a high concentration of protein, sugar, vitamins, and amino acids [205]. This wastewater is acidic and colored and difficult to remediate. Molasses wastewater contains a high concentration of *COD*, which prevents adequate treatment and generation of energy. Molasses wastewater facilitates the growth of microbes due to the presence of nutrients such as phosphate, polysaccharide, and nitrate [206].

A study was conducted to evaluate the efficiency of a double chamber MFC using complex substrates such as molasses and black liquor [35]. The study showed the positive effect of activated sludge by increasing the efficiency of electrogenic bacteria. Efficiency parameters such as PD_{max} of 2.425 W m^{-2} and *COD* removal of 67% were observed with molasses, whereas PD_{max} of 3.55 W m^{-2} and *COD* removal of 78% were observed with black liquor. Increasing the surface area of the PEM increased the voltage generated by 5–8 times [35]. Similarly, a dual-chamber MFCs performance was evaluated using simulated molasses wastewater, with MnO_2 as the cathode catalyst for carbon cloth cathode or carbon felt cathode [51]. The study shows that the use of catalysts increased the PD_{max} by 51% in the case of carbon cloth (from 0.006 to 0.010 W m^{-2}), whereas an increase of 771.4% was observed in the case of carbon felt (0.003–0.031 W m^{-2}). Using the MnO_2 catalyst enhanced the performance of the MFC [51].

3.4.8. Cassava mill effluents

Cassava starch is an agro-industry product in tropical Africa, Southeast Asia, and Central America. One ton of fresh cassava roots produces 0.2 tons of starch as a product, 10.7 m^3 of wastewater, and 0.4–0.9 tons of residual materials [210]. The wastewater from cassava mills contains high organic matter (mainly low nitrogen content and high carbohydrates), *BOD*, *COD*, and total solids for electricity generation using MFCs. Cyanoglycosides, which are present in cassava, are released during the

formation of starch. Cyanides, formed by hydrolysis of cyanoglycosides, inhibit the biological treatment of wastewater. This wastewater can be treated by using MFCs and producing electricity simultaneously.

Cassava mill effluents are rich in organic matter and thus are a probable substrate source for MFC. An OCV_{max} of 275 mV, current of 2.75 mA, and PD_{max} of 189 mW m^{-2} were observed in the MFC, showing the possibility of electricity generation from this effluent [209].

3.4.9. Potato chip processing wastewater

The industries that process potato chips are spread worldwide and use fresh water for washing, blanching, slicing, shredding, and peeling potatoes. The wastewater produced from the potato chip manufacturing industries contains a high amount of suspended solids, *BOD*, total suspended solids, and *COD* due to starches, carbohydrates, sugars, vitamins, proteins, and pectin [211]. Potato chip processing wastewater consists of a high amount of total suspended solids (9700 mg L^{-1}) resulting from the cutting, fluming, and washing of potatoes [212].

Bioelectricity generation from potato chip wastewater was evaluated using dual-chamber MFC [213]. PD_{max} was observed to be 95.7 mW m^{-2}, and *COD* removal of 90% was reported at room temperature. The study also established a model for the optimum use of the MFC [213].

3.4.10. Tofu

Among the food industries, tofu is one industry that causes environmental pollution. The tofu industry generates two types of waste: liquid and solid waste. The coagulation of soy milk protein produces liquid wastes, and soy milk extraction produces solid wastes. The presence of an excess amount of organic content in the liquid tofu wastes is a major environmental concern. The *BOD* (6,000–8,000 mg L^{-1}) and *COD* (8,000–11,400 mg L^{-1}) of liquid tofu waste is also very high. Tofu wastewater contains mainly phosphate, is nitrate-rich in organic content, and can be used as a substrate for bioelectricity generation.

A single-chamber MFC was operated using biocatalysts such as *Escherichia coli, Saccharomycopsis fibuligera*, and mixed cultures [3]. The results show that the mixed culture of both microbes shows better performance, with *BOD* removal of 76.57%, *COD* removal of 77.22%, current of 5.49 mA, OCV_{max} of 757 mV, and electrical energy of 9.216 $\times$ 10^{-5} kWh.

3.4.11. Wood industry wastewater

Because wood is considered a sustainable substrate, wastewater generated from wood industries was not treated. However, industries that produce wood panels use 0.1–1.5 m^3 of water per m^3 of fabricated panels [214]. Thus, the amount of wastewater generated annually from the wood industry around the globe is high. The wood industries generate around 30,000 mg L^{-1} of *COD* in their wastewater based on the washing method. This cleaning water is mixed with potable water and treated in the treatment plant.

The wood industry wastewater-based MFC has been considered a feasible option for bioelectricity generation [215]. Raw industrial wastewater from hydrothermal treatment of wood was used as a substrate and source of electrogenic bacteria in a first attempt to use raw wastewater from the wood industry. The bacterial genera in the cathode and anode biofilm were assessed [208]. The *COD* removal efficiency was 87% $\pm$ 5, and *CE* was 18% $\pm$ 2. PD_{max} was observed to increase from 70 to 360 mW m^{-2} after introducing municipal wastewater. Thus, the feasibility of wood industry wastewater as a substrate for MFCs was successfully established.

Table 4 provides an account of MFC performance using different agricultural wastewater emissions as substrate sources.

3.5. Animal origin waste

3.5.1. Fish market

Wastewater is produced due to washing, cutting, and cleaning of fish in the fish market and fish processing industries. These activities generate

Table 4. An account of agricultural wastewater explored in MFCs.

Substrate type	Inoculum	Type of MFC	Working volume (mL)	Anode	Cathode	CD_{max} (mA m^{-2})	PD_{max} (mW m^{-2})	OCV_{max} (mV)	*CE* (%)	*COD* removal (%)	References
Sugar industry wastewater	NA	Double-chamber air-cathodic MFC	60	Carbon cloth	Carbon cloth	320.9	160.16	890	46	85.4	[204]
Lignocellulosic hydrolysates	Mixed microbial culture from wastewater	Single-chamber air cathode MFCs were	12	Carbon cloth	Carbon cloth with platinum	NA	1995	600	32	88	[188]
Retting wastewater	Pre acclimatized culture from MFC	Continuous upflow MFC	500	Plain graphite sheets	Plain graphite sheets	NA	254	880	8	70	[194]
Sugar mill wastewater	*Saccharomyces cerevisiae*	Double Chamber MFC M	3000	Carbon cloth	Carbon cloth	0.784[b]	768	970	NA	NA	[207]
Wood industry wastewater	Wastewaters	Single-chamber MFCs	28	Carbon- fiber brush	Carbon paper with Pt catalyst	7.96[a]	71	430	18 $\pm$ 2	87 $\pm$ 5	[208]
Molasses wastewater	Mixed culture from activated sludge	Dual-chamber MFC	512	Carbon cloth	Carbon cloth with MnO_2	~15	31.37	NA	NA	36	[51]
Cassava mill effluent	Mixed microbes	Dual chamber MFC	700	Carbon rods	Carbon rods	2.75[£]	189	275	NA	NA	[209]

[a] - Volumetric current density (mA m^{-3}).
[b] Total current (mA).

odor and a large amount of wastewater, and the treatment plants for this water have a large footprint [216]. MFC, a technology with a lesser footprint, is used to treat fish market water and produce electricity.

Fish market wastewater was treated in an air-cathode MFC using the hydrothermally synthesized catalyst V_2O_5-MFs (vanadium pentoxide-microflowers) and compared with the efficiency of MnO_2- nanotubes (MnO_2-NTs) [55]. MFCs operated using V_2O_5-MFs cathode catalyst show a 31% increase in *CD*, a 52% increase in *CE* efficiency, and a 32% decrease in charge transfer resistance compared to MnO_2-NTs catalyst. PD_{max} was 6.06 W m^{-3}, *CE* was 17%, and *COD* removal was 80%. The results show that V_2O_5-MFs can be used as a catalyst for fish market wastewater treatment.

3.5.2. Swine wastewater

Animal manure wastewater produced by breeding industries contains highly concentrated phosphorus, organic materials, and nitrates. This wastewater must be treated to eliminate the rich organic contents before it is dumped into the environment [217]. Although there are different chemical processing methods, physical methods, composting, and biological processing methods available, these methods are expensive, and aeration treatment generates a large amount of sludge. Thus, this high-strength organic wastewater is a promising substrate for MFCs to generate bioenergy.

Swine wastewater rich in organic waste has been used as a substrate for bioelectricity generation via MFCs [218]. Three multi-electrode-embedded MFCs fed by an anaerobic digester was operated using swine wastewater [219]. The MFCs were connected sequentially in series and parallel modes. The anaerobic digester resulted in 71.2% *COD* removal and 0.8% ammonia removal. Further, it was observed that in parallel connection, the PD_{max} of 25 Wm^{-3} was 18% higher than in series connection, suggesting the positive aspect of using the parallel connection [219]. In another approach, constructed wetlands coupled with MFCs were used to treat swine wastewater [220]. *Canna indica, Acorus calamus,* and *Ipomoea aquatic* (common macrophytes) were employed. The results show that the *COD* removal rates in control and planted (using the three macrophytes) MFCs were 80.20%, 88.07%, 84.70%, and 82.20%, respectively, while the ammonia removal rates were 80.20%, 88.07%, 84.70%, and 82.20%, respectively. The OCV_{max} was 520 ± 42, 715 ± 20, 660 ± 27, and 752 ± 26 mV, respectively and PD_{max} was 0.2230, 0.4136, 0.3614, and 0.4964 W m^{-3}, respectively. The study shows the positive effect of high removal of contaminants and high energy production as well as the benefits of the constructed wetland coupled MFC. In another study, swine wastewater with 3300 mg L^{-1} of total *COD* was used as a substrate for a two-chambered up-flow MFC in which the effect of variable hydraulic retention time was evaluated [221]. The maximum $TCOD_{max}$ removal rate of 83% was observed at a 20 d hydraulic retention time, and a CE_{max} of 7.1% was observed at a 13 d hydraulic retention time. The PD_{max} values observed at 13 and 14 d hydraulic retention time were 12 and 13 mW m^{-2} respectively.

Pig farm wastewater was used in an MFC with a spherical cathode compartment, and a benthic strain of proteobacteria showed increased electrogenic abilities [222]. Four individual MFCs were stacked in an anaerobic condition to treat piggery wastewater with series or parallel arrangements [223]. The results show an increase in influent *COD* load from 0.2 to 4 g L^{-2}, and the voltage output decreased in the series (71.7%) and parallel (30.7%) connection. *CE* was also reduced in both the series (96.7%) and parallel (94.3%) connections.

3.5.3. Seafood processing wastewater

Fish, crabs, shrimp, and squids are processed and packaged in the seafood processing industry. This seafood processing wastewater contains a high amount of organic contents because of contamination by intestinal remains of fish, flesh, fish heads, and blood. The type of fish and type of processing dictates the quality of the effluents. For instance, oily fish creates more pollution than white fish. This wastewater pollutes the coast and causes eutrophication. This wastewater is used as a substrate in MFCs for both treatment and energy production.

Seafood processing wastewater was used as a substrate for a tubular up-flow MFC. The results show that at an organic load rate of 0.6 g d^{-1}, the *COD* removal was 83% and 95% for total and soluble *COD*, respectively [58]. An OLR of 2.57 g d^{-1} shows a PD_{max} of 105 mW m^{-2}; 2210 mW m^{-3} (volumetric). The bacteria of *Stenotrophomoas* genus were found to be predominant in the anode biofilm. The results show a positive response to the treatment of seafood processing wastewater using MFC.

3.5.4. Slaughterhouse wastewater

Wastewater from slaughterhouses contains a high concentration of organic materials such as blood, lard, fat, and proteins [224]. The total organic carbon and *COD* of wastewater from slaughterhouses are 1200 and 15900 ppm, respectively. Granular anaerobic sludge or anaerobic bacteria is added as a source of microbes and used as substrates in MFC. Additionally, the energy output of MFCs is improved by adding the rumen microbe obtained from ruminant animals to this type of wastewater. This wastewater also contains pathogens and detergents.

A polymer inclusion membrane was used in an air-breathing cathode MFC to treat slaughterhouse wastewater with high *COD* values [225]. The OCV_{max} was 200 mV, *COD* removal was 72%, and PD_{max} was 32 mW m^{-3}. Also, the nitrate, sulfate, ammonium concentrations were reduced to half. The study shows that this MFC could effectively treat slaughterhouse wastewater. Similarly, a self-fabricated MFC with different electrode materials and sizes for treatment of slaughterhouse wastewater showed that a combination of a graphite-copper electrode and 1:10 ratio of the substrate with microbes revealed the best output, with a voltage of 2.4V, PD_{max} of 700 mW m^{-2}, CD_{max} of 318 mA m^{-2} and *COD* removal of 67.9% [59].

3.5.5. Poultry droppings wastewater

Poultry droppings combined with rice husks are a source of organic matter and can be used as an MFC substrate. Poultry droppings are rich in organic contents and are a good substrate for MFCs. A laboratory-scale MFC was designed to evaluate the efficiency of this substrate [226]. The *COD* removal efficiency of a rice husk charcoal electrode compared to carbon cloth was 40%, and the PD_{max} was 6.9 ± 3.1 W m^{-3}. The study suggests the possible use of poultry droppings for electricity generation and simultaneous biodegradation.

3.5.6. Biogas slurry

Biogas is generated by anaerobic digestion of lignocellulosic biomass by biogas plants. These biogas plants produce a high amount of biogas slurry as waste. Biogas slurry contains heavy metal remains, antibiotics, pathogenic bacteria, ammonia, and other volatile materials. It is used as fertilizer for crops [227]. Biogas slurry, a by product of anaerobic digestion, consists of organic matter (4.5%), water (93%), inorganic matter (2.5%), metals, and dry matter (7%).

Corn stover from biogas slurry was used as an anode substrate for MFC to treat biogas wastewater with domestic wastewater as the inoculum [61]. The PD_{max} was 296 mW m^{-2}, *COD* removal was 72%, and nitrogen ammonium removal was 43.9%.

3.6. Fruit waste

3.6.1. Lemon peel

Lemon is a common citrus fruit that produces residues in the form of seeds, pulp, and peels after the extraction of juice. These residues can be fermented to furnish volatile fatty acids. Moreover, they can be used as substrates in MFC to generate bioelectricity.

The use of lemon peel waste as a carbon source substrate for MFCs was evaluated [232]. A double-chamber MFC was used, and the results show that at a peel waste concentration of 1 g L^{-1}, the PD_{max} was 371 ± 30 mW m^{-2}, CD_{max} was 994 ± 41 mA m^{-2}, *CE*% was 32.3%, and the

internal resistance was 143 Ω. The results showcase the possibility of using lemon peel for electricity generation via MFCs.

3.6.2. Orange peel

Orange, another citrus fruit, is consumed as juice or in peeled form, and it occupies 52% of the total production of citrus food throughout the globe. A large volume of wastes, such as seeds, peel, membrane residues, are generated during the production of orange juice. This waste is disposed of in soil or used as animal fodder. Ethanol and methane gas have also been produced from orange peel waste. Orange peel waste is also used as a substrate in MFCs to generate bioelectricity.

Similarly, orange peel was used as a substrate without pretreatment [28]. The results show a maximum voltage output of 0.59 ± 0.02 V, PD_{max} of 358.8 ± 15.6 mW m^{-2}, and CD_{max} of 847 ± 18.4 mA m^{-2}. The activity of pectin and cellulose was also assessed, which shows an absence of cellulose-degrading bacteria. Further studies are needed to optimize the efficiency of MFC using fruit peel waste.

3.6.3. Cashew apple juice

The cashew apple is a significant tropical plant, and 90% of the fruit generates waste after removal of the nuts. Bioethanol is produced from cashew apple pulp, and its fermentation generates important products such as mannitol, lactic acid, wine, dextran, etc. Cashew apple juice has been used as a substrate for an MFC. An MFC operated on clarified cashew apple juice as a substrate showed an OCV_{max} of 0.4V, PD_{max} of 31.58 mW m^{-2}, and CD_{max} of 350 mA m^{-2} [29]. The results show a possible use of this agricultural waste for electricity generation.

3.6.4. Fruit peeling leachate

Fruit peeling leachate was used as an electroactive biofilm over a carbon-felt bio-anode and later scraped for future use. The results show that repetitive use of the biofilm enhanced the efficiency of the MFC [233]. For the first level of biofilm, a 65 mV voltage was produced in 10 days, and the second use showed an OCV_{max} of 276 mV. The study shows an effective technique for optimizing the efficacy of MFC [233].

Table 5 presents MFC performance using agricultural, animal, and human waste as substrate sources.

3.7. Chemical waste

Wastewater from different industries contains a large amount of organic-inorganic chemicals along with heavy metals.

3.7.1. Sulfide and nitrate waste

Nitrate can be removed in the cathode chamber as an electron acceptor while sulfide is used as the electron donor in the anode chamber [234]. Thus, both sulfide and nitrates can be removed from wastewater by using them as substrates in MFCs.

A MFC was constructed to treat sulfide and nitrate, and electricity generation was operated in four different conditions (continuous mode with a graphite rod, batch mode with a graphite rod, continuous mode with graphite felt, and batch mode with graphite felt) [235]. The results show that the *CDmax* was unstable in batch mode, but it was stable in the continuous mode. Bacterial species richness analysis shows a strong positive correlation between anode biofilm bacteria and electricity generation.

3.7.2. Ethanolamine wastewater

Ethanolamine is commonly used in nuclear power plants to inhibit the corrosion process. The concentration of *COD* is raised due to condensed ethanolamine in wastewater. However, a lower ratio of *BOD* to *COD* in this wastewater reduces its biodegradability and hinders its elimination [63]. Ethanolamine (ETA) is an organic chemical used to produce various intermediates, detergents, etc. Studies have realized the significance of ETA wastewater as a substrate for MFCs [63]. A single air-cathode MFC was operated using ethanolamine wastewater as the

Table 5. An account of agricultural wastewater, including animal, fruit waste, explored in MFCs.

Substrate type	Inoculum	Type of MFC	Working volume (mL)	Anode	Cathode	CD_{max} (mA m^{-2})	PD_{max} (mW m^{-2})	OCV_{max} (mV)	*CE* (%)	*COD* removal (%)	Reference
Swine Wastewater	NA	A two-chambered upflow MFC	800	Carbon cloth	Carbon cloth	NA	13	92.8	7.10	83%	[221]
Dairy Industry Wastewater	Activated sludge	Catalyst-less and mediator-less membrane MFC	2000	Graphite plate	Graphite plate	3.74 mA	621.13	0.856	37.16	90.46	[228]
Slaughterhouse Wastewater	NA	Single-chamber MFCS	250	Graphitic granules	Carbon cloth with platinum catalyst	NA	32[a]	200	~5	72%	[225]
Slaughterhouse Wastewater	Mixed culture from rumen	Dual-chamber	1000	Graphite	Zinc, graphite, and copper	318	700	2400	NA	67.9%	[59]
Biogas Slurry of Corn Stover	Domestic water	Dual-chamber MFC	500	Carbon felts	Carbon felts	NA	296	622.7 ± 30.3	4.1	72.0	[61]
Orange Peel Waste	Anaerobic consortia	Dual-chamber MFC	200	Graphite felt	Platinum-coated graphite cloth	847 ± 18.4	358.8 ± 15.6	590 ± 20	15.50	78.3	[28]
Cashew Apple Juice	NA	Dual-chambered MFC	300	Carbon coated on carbon cloth	Carbon coated on carbon cloth with platinum	350	31.58	400	NA	NA	[29]

[a] Volumetric power density (mW m^{-3}).

carbon source, and three different types of separators, namely, a cation exchange membrane (CEM), polypropylene (PP) felt, and proton exchange membrane (PEM) [236]. The results show the best output of MFC with PP felt as the separator in biodegradation, where *COD* removal of 94% and ammonium removal of 52% were observed. In terms of electricity generation, the cation exchange membrane MFC shows a PD_{max} of 583.7 mW m^{-2} and CD_{max} of 0.15 mA cm^{-2}, but the *CE* of 25.1% was the highest for the PEM MFC. A study was performed to scale up stacked MFCs using real ethanolamine wastewater sources [64]. The research shows that a stack of eight MFCs in series shows the best performance with *COD* of 96.5% and ammonia removal at 97.3%.

3.7.3. Acid mine drainage

The flow of acidic water from mines that mixes with the surface water is termed acid mine drainage and consists of a poor amount of organic compounds. Acid mine water is obtained from mines that are environmental hazards because they have a high concentration of heavy metals and are highly acidic [237]. The solution with pyrite oxidation is the solution with the highest acidity and increases the solubility of the heavy metals. The bio-oxidation and oxidation of pyrites naturally produce acid mine drainage.

Acid mine drainage (AMD) lacks organic content, and thus, the use of sewage sludge (rich in organic components) as a substrate in MFC for treating AMD was evaluated [238]. The cathode biofilm was a sulfate-reducing mixed culture of bacteria. After ten days of operation in anaerobic conditions, the results show a 30% (v/v) sludge concentration. The MFC could degrade 71.2% sulfate, 51.6% *TCOD*, and 99.7% heavy metals and showed a PD_{max} of 51.3 mW m^{-2}. Thus, the inoculation of a MFC with sewage sludge enhanced degradation and bioelectricity generation during AMD treatment.

3.7.4. Isopropanol wastewater

Isopropanol wastewater is produced from the cosmetics, electronics, and rubber industries and contains high *COD* and toxic organic compounds. Isopropanol (IPA) wastewater treatment is a must to reduce environmental pollution. This wastewater is treated using different biological methods and physicochemical techniques. However, these techniques consume a large amount of energy and lead to secondary pollution [239]. Hence, the best method is to convert this wastewater to electricity using MFCs.

Wastewater containing isopropanol was decontaminated using an anaerobic fluidized bed MFC filled with adsorptive resin acting as a bio carrier that generated a substantial amount of current [67]. An anaerobic fluidized bed MFC was filled with a microporous adsorptive resin (MAR) and used for IPA wastewater treatment and bioelectricity generation. A PD_{max} of 135.73 $\pm$ 0.17 mW m^{-2} and *COD* removal of 68.21 $\pm$ 0.24% were observed after 21 h with an initial concentration of IPA at 483.49 mg L^{-1}.

3.8. Pharmaceutical wastewater

The pharmaceutical industries and the use of pharmaceutical products at home lead to pharmaceutical-contaminated wastewater. Moreover, wastewater treatment plants also release pharmaceutical wastes. The long-term presence of these pharmaceuticals causes chronic damages, tissue accumulation, cell proliferation retardation, etc. and other chemicals present in the effluents induce their activities further. These pharmaceuticals can be disintegrated by use as substrates in MFCs.

Pharmaceutical wastewater consists of complex organic materials, drugs, and antibiotics. The strength of this wastewater is high based on the presence of different complex materials. MFCs have been used to treat pharmaceutical products such as ibuprofen and naproxen [240]. A dual-chamber MFC was operated with a granular activated carbon (GAC) cathode using pharmaceutical wastewater as a substrate and compared with high-density polyethylene (HDPE) as an alternative [31]. The PD_{max} was 204.9 mW $m^{-2,}$ and *COD* removal was 83% in both cases, showing that the adsorption of organics did not affect the efficiency of the MFC.

3.8.1. Sulfamethoxazole

Approximately 50,000 tons of antibiotics are annually discharged into aquatic habitats. Sulfamethoxazole is a strong broad-spectrum antibiotic and is released in high concentrations in hospital wastewater. If this antibiotic is released on such a large scale to the environment, it can create genes that are resistant to the antibiotic. The transfer of these genes between non-pathogenic and pathogenic bacteria is risky [241], and these genes might contaminate aquatic life. Thus, sulfamethoxazole should be removed from the environment by using it as a substrate in MFCs.

The removal efficiencies of tetracycline and sulfamethoxazole, the two most common antibiotics, were evaluated using a constructed wetland coupled MFC [242]. The results show that the configuration of an MFC integrated with a constructed wetland (CW-MFC) could reduce the concentration of these antibiotics, and the highest accumulation of antibiotic resistance genes was observed at the cathode. The study demonstrates the efficacy of CW-MFCs in treating wastewater containing antibiotics. Another study revealed that a concentration of 200 ppm of sulfamethoxazole could be treated with an MFC [230]. Microbial analysis of the biofilm revealed the presence of sulfamethoxazole scavengers.

3.8.2. Gelatine wastewater

Gelatin is a heterogeneous mixture of proteins obtained from the bones and collagens of animals and is used in the pharmaceutical, cosmetic, food, wine fining, and photography industries [243]. Wastewater obtained during gelatin production consists of calcium, nitrogen, and phosphorus. Conventional methods cannot treat gelatin wastewater [244]. Thus, gelatin can be effectively degraded by using it as a substrate in MFC.

Bioelectricity production using gelatine wastewater as inoculum was evaluated using a single-chamber MFC [245]. Different microbial concentrations (0%, 1.25%, 2.5%, and 5%) were used, and the results show that a concentration of 1.25% produced the highest performance with a maximum *BOD* removal of 20.91 $\pm$ 0.95 mg L^{-1} and *COD* removal of 81.64 $\pm$ 0.01 mg L^{-1}. The performance of the SMFC fluctuated due to competitive interactions among the different microbes in the effective microorganisms (*Escherichia coli*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Lactobacillus bulgaricus*).

3.9. Dairy waste

Dairy wastewater consists of different carbohydrates, proteins, and fats and can be easily biodegraded and used as a substrate in MFCs [246]. Dairy-based wastewater sources have been used as a substrate for a single-chamber MFC with optimization of the configurations to enhance output [68,228]. A continuous MFC was operated, producing an OCV_{max} of 576 mV, PD_{max} of 92.2 mW m^{-2}, *COD* removal of 63 $\pm$ 5%, and *CE* value of 24.2 $\pm$ 1.5% [246]. A double-chamber MFC was used to optimize the treatment and electricity generation of dairy industry wastewater [247]. The production of bioelectricity using ferrite electrodes was enhanced significantly. At a 0.10 m salt bridge length and 10% agar concentration, the efficiency parameters were PD_{max} of 1.0 W m^{-2}, CD_{max} of 1219.69 mA m^{-2}, power of 14.27 mW, OCV_{max} of 886.34 mV, current of 16.10 mA, and *COD* of 86.3 %. In another study using a similar configuration, the *COD* removal rate was 92.2%, *BOD* removal was 88.02%, TDS removal was 76.3%, and OCV_{max} was 644mV [248]. A double-chamber MFC devoid of a membrane generated more current using copper electrodes than stainless steel electrodes [248]. An up-flow tubular air-cathode MFC inoculated with two bacterial consortia, i.e., *Shewanella oneidensis* and *Clostridium butyricum,* was operated to treat dairy wastewater [249]. High biodegradability was observed, with *COD* removal of 94%, *BOD* of 96%, organic nitrogen removal of 47%, phosphorus removal of 95%, sulfate removal of 75%, and nitrogen removal of

100%.The MFC show high electricity generation with PD_{max} of 3.5 Wm^3 and a CD_{max} of 2.4A m3 [249]. In another study, copper-doped iron oxide nanoparticles were used in the anode [250]. The *COD* removal efficiency using nanoparticle-coated anode increased to 75% compared to 64.2% using a graphite anode.

3.9.1. Cheese whey

The watery by product of cheese making that is rich in lactose is known as cheese whey and consists of lipids, soluble proteins, and mineral salts. Because lactose contributes to a high *COD* value, the disposal of cheese whey creates an environmental concern. Although cheese whey can be anaerobically digested, raw whey cannot be treated anaerobically because it is slightly alkaline. Thus, it can be used as a substrate in MFCs.

Cheese whey was used as the electron donor in an air-cathode single-chamber MFC that was assessed using a filtered sterilized raw and pre-treated (fermented for 48 h at mesophilic temperature) cheese whey as the substrate [69]. The investigation showed a PD_{max} of 3.26 W m^{-3} with filter-sterilized cheese whey, but in the case of pretreated cheese whey, the efficiency was lower.

3.9.2. Yogurt wastewater

Wastewater obtained from yogurt production contains high *COD*, suspended solids, and *BOD* concentrations. NaOH is used to wash yogurt bottles, thereby enhancing the pH to 11 [251], and the *COD* of yogurt wastewater can reach 136 g L^{-1}. Thus, yogurt wastewater is used as a substrate for MFC based on its high *COD* concentration and alkalinity.

Yogurt wastewater (pH = 6 to 11), an MFC substrate source for MFC under alkaline conditions (pH 10.5), was assessed using different initial *COD* concentrations. The results showed a PD_{max} of 1.04 W m^{-2}, *COD* removal of 87%, and ammonia removal of 74%, indicating a positive effect of alkaline conditions in electricity generation [70].

3.10. Human waste

3.10.1. Rumen fluid/human waste

Unique and diverse microbial communities are present in cattle rumen fluid, which enhances the efficacy of substrate degeneration. The microbial ecology of the adult cattle rumen is still not known, and it contains 109 to 1011 bacteria per liter of fluid. Only 10% of the microbes present in the rumen have been identified [252]. Plant polysaccharides are digested by the microbes and fermented to produce sugar in the rumen. The rumen is used both as inoculum and substrate. Rumen fluid is eliminated from slaughterhouses and contains a high amount of waste organic constituents.

Rumen fluid from a slaughterhouse was used as a substrate in a double-chamber MFC, where operational parameters such as pH and electrodes were varied [60]. The study shows that the respective OCV_{max} and current values were 610 mV, and 530 mA from a carbon substrate, 470 mV and 140 mA from acetic acid, and 470 mV and 20 mA from spinach. A pH of 7 produced an OCV_{max} of 590 mV with 420 mA current [60].

3.10.2. Urine

Urine has proved a promising fuel for electricity production in MFCs [253,254]. The average production of urine by an adult is 2.5 L per day, which leads to generation of 17 billion L of urine by the global population.

A ceramic-based MFC was fed with human urine, and electroactive bacterial growth was assessed in the absence of sludge inoculum [255]. The study shows that even without sludge, the power output was equivalent to that in the case of sludge, and the start-up time was reduced by 3 days. An analysis of the bacterial community revealed the presence of electroactive bacteria. Another study was conducted using synthetic urine-containing wastewater in a three-chamber resource recovery MFC [256]. The results included efficiencies in treating various contaminants such as 99% urea, 91% creatinine, 99% sodium acetate, and 97% *COD* removal. Another study evaluated microbial diversity and biodegradation with decreasing dilution levels [231]. The results show that in undiluted urine, *COD* removal was 75.5 ± 0.7 %, *CE*% was 26.5 ± 0.7%, and CD_{max} was 495 ± 16 mA m^{-2}. The study concludes that undiluted urine mixed with the microbial community can be an efficient substrate for MFC, with 80% higher COD_s than undiluted urine samples devoid of added microbes.

Table 6 provides an account of MFC performance with chemical, pharmaceutical, and human waste as substrate source.

4. Influence of design parameters on MFC performance

Several studies have been conducted to evaluate the performance of MFCs by varying key operational parameters. These parameters include substrate concentration, microorganism load, conductivity, pH, and electrode material [117,257]. An H-type MFC used a mixture of distillery wastewater diluted with sewage wastewater [258]. The study showed that with an equal ratio of both wastewater types, the total dissolved solids removal was 40%, the total suspended solid removal was 97%, PD_{max} was 836.81 mW m^{-2}, and OCV_{max} was 745.13 mV. The study concluded that the optimum ratio could enhance performance. Another study evaluated the impact of pH, concentration, temperature, and electrodes on efficacy [117]. The performance of a *Rhodococcus pyridinivorans* (HR-1)-inoculated MFC was evaluated using the cationic binder salinomycin [259]. The MFC with 5 mg L^{-1} salinomycin shows a 63.9% increase in PD_{max} and a 28.1% increase in CD_{max}, compared to an MFC with the unfunctionalized electrode. Fruit and vegetable residue was treated in twelve single-chambered air-cathode tubular MFCs [260]. The study suggests the use of graphite-based porous cathodes made of ceramic for cost-effective scale up of MFCs. The following paragraphs highlight the various studies conducted to assess the effect of operational parameters on MFC performance.

4.1. Effect of recirculation

The efficiency of a bioreactor can be enhanced by increasing the flow rate [83]. A recent study evaluated the effect of recirculation rates in a two-chamber up-flow MFC. The results show that at a recirculation rate of 4.8 RV h^{-1}, PD_{max} of 356 ± 24 mW m^{-2} and *CE* of 21.3 ± 1.0% were observed. The effect of anolyte recirculation was evaluated in a two-chamber MFC that treated food waste leachate [131]. The results show the enhanced MFC performance due to recirculation, with CD_{max} of 150 mA m^{-2}, PD_{max} of 29 mW m^{-2}, and *CE* at a *COD* of 1250 mg L^{-1}.

4.2. Influence of operational parameters on performance

Several studies have been conducted to test the effect on efficiency of changing the pH or organic load [117,133,136,261,262]. Different substrate concentrations were used in a double-chamber MFC to treat sugar-industry wastewater for electricity generation [207]. The organic load (substrate concentration) was varied from 100 g L^{-1} to 300 g L^{-1}, the aeration rate was varied from 100 to 250 mL min^{-1}, and the pH was varied from 4.5 to 6.5. An operational value of CD_{max} of 820 mA was produced at a pH of 6, aeration rate of 200 mL min^{-1}, and substrate concentration of 200 g L^{-1} [207]. In another study, dual-chamber MFCs were operated on palm oil mill effluent (POME) using a controlled inoculum (CI) containing microbes from palm oil anaerobic sludge at the anode [263]. The study showed that using a polyacrylonitrile carbon felt anode, a PD_{max} of 0.10 W m^{-2} (twice as high as in the inoculated MFC) and *CE*% of 74% (50% higher than AS) were achieved. However, in the case of *COD* removal, the efficiency of CI was lower. The study shows the positive effect of CI in enhancing MFC output. A double-chamber MFC was operated under three different pH values (4,7 and 8) using POME as the substrate source [264]. The results showed that the highest *PD* was

Table 6. An account of chemical, pharmaceutical, and human waste explored in MFCs.

Substrate type	Inoculum	Type of MFC	Working volume (mL)	Anode	Cathode	CD_{max} (mA m^{-2})	PD_{max} (mW m^{-2})	OCV_{max} (mV)	*CE* (%)	*COD* removal (%)	Reference
Dye Wastewater	Pre-enriched anode	Microbial-fuel-cell-coupled constructed wetlands (CW-MFCs)	NA	Granular activated carbon	Air cathode	NA	0.88 W m^{-3}	560	2.93	312.17 mg/L	[177]
Isopropanol (IPA) Wastewater	Acclimated anaerobic sludge	Anaerobic fluidized bed microbial fuel cell (AFB-MFC)	1280	Graphite rod	Wet-proof Carbon cloth	135.73 ± 0.17	135.73 ± 0.17	NA	NA	68.21 ± 0.24%	[67]
Purified Terephthalic Acid Wastewater	Anaerobic sludge	Single-chamber	250	Stainless steel mesh	Wet-proof Carbon cloth with platinum catalyst	~300	65.6	130	NA	NA	[229]
Sulfamethoxazole	Anaerobic sludge	Two-chamber	115	Carbon felt	Carbon felt	NA	NA	640 ± 40	NA	70%	[230]
Human Urine	Anaerobic sludge	Dual-chamber MFC	NA	Carbon felt in contact with carbon paper	Modified graphite inserts in contact with carbon paper	495 ± 16	227	500	26.5 ± 0.7	75.5 ± 0.7	[231]

achieved with neutral pH, while further changes to the pH of the substrate enhanced the *COD* removal efficiency of the MFC.

Three bacterial strains (*L. sphaericus SN-1*, *L. sphaericus SN-2,* and *B. safensis SN-3*) isolated from wastewater from the alcoholic distillery industries were used as a biocatalyst to assess their effect on power generation and biodegradation in a MFC running on distillery wastewater as a substrate [265]. Furthermore, the impact of changing pH in the range of 6–8 and *COD* concentrations in the range of 3200–6400 mg L^{-1} was assessed for the MFC. The results show that using *L. sphaericus SN-2* as the biocatalyst gave the best performance of the MFC, with an OCV_{max} of 646 ± 5 mV, a PD_{max} of 104 ± 3 mW m^{-2} and treatment efficiency of 63.4 ± 0.5% at pH 8. Using the same strain, the MFC operated efficiently with PD_{max} of 88.8 ± 5 mW m^{-2}, CD_{max} of 287 ± 12 mA m^{-2}, and *COD* removal of 57.4 ± 0.4% at a pH of 8, whereas in the same conditions, varying the *COD* concentration to 6400 mg L^{-1} resulted in PD_{max} 123.5 ± 3 mW m^{-2} and CD_{max} of 323.4 ± 4 mA m^{-2}. Variable substrate pH and buffering conditions were used in an MFC that treated distillery wastewater [135]. The anode chamber pH ranged from 5.4 to 10, while the pH in the cathode chamber was fixed at pH 7.5. The best performance was achieved at a pH of 8, yielding PD_{max} of 168 mW m^{-2}, CD_{max} of 580 mA m^{-2}, *COD* removal of 68.2%, color removal of 26.4%, and TDS removal of 15.4%. Furthermore, the use of a borate buffer also enhanced the PD_{max} to 194.7 mW m^{-2}. The study shows that pH 8 and the use of borate buffer highly influenced the system performance. Variable substrate concentration, aeration rate, and pH were used together in an MFC operating on distillery wastewater as the substrate [257]. The use of a biocatalyst (*Saccharomyces cerevisiae*) was also explored. At a substrate concentration of 175 mg L^{-1}, a PD_{max} of 69 mW m^{-2}, CD_{max} of 82.48 mA m^{-2}, OCV_{max} of 770 mV, power of 0.6391 mW, and current of 0.83 mA were produced. Thus, by varying the parameters, the optimum efficacy of the MFC was attained. Another study showed that increasing the pH to 8 produced PD_{max} of 63.8 ± 0.65 mW m^{-2} and *COD* removal of 63.5 ± 1.5% for an MFC running on distillery wastewater [266]. Furthermore, a *COD* concentration of 3200 mg L^{-1} and conductivity of 9.7 mS cm^{-1} shows a PD_{max} of 202 ± 6 mW m^{-2} and a CD_{max} of 412 ± 12 mA m^{-2}. Different organic load rates (OLR) (435–870 mg *COD* L^{-d}) were used in a dual-chamber MFC that treated domestic wastewater [267]. The results show that an OLR of 435 mg *COD* L^{-2} was optimum with a PD_{max} of 253.84 mW m^{-2} and *CE* of 25.01%. Furthermore, the study also shows that an increase in the organic loading rate negatively affected the ammonia and phosphate recovery.

4.3. Effect of substrate type and concentration

Various groups have evaluated the effect of substrate type on the performance of the MFCs. In one such study, variable concentrations of acetate and peptone were used in a dual-chamber MFC [89]. The results showed that 500 mg of dissolved acetate or peptone produced PD_{max} values of 0.11 W m^{-2} and 0.11 W m^{-2}, respectively. Furthermore, the substrate removal rate was 44 g DOC m^{-2} h^{-1} in acetate and 52 g DOC m^{-2} h^{-1} in peptone. A double-chamber anoxic MFC was operated using wastewater from a primary clarifier stream effluent, and its efficiency was assessed for different added glucose concentrations [268]. The OCV_{max} of 96.4 mV was observed at 5.56 mM. It was found that the increasing glucose concentrations results in lower the *PD* by 46%. Another study evaluated the efficacy of co-substrate for increasing MFC efficiency in treatment of refractory water [269]. 4-chlorophenol was the refractory pollutant, and acetate was used as a co-substrate. The study shows a 4.3-fold increase in power generation, and the removal rate of 4-chlorophenol increased by 53%. Also, additional bacteria that degrade 4-chlorophenol were found to be present.

A study assessed the removal of neomycin sulfate antibiotic using MFCs. The findings show that an EAB-based MFC degraded the antibiotic and detected its minimal levels in the wastewater using LC-MS/MS in parallel with *COD* and total carbohydrate removal [270]. A miniaturized MFC was operated using various substrates such as acetate, glucose, lactate, and octonate [271]. The results show that biodegradability using any of the four substrates was the same at 650 mg *COD* L^{-1} d^{-1}. In terms of bioelectricity generation, acetate MFC gave the best performance with CD_{max} of 20 A m^{-2}, PD_{max} of 2 W m^{-2}, OCV_{max} of 0.376 V, and *CE* of 12.6% [271]. Variations of the substrate concentrations and three different cathode conditions (dissolved oxygen, ferricyanide, and air cathodes) were used to evaluate the efficacy of current generation by waste products using a bioethanol effluent [272]. The results reported the highest substrate load of 2 g *COD* L^{-2}. The ferricyanide cathode MFC shows a CD_{max} of 1630 mA m^{-2}; however, the air cathode MFC was the most suitable option for bioethanol effluent. A double-chamber MFC that treated glycerol was assessed for its performance by varying the substrate (glycerol) concentration in the range of 0.5–5.2 g *COD* L^{-1} [85]. The best performance was observed at a glucose concentration of 3.2 g *COD* L^{-1}, where the *CE* value was 34.1%, *COD* removal was 99%, and the PD_{max} was 0.065 Wm^{-2}. In another study, the concentration of acetate was varied [11]. The results show that an acetate concentration as low as 1

mmol L^{-1} did not reduce power. The performance of an anoxic-aerobic MFC that treated spent caustic wastewater was evaluated using different hydraulic retention times (HRT) (7–9 days) and mixed liquor suspended solids (MLSS) (1500, 2000, 2500 mg L^{-1}) [30]. For all MLSS concentrations, the MFC performed best at a hydraulic retention time of 9 days, with *COD* removal of 98%, sulfide removal of 98.99%, and OCV_{max} of 82.1 mV. At an MLSS concentration of 1500 mg L^{-1}, the highest *COD* removal of 94.7% and sulfide removal of 89.01% were observed, whereas an OCV_{max} of 36.7 mV was obtained at 2500 mg L^{-1} MLSS [30], and other figures of merits were reduced.

4.4. Influence of wastewater conductivity

The ionic conductivity of wastewater has a substantial impact on MFC performance. An enhancement in NaCl (20 g L-1) concentration in synthetic wastewater raised the PD_{max} value to 35 W m^{-3} from 27 W m^{-3} [119]. Conversely, the PD_{max} value decreased from 35 W m^{-3} to 18 W m^{-3}, enhancing the NaCl concentration from 20 g L-1 to 40 g L^{-1} in wastewater from food leachate [120]. In another study, highly conductive landfill leachate (73 mS cm^{-1}) generated a low PD_{max} value of 0.3 W m^{-3} [273]. The high amount of total dissolved solids (57 g L-1) exhibited a PD_{max} value of 1.5 W m^{-3} [274]. A small PD_{max} value of 9 W m^{-3} was obtained with highly conductive seafood wastewater (7.95 mS cm^{-1}), despite exhibiting 15% of *CE* and 80% of *COD* removal efficacy [275]. Alternately, another study reported an enhancement of MFC power densities by boosting the ionic conductivity (20 mS cm^{-1}) [276].

4.5. Summary of recent progress on substrate utilisation

Power generation using MFC devices has been regarded as a sustainable source of energy from waste organics that could positively impact environmental well-being [277]. Several studies concluded that the SCMFC had been effectively used for power generation from domestic, dairy, distillery, brewery sources, etc. [278]. An up-flow anaerobic sludge blanket reactor MFC-biological aerated system (UASB-MFC-BAF) was designed to manage wastewater obtained from molasses with simultaneous power generation [279]. Another MFC system was designed on a pilot scale and was equipped with a multi-anode/cathode system configuration to treat wastewater [280]. Ideally, the MFCs should be stable and efficient in long-term applications for practical purposes. However, the use of Pt in cathodes, the need for mediators, and the proton exchange membranes result in high construction costs. This has led to the development of SCMFC in which the proton exchange membrane has been substituted by biocathodes [281].

In a DCMFC, the organic pollutants are treated abiotically within the cathode chamber, and the electron acceptors are the pollutants. The double-chamber MFC has been used to treat wastewater generated from slaughterhouses, food processing industries, and distilleries. The water contains contaminants like polyaromatic hydrocarbons, azo dyes, and metals (Cu, electroplating water, and V) are also purified using DCMFC [282]. Domestic sewage and industrial wastewater consist of different contaminants that a single treatment cannot remove. Thus integrated and hybrid MFCs are essential for adequate treatment of wastewater, including sediment MFC, wetland MFC, Fenton type MFC, earthen-pot MFC, and macrophyte-based MFC [282]. Wastewater was treated in two stages using a hybrid SCMFC combined with an anaerobic fluidized bed membrane bioreactor [283]. The initial phase of treatment was performed by the MFC, while the next step of the treatment was carried out by the bioreactor, eliminating *COD* and suspended solids by up to 92.5%. Another work reported integrating a hydrolytic up-flow sludge blanket with a constructed wetland-MFC to remove pollutants from wastewater. The wastewater was treated initially by the sludge blanket, followed by CW-MFC treatment operating in horizontal subsurface flow mode in the second stage [282]. The combination of more than 1 unit of MFC in a series or parallel configuration also enhanced bioelectricity production. However, the current output is greater when the MFC units are connected in parallel. The efficacy of the MFC was enhanced by introducing submergible electrode modules in which the electrode assemblies could be removed and maintained individually [284]. The existing facilities can be modified, and the MFC can function in primary clarifiers or sludge storage tanks that are empty. Thus, another submergible MFC was designed to treat municipal wastewater for enhanced efficacy; however, it was prone to biofouling due to high salt concentration [105]. Cu-doped iron-oxide nanoparticles were designed as an anode for enhancing the MFC efficacy for treatment of dairy effluents containing carbohydrates and proteins [250].

MFC performance fluctuation is attributed to altering the concentration of the feed solution. The concentration of the carbon source and the rate at which the microorganisms utilize the substrates dictate power generation. Biofilm formation occurs on the surface of the anode under the open-circuit mode, enhancing current production. Moreover, a higher organic loading rate sometimes decreases the amount of current generated because the low concentration of *COD* aids in the current generation. A high organic loading rate tends to foul the membrane, affecting the electrochemical performance of the system. A high organic loading rate also initiates the deposition of volatile fatty acids, contributing to diminishing current. Thus, there is always an optimal value of the organic loading rate for maximum power generation. The power production in MFCs is also affected by the *COD* concentration because the decrease in the concentration of the influent *COD* reduces the power output. However, the *COD* removal efficiency may be stable, but the power generation is not stable because it depends on the microbe conversion routes [285]. A series of MFCs equipped with an algae cathode were connected with different HRT to purify landfill leachate [166]. Wastewater containing dye liberated from the textile industries was treated with 3 MFC units connected in parallel and series connections. The study revealed that the bioelectricity generated from the parallel connection was much higher than that of the series connection [187].

5. Best performing substrates—a path to implementation

In the last decade, numerous substrates and wastewater sources have been tested in MFCs for power generation and wastewater treatment. However, the field has already explored hundred of substrates, so it is time to shortlist the best-performing substrates in each category for power generation or treatment, depending upon the desired outcome from the systems. We have shortlisted the best candidates with consideration of the highest *CD, PD, CE* recovery, and *COD* removal (Figure 2a-d, respectively) within the last five years. Here we see that domestic waste, including kitchen, human urine and sewage, appear regularly in as top 7 candidate substrate sources for these categories. Thus, MFCs applied to household waste shows much promise, and one can imagine small-scale MFCs being integrated as an important component in future homes. Other waste streams that appear to be promising sources of substrates for MFCs include those being produced from distilleries, sugar industry, slaughterhouses, and those producing lignocellulosic hydrolysates.

5.1. Summary of recent progress and gaps for industrial implementation and fundamental studies

Following the exhaustive review of MFC performance by substrate, it is worthwhile to close with some overarching technological considerations, independent of which substrate is used. For example, significant development is continuously underway to realize low-cost, high-performance and low-maintenance electrodes [10,286]. Another avenue to implementation is in scaling up. The reader is directed to a recent review on pilot-scale MFCs for an up-to-date snapshot of the current stat od the field [287]. The use of a large scale (85 L) MFCs fed with a recirculating domestic wastewater anolyte led to power densities of 0.12 W m^{-2} and COD removal rate of approximately 80%. A 260 L MFC applied to domestic wastewater produced lower power densities (15 mW m^{-2}) but

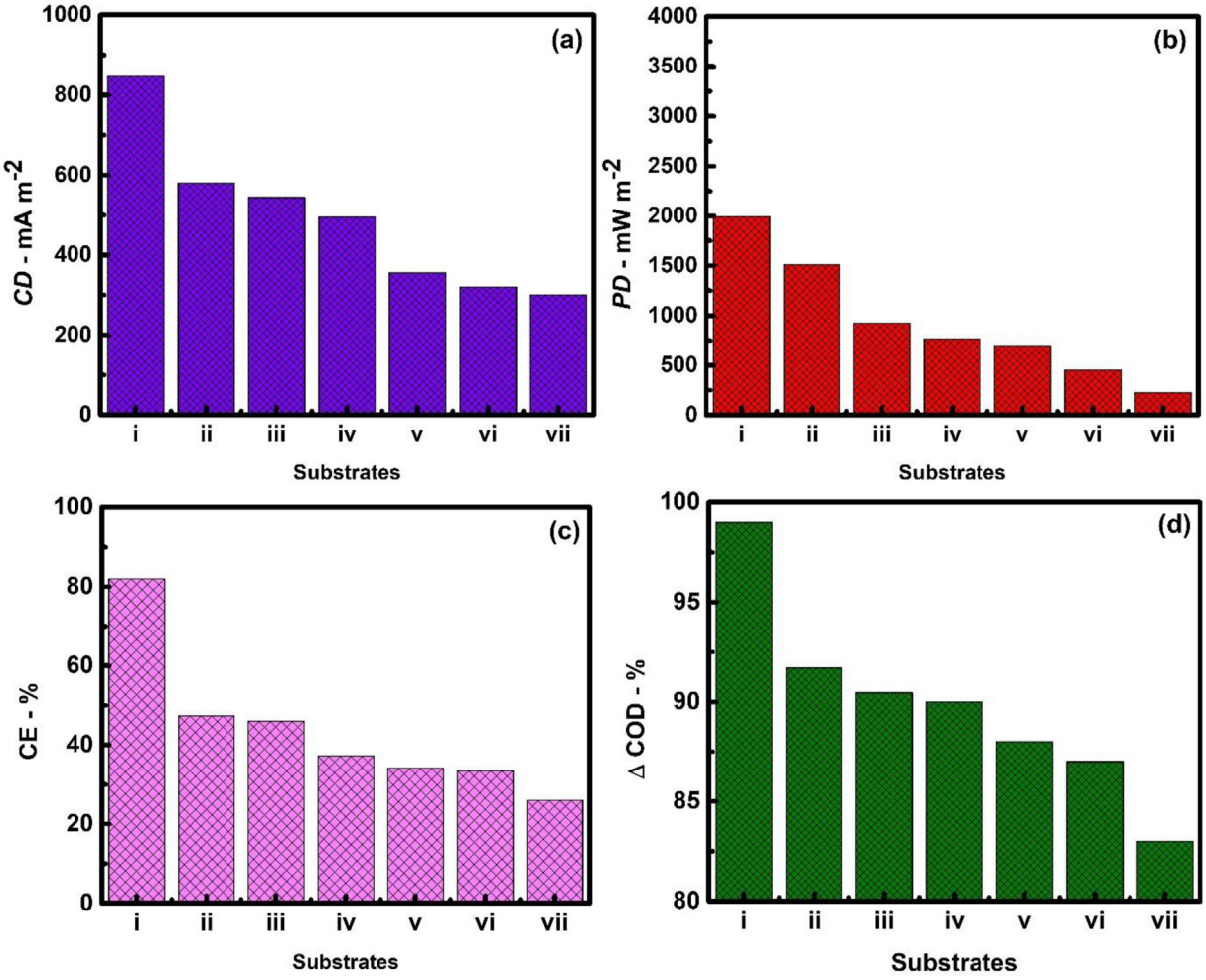

Figure 2. Seven best results organized by metric from best (i) worst (vii). (a) Best current densities (CD) achieved to date – (i) Domestic wastewater, (ii) sugar industry wastewater, (iii) synthetic wastewater with xylose, (iv) human urine, (v) petroleum refinery wastewater, (vi) distillery wastewater, (vii) orange peel waste. (b) Best power densities (PD) to date – (i) Human urine, (ii) glucose-based synthetic wastewater, (iii) slaughterhouse wastewater, (iv) sugar mill wastewater, (v) kitchen wastewater, (vi) landfill leachate, (vii) lignocellulosic hydrolysates. (c) Best Coulombic efficiencies (CE) to date – (i) Human urine, (ii) domestic wastewater, (iii) glycerol, (iv) dairy industry wastewater, (v) sugar industry wastewater, (vi) distillery wastewater, (vii) synthetic wastewater. (d) Best carbon removal efficiencies to date – (i) Swine wastewater, (ii) wood industry wastewater, (iii) lignocellulosic hydrolysates, (iv) chocolate industry wastewater, (v) dairy industry wastewater, (vi) domestic sewage (vii) glycerol.

almost identical COD removal rate of 80%. Thus, MFCs as a tool for wastewater remediation seems promising with scale up. Nevertheless, pushing the COD removal efficiency to higher values is required to avoid costly secondary treatment steps before environmental discharge. The literature shows that COD removal can be efficient in bulk-scale MFC down to concentrations as low as 100 mg L^{-1}, and that current densities become reduced and stop outright at 50 mg L^{-1} [72,73]. Focusing on power continues to be the priority for researcher. Recently, Rossi et al., 2020 reported a PD_{max} of 7.1 ± 0.4 W m^{-2} by accurately controlling buffer concentration in localized pH [288]. At that time this was the highest PD_{max} ever recorded for an MFC. Since then, the same authors improved on this with the current record output (8.8 W m^{-2}) which was produced with a "near-zero" electrode gap distance across an anionic exchange membrane [289]. We anticipate the incorporation of more micro-scale MFC features will be an avenue to future development. For now, fully microfluidic systems have been the subject of significant development as tools for better understand fundamental aspects of electroactive biofilms [74,290,291,292] as well as toward practical considerations including fine-tuning of electrode spacing, hydrodynamic effects, and the associated resistance distribution [293,294,295,296]. Moreover, it is anticipated that certain micro- and milli-fluidic elements can be integrated into otherwise large-scale MFC systems. One avenue that shows promise is in the ability of microflows to drastically enhance mass transport. A recent study showed that microfluidic sub-systems may be possible to remove remnant COD from bulk-scale MFCs [74]. Apart from power generation, these systems can be used for various environmental sensors [294].

6. Conclusion and future directives

This review showcases the recent progress of substrate exploration and its performance in MFC systems and highlights the success stories of various configurations and the operating parameters that affect the efficacy of the MFCs. It also emphasizes the gaps that need to be addressed in the near future with the proposed cutting-edge research methodologies.

There have been promising improvements in the MFC performance, and several groups have already reported field trials. A few tests are currently underway to understand the practical applications of this technology. It is well understood that the overall performance is associated with bacterial kinetics, and therefore, it has intrinsic limitations. However, to enhance MFCs and their performance for practical applications, more studies need to be conducted with the best-performing substrates for the purpose of deploying systems in real-scale

wastewater treatment plants. In our opinion, apart from wastewater and power generation, MFCs can be used in several other applications, depending upon the system architecture and utilization of EABFs. A few areas need to be explored and are further described below:

i) Reactor configuration and system architecture

The MFC is suitable for treating diverse wastewater sources. However, due to their lower efficiency, they can be installed in a hybrid bioelectrochemical wastewater treatment plant. The hybrid process train can be composed of several stages. The stages include an anaerobic digester and an electrochemical oxidative reactor, followed by a MFC stack. For this configuration, highly efficient electrodes need to be developed and investigated for cost-effective scale up of MFCs.

ii) Separator

The proton exchange membrane has been generally explored to test the various configurations of the MFCs; however, polymer-based membranes cannot be the best option for building large-scale device fabrication. The major concern is that the polymer membrane cannot withstand high hydrostatic pressure, and therefore, it is essential to refocus efforts on ceramic-based membranes for MFC applications.

iii) Cathode catalyst

The catalyst plays a pivotal role in MFC systems, and several high-performing non-precious metal catalysts have already been examined. The best candidates should be tested in the ceramic-based electrode assembly for a performance and longevity study. Photosynthetic bacteria could be used to make self-sustained cathodic compartments by feeding CO2 generated by an anode chamber. The identification of novel bacteria that enhance the MFC performance will also benefit from the practical utility of the device.

iv) Biocatalyst and sensors

Apart from power generation and wastewater treatment, the MFC has a potential application in environmental sensors. There has been a great focus on making various amperometric MFC sensors for online *BOD*, toxicity, etc. However, these sensors have relied on bacterial kinetics, and bacterial composition can be easily altered upon slight variation of the substrate, pH, and operating parameters, resulting in inaccurate measurements. 3D biofilm printing of a pure EAB can be a promising approach to making highly reliable sensors that can address this problem. A 3D printed biofilm is prone to the issues mentioned above; however, 3D biofilm printing research is in the early stages and needs extensive work for 3D printed biofilms in MFC-based sensors for precise response. 3D biofilm printing technology can be a promising solution for desired bacterial catalysts, including the loading of bacteria and a highly defined biofilm layer that may enhance power generation and wastewater treatment.

Declarations

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