

9

Bioelectricity Generation from Organic Waste Using Microbial Fuel Cell

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Abstract

Organic waste is a huge challenge and the scientific community is constantly striving to reduce organic waste emission. The moto of scientific community is "waste to watt" or "waste to energy." This chapter emphasizes the application of microbial cells as electrochemical platforms for the conversion of organic waste for the production of fuels. Microorganisms play the most prominent role that is used to degrade the contaminants or substrates into harmless and valuable resources under mild operating conditions. In this technology, microorganisms act as biocatalysts to oxidize the substrate in the anode chamber from where the electrons are directed to the cathode as a result of electrical flow. Electricity generation by microbial fuel cell (MFC) is a pioneer in this issue. Like a battery, MFC uses chemical energy to generate electricity by using a natural process of cellular respiration of microorganism. MFCs have two electrodes each in the anode and cathode and they are held in separate chambers. The chambers can be with or without membrane. The anode chamber contains the anaerobic bacteria and the cathode chamber is aerobic. One of the best advantages of bacteria is that they can practically use nutrient that may be organic or inorganic. The oxidation process occurs within the bacteria living in the anode chamber. Electron bonds hold the food molecules together that bacteria eat. The bacteria break these bonds to release the electrons. The electrons released are captured to maintain a constant power density. Although the amount of fuel generated is low, nevertheless the technology is a hope for mitigating waste.

Keywords

Microbial cell fuel · Bioelectricity · Electrode · Microbe · Organic waste

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9.1 Introduction

The global organic waste produced is alarming; most of the waste collected is dumped into landfills. This is not an effective way of disposing of the waste as this may further enhance the greenhouse effect by producing methane gas. The rapid consumption of non-renewable energy resources has led to the depletion of fossil fuels, an increase in CO₂ emission, and global warming, forcing the new world to look into alternative energy sources (Dhulipala et al. 2020). Organic waste and waste water are becoming a unique investment choice for developing biofuels because of the high organic contents, which could reduce the cost of biofuels production effectively (Owusu and Asumadu-Sarkodie 2016; Rai et al. 2020). Some specialized microorganisms have the ability to transfer electrons from the inner to the outer membrane of the cell via the electron transport chain. Researchers have used this phenomenon to explore new renewable energy generation methods based on microbial fuel cells (MFC) (Madakka et al. 2020). MFC is based on dual benefits for treating waste and producing energy from waste (Zhang et al. 2008). The entire concept of microbial fuel was an initiative by Michael Cresse Potter in 1911, wherein the first employed Saccharomyces cerevisiae and bacteria Escherichia coli for power generation in MFC (Potter 1911). For the last century, microbial fuel cell (MFC) has been used as an instrument in recovering resources from organic waste in generating biogas, dyes, electrical energy, biosurfactants, biofertilizers, bioplastics, pesticides, phenolic compounds, polyhydrocarbons, pharmaceutical products, textile, and removal of heavy metal (Sharma et al. 2020; Rai et al. 2020; Suresh et al. 2022). MFCs are also used in fertilizer production from human excreta and urine (Sabin et al. 2022).

Exo-electricigens are bacteria that can transfer electrons exogenously (outside the cell) to a terminal electron acceptor. A terminal electron acceptor's higher positive redox potential results in a higher energy gain. The process of generating electrons is known as electrogenesis, and the system or reactor is known as a microbial fuel cell (Logan 2009). The MFC architecture varies widely depending on the designer's need. A simple microbial fuel cell (MFC) consists of two compartments separated by a membrane or not, which allows the flow of electrons during the process (2004). The compartment consists of two electrodes, an anode and a cathode inoculated with microbes, and organic waste is added to the anode (Xu et al. 2017). Microorganisms use the organic matter contained in waste for their growth, nutrient, and reproduction. The metabolic processes by microorganisms produce several byproducts, such as protons and electrons that can be converted into energy (Clark and Pazdernik 2016). MFCs cannot operate at very low temperatures as the reactions inside the reactor take place at a very slow rate. Raw materials such as glucose, alcohol, butyrate, acetate, sodium acetate, sodium butyrate, and propionate are used as substrates for the organism to produce bioelectricity by chemical reduction (Logan 2008; Harnisch and Schröder 2010). Currently, the technology suffers in scaling-up with respect to designing and optimizing the physical and electrochemical parameters. Although microbes (biocatalysts) have a faster generation time, it is a poor conversion rate. Factors swaying MFC are type of microbial diversity,

electrodes, electron donor/acceptor, series and parallel connection, metal oxide, structure and concentration of organic pollutants, nature and resistance of electrolyte, circuit connection type (closed and open circuit), pH, temperature, and carbon source (Suresh et al. 2022).

9.2 MFC Working Principle and Electron Transfer

The significant components of MFCs are anode, cathode and membrane or separators. In MFCs, the anode chamber consists of organic matter and the exoelectrogenic bacteria that adhere to the anode surface and decompose the organic matter by oxidation of the substrates to produce CO_2 , protons, and electrons by the anaerobic process (Verma et al. 2018). The electrons generated from the metabolic activity of microorganisms are collected by cytochrome or redox protein and transferred to the cathode to react with a terminal electron acceptor (oxygen) through a copper electrical circuit and resistor. The flow of electrons through the external electric circuit is responsible for generating electric current (Logan and Regan 2006). Zhang et al. (2017) found that operating MFCs at a higher external resistance (1000 Ω) was feasible and then gradually switching to lower external resistances to facilitate higher current, increased energy output, and maximum power density. Concurrently, the H⁺ ions flow through the semipermeable membrane combined with dissolved oxygen to form water molecules at the cathode. This process is driven by the electrochemical gradient resulting in a higher concentration of H⁺ ions near the anode. The anode material acts as a catalyst for the transfer of the reaction while maintaining conductivity (Logan 2009).

Anode : organic waste
$$+ H_2O \rightarrow CO_2 + H^+ + e^- + O_2 \uparrow$$
 (9.1)

Cathode :
$$e^- + H^+ + O_2 \rightarrow H_2O$$
 (9.2)

The bacterial cells gain energy from pumping protons across the bacteria's inner membrane. This is responsible for forming a proton gradient, which produces ATP from ADP through ATPase and provides metabolic energy for the bacterium. The maximum current that an MFC can produce depends on the actual rate of substrate biodegradation and electron donor. The higher the positive redox potential of a terminal electron acceptor, the higher the energy gains for an organism (Harnisch and Schröder 2010).

9.2.1 Role of Microbial Fuel Cell (MFC)

- MFC system produces low amounts of sludge
- Recovers chemical energy from renewable sources like wastewater and organic matters

- Human waste is being reconnoitered as an efficient source to produce bioenergy or bioelectricity
- The concept used is "waste-to-energy"
- Generating electro carbon compounds from sequestration of CO₂ by employing anaerobic electrotrophic microbes as biocatalysts
- · Onsite generation of biohydrogen and power in remote areas
- Potential application in groundwater to remove petroleum contamination
- · It can be operated at ambient temperature and atmospheric pressure
- MFCs are used for the simultaneous removal of sulfide and nitrate from wastewater
- · MFCs have desirable features of secondary storage batteries
- As biosensors in in situ monitoring and control for pollutant analysis, the advantages of using biosensors are miniaturization, easy operation, low cost, and safety

9.2.2 Limitation in Microbial Fuel Cell (MFC)

Besides the advantages of this technology, it still faces practical barriers such as low power, low efficiency, and current density that may be attributed to low-quality materials being used as anodes or material cost issues, especially the cathode and membranes if used (Yaqoob et al. 2020). The technology can have a brighter side as a new source of bioenergy, as researchers are extensively working on designs and configurations of electrodes and kinetics models for biofilm formation and planktonic performance (Solanki et al. 2020). Large-scale commercialization of MFC is the biggest obstacle due to its architecture (Logan and Regan 2006), membrane resistance during transportation of protons and problems in both chambers (Yaqoob et al. 2020). New materials, factors affecting the performance (electron transfer mechanisms, material and surface area of anode, cathode electrode, membrane, distances and flexibility), applications and cost-effectiveness for manufacturing MFCs have to be considered to extenuate electricity generation (He et al. 2017). Existing literature has pointed to greater power outputs between 2 and 5 W/m² and volumetric power over 100 W/m³ if a smaller microbial fuel cell reactor is used for operation. Smaller the MFC, the better the operating condition (higher temperature and better conductivity). Reducing the distance between the anode and cathode can prevent fermentation and ohmic losses (Behera and Ghangrekar 2011; Yang et al. 2020a).

9.2.3 Mediators and Non-mediator MFCs

9.2.3.1 Mediator-Less or Direct Electron Transfer Between the Cell Surface and the Electrode

It's the perfect alternative for producing electricity, mediator-less MFCs are operated with a dissimilatory metal-reducing microorganism primarily to the families of Shewanella, Rhodoferax, and Geobacter. Here the electron transport proteins present within the microbial cell transfer electrons from the cytoplasm to the outer membrane and finally to the anode. Electron transfer occurs through the outer membrane cytochrome or transmembrane and nanowires on the anode surface without any electron mediators. Bacterial nanowires are electrically conductive appendages composed of stacked cytochromes produced notably from the Geobacter and Shewanella genera and can form biofilms on the anode. The nanowires allow electricigens to use an electrode that is not in direct cell contact as the electron acceptor (Gorby et al. 2006). Chaudhuri and Lovley (2003) first reported a stable and long-term power generator by a mediator-less MFC using *Rhodoferax ferrireducens*, that oxidized glucose to CO_2 and quantitatively transferred electrons to graphite electrodes. The H⁺ diffusion also improved in the electrolyte fed with salt, thus enhancing current generation in membrane-less cathode chambers (Liu et al. 2015).

9.2.3.2 Mediator or Indirect Electron Transfer Mediator

Here, a soluble mediator eliminates the direct interaction between the cells and the electron acceptor. In order to generate electricity, electro-active metabolites are used since microorganisms are electrochemically inactive for transferring electrons to the anode electrode. The electron mediators enter the bacteria cells, extract the electrons from the metabolic reactions of the electricigens, and supply these electrons to the anode of the MFC (He et al. 2017). Lactococcus lactis produces a natural mediator that produces guinones which are able to mediate electron transfer to extracellular electron acceptors such as Fe^{3+} , Cu^{2+} and hexacyanoferrate (Freguia et al. 2009). Depending on the microorganism species, involved mediators such as phenazine and pyocyanin may be natural. Neutral red, potassium ferricyanide and sulfate/sulfide anthracenedione, thionine, humic acid, meldola's blue (MelB) and 2-hydroxy-1,4naphthoquinone (HNQ), riboflavin and methylene blue are used to increase the efficiency of microbial fuel cells and to reduce the activation energy (Li et al. 2014). However, as Cao et al. (2019) reported, the addition of mediators has attracted drawbacks to the working of MFCs as it could lead to relatively low current densities, expensive and toxic to the microorganisms. Also, separating these mediators from the solution is difficult as the mediators are water-soluble phenolic compounds. Some of the properties of mediators, as reported by Shukla et al. (2004), are (1) they should not interfere with the metabolites in the bacteria; (2) the mediators should be in an electrolyte solution and not adsorbed onto the microorganism; (3) the reduced mediator should easily diffuse out of the cell and move to the anode where they are oxidized; (4) the oxidized or reduced states of the mediator should be chemically stable and must be fast in the electrolyte solution.

9.3 Materials and Architectures of Different Types of MFC

Depending on the availability of the substrate and microorganisms to metabolize the substrate, the power produced by MFCs may vary. The reactor is also affected by the rate of electron transfer from bacteria to the anode, cathode performance, the



Fig. 9.1 Single chamber microbial fuel cell

electrolyte, circuit resistance, proton mass transfer within the liquid, and the ion exchange (Liu et al. 2015). The anodic and cathodic chambers may or may not be separated by a proton exchange membrane (PEM), and different types of electrode material are being commercialized. Moreover, there are various influential factors for the performance of the MFC, such as temperature, pH, nutrients, and fuel cell configuration (Yaqoob et al. 2020). Figure 9.1 is a schematic illustration of single chamber microbial fuel cell.

9.3.1 Double-Chambered Fuel (DCF)

Double-chambered fuel is the most commonly used MFC. They are H shaped in structure and consist of a double-chamber with an anode and cathode chamber separated by a proton exchange membrane (PEM) or salt bridge. Oxidants such as ferricyanide and permanganate are used as a source of oxygen. In double-chambered MFC, the two chambers are connected by a circuit, and the sum of cations apart from H⁺ transferred from the anode chamber to the cathode chamber is equal to the sum of e^- transported through the circuit (Yap et al. 2020). The membrane (PEM) prevents oxygen diffusion into the anode and facilitates proton transfer from the anode to the cathode. The electrodes are in close proximity with the membranes resulting in higher oxygen diffusion from the cathode to the anode, thus increasing power



Fig. 9.2 Double chamber microbial fuel cell

production and density (Choi et al. 2013). They are typically in batch mode and used in waste water treatment rather than electricity production (Du et al. 2007). Figure 9.2 schematic illustration of double chamber microbial fuel cell.

9.3.2 Single Chamber Fuel Cell (SCFC)

This fuel cell consists of a simple carbon electrode as an anode chamber and porous carbon exposed to air as a cathode. The cathodes are normally coated with graphite, in which electrolytes are poured into a steady state that prevents them from drying out. Single-chamber MFCs can achieve better performance than a two-chamber system due to the high mass transfer rate and oxygen concentration in the air compared to water (Fan et al. 2007a). A single chamber microbial fuel cell has an external cathode wall that is exposed to the atmosphere and eliminates oxygen (aeration) pumping to the cathodic chamber, thus reducing the cost (Cheng and Logan 2011). The advantage of using SCFC is less frequent oxidative media, aeration changing, and higher power generation (Logan et al. 2019). Eliminating the membrane in the chamber not only reduces the cost and complexity of MFCs but also increases the power density due to a decrease in internal resistance and is simpler to use than DCF.

9.3.3 Stacked MFC (SMFC)

In stacking, multiple cells are positioned in series or parallel connections. The voltage and current increase depending on the connection mode (parallel or series). The factor affecting the electricity production in stacked MFC is a microbial community, resistance, composition of the substrate, module configuration, anolyte and operation mode such as batch or continuous. It is possible to achieve improved voltage or current output by connecting several MFCs in series or parallel (Zhuang et al. 2012). Zhao et al. (2016) observed that when glycerol was used as a substrate, it is degraded faster in parallel connection than in series; they also noted that maximum power density increased with the increasing glycerol concentration in either of the connections. Generally, when MFC units are stacked in series, the voltage increases, whereas a parallel connection enhances the current (Aelterman et al. 2006).

Furthermore, switching from one connection mode to the other, the voltage output and microbial communities changed. For instance, when stacks were connected in series and then in parallel, microbial communities remained stable, but microbe abundance was affected when operated in parallel. The limitation of stacked MFC is a voltage drop due to voltage reversal, a cathode electrode and ionic conduction (Estrada-Arriaga et al. 2018).

9.3.4 Magnetic Fields Ceramic Microbial Fuel Cell (CMFC)

Ohmic losses are often a severe problem in the MFC reactor, sorted by ceramicbased stack MFC operating in super capacitive mode. This boosted power output and conductivity to a maximum of 27.4 W/m³ with an electrolyte solution of 40.1 mS cm⁻¹, thus reducing the overall system ohmic loss (Santoro et al. 2018). In another study, the efficiency of electricity generation was improved by replacing proton exchange membranes with ceramic membranes using microalgae Spirulina *platensis.* The results showed that the power generation could be boosted by 61% when implementing a 200 mT magnetic field (MF). The magnetic field affected the microorganism in both anode and cathode and improved the power density up to 35.9 mW/m² and the current density of 158.7 mA/m². Ceramic microbial fuel cells (c-MFC) using diatoms have high energy conversion efficiency. The uniqueness of diatoms is they can fix 25% of atmospheric CO₂, hence releasing oxygen at longer hydraulic retention times (HRT). The hydraulic retention times (HRT) was 32.2 W/ m³ at 24 h with constant power performance. These ceramic membranes are inexpensive when compared to other membranes (Walter et al. 2022). Though this technique is cost-efficient, it still suffers from calcium carbonate fouling (Chu et al. 2020).

9.3.5 Plant Microbial Fuel Cell (P-MFC)

Alternative approaches for power generation are being considered, such as plant microbial fuel cells (P-MFC). It is a novel technology that converts solar energy into electrical energy and is widely used in highly water-saturated ecosystems to produce sustainable energy. P-MFC is a reactor combining a microbial-based energy generation system and plants. Plants that can withstand waterlogged conditions, such as prickly pear, Pachirama crocarpa, Populus alba, Opuntia species (succulent plants), are widely utilized for sustainable electricity generation via plant-based biobattery technology. Despite the technology being initiated almost a decade ago, it is still considered in its infancy (Apollon et al. 2020; Lu et al. 2020). However, recent studies have revealed the beneficial roles of wetland plants in enhancing bioelectricity production within constructed wetland microbial fuel cells (CW-MFC). This enhancement can be attributed to the exudation of root oxygen, root exudates, and the removal of pollutants (Yang et al. 2021b). Performance of plant-MFC is governed by various parameters, such as selection of plant species, microbial flora in rhizosphere, design of MFC, electrode properties, inoculum characteristics, wastewater properties, factors like light intensity, and carbon dioxide concentration in air (Jadhav et al. 2021). Sharma et al. (2021) compared the cathode performance of wastewater containing plant Canna indica (PMFC) and the other having alga Chlorella vulgaris (AMFC). PMFC was deemed superior since its power output was six times higher (22.76 mW/m^2) than the AMFC (3.64 mW/m^2) . Nguyen's studies have shown purple guinea grass cultivated in waterlogging could provide power densities of 10.13 mW/m² two at the anode area. Soil water contents, ambient temperatures, photosynthesis, and photo-period were accredited to have a substantial role in controlling power and current outputs. At a lower temperature range of 27-34 °C, a power density of 0.6 mW/m² was obtained in waterlogging. The authors attributed the lower performance at low temperatures to the electroactive bacteria activities in the anode and the carbohydrate metabolism of plants (Nguyen and Nitisoravut 2019).

9.3.6 Photosynthetic Microbial Fuel Cell (Photo-MFC)

Photo-MFC can be considered the next-generation fuel cell for bioelectricity generation. Phototrophic prokaryotes (Anoxygenic phototrophic bacteria (APB)) are used to convert light energy into electricity through photosynthesis. As reviewed by Qi et al. (2018), at the anode, APB contains two pathways: APB can produce electrons by anoxygenic photosynthesis or endogenous respiration; hydrogen from APB photosynthesis is used as a medium for electron generation. The most frequently used APB were *Rhodospirillum*, *Rhodobacter*, *Rhodopseudomonas*, *Rhodovulum*, and *Chlorobium*. Photosynthetic MFCs provide treatment of biodegradable wastes by bacteria in the anode and remove carbon dioxide, phosphorus, and nitrogen in the cathode. The organic matter in the cathode could serve as nutrients for the algae, improving photo-MFC competence (Aiyer 2021). Sogani et al. (2021) investigated the influence of a hybrid photo-assisted microbial fuel cell using *Rhodopseudomonas palustris* for the biodegradation of ethinylestradiol (EE2). An essential component of oral contraceptives that causes micropollutants in various wastewaters is highly recalcitrant. Degradation of EE2 to 89.82% with a maximum power density of $0.633 \pm 0.04 \text{ mW/m}^2$ occurred at the bottom photo MFC along with top 63% bio-hydrogen production as a co-catabolite along with glycerol (Sogani et al. 2021).

9.4 Electrodes

The performance and cost of electrodes are the most critical aspects of designing an MFC. In recent years, a wide range of electrode materials and configurations have been tested and developed to enhance the performance of MFCs and lower material costs. The current trend in electrode modification with nanoparticles has become a new buzz to improve the performance of power outputs. According to Logan and Regan (2006), for an electrode to be ideal, the materials should possess certain features: (1) satisfactory conduction of electricity and little resistance; (2) corrosion resistance and chemical stability; (3) biocompatibility; (4) suitable toughness and mechanical strength; (5) high surface area. Figure 9.3 shows the factors affecting the microbial fuel cell.



Fig. 9.3 Factors influencing microbial fuel cell performance

9.4.1 Cathode Electrode

The cathode electrode plays an important role in power generation. There are two potential methods of reducing cathode fuel oxygen levels. A direct 4-electron pathway can reduce oxygen to water or a 2-electron pathway to peroxide. The most desirable one is the 4-electron pathway (Panomsuwan et al. 2016). The drawback at the cathode is a low oxygen reduction reaction (ORR) kinetics which is improved by noble metals such as platinum (Pt), gold (Au), and palladium (Pd) (Khilari et al. 2015). Noble metals have outstanding electro-catalytic performance and four-electron transfer routes (Shabani et al. 2020). However, as reported, these noble metals come with a high cost, limited availability, poor stability, and surface poisoning. To overcome these problems, researchers have identified alternative solutions using tin oxide (SnO₂), nickel-based composite, and sodium hexahydroxostannate (Na₂Sn(OH)₆) (Das and Jayaraman 2014). The cathode electrocatalyst developed using Na₂Sn(OH)₆ synthesized with a higher concentration of NaOH (2.0 M) showed higher ORR activity in terms of higher power density, the onset of potential and current density with a four-electron transfer process using pure and mixed inoculums. It proved to be a more cost-effective material for energy recovery in the MFC than SnO_2 (Rout et al. 2020). The nickel-based composite showed promising high-effective oxygen reduction performance and outstanding power output with a power density of 1421.4 mW/m^2 (Li et al. 2020a). Different approaches have been developed to enhance the activity of MFC by using earthenware and clayware as a membrane (Dhulipala et al. 2020; Suransh et al. 2020). Filtration types of membrane electrodes with Prussian blue (PB) doping and PVDF-PVC-PEG triblock copolymers prepared by the phase inversion process also exhibited superior ORR activity with the highest electrochemical activity and lowest charge transfer resistance (Yu et al. 2020). Current densities could be increased by utilizing modified polyaniline (PANI) polymers, such as fluorinated PANI (Yaqoob et al. 2021). Similarly, metal-free N/B-co-doped carbon-based catalyst (denoted as PANI/B-8) developed by pyrolysis of polyaniline and boric acid mixtures showed extraordinary enhanced kinetic activity toward ORR in alkaline electrolytes. This asymmetric neutral-alkaline microbial fuel cell (ANA-MFCs) markedly delivered an output power density twice as high than the symmetric MFCs (Hu et al. 2021). Among different types of co-catalysts, ten (weight %) hydrophobic Fe-N4/AC (activated carbon) air cathodes showed a simultaneous increase in the power density and Coulombic efficiency for electricity generation (Yang et al. 2020a, b). In openair cathode MFCs, cation transfer through the membrane reduces the cathodic redox reactions by forming thick layers of carbonate salts on the surface of the electrode (Pham et al. 2003). Wetland-microbial fuel cells (CW-MFC) have shown to be extenuating to greenhouse gases. For instance, the roots of wetland plant Acorus Calamus L., when placed in anode, showed better microbial ecosystem for power generation. Correspondingly, carbon fiber felt (CFF) cathode showed lowest emission of methane 0.77 ± 0.04 mg/(m²/h) and nitrous oxide 130.78 ± 13.08 µg/(m²/h). The maximum power density was 2.99 W/m³. Thus proving to be eco-friendly in mitigation of greenhouse gases (Liu et al. 2022)

Air cathodes efficiently use oxygen from the air and avoid the need for aerating water or chemical catholyte (Fan et al. 2007a). Similarly, the addition of acetylene black (AB) into exfoliated porous graphitic carbon nitride (ep-GCN) cathode catalyst indicated excellent oxygen reduction reaction activity and was less costeffective (Chakraborty et al. 2020). Copper (II) oxide (CuO) has shown extraordinary characteristics as the electrocatalyst for ORR in the cathodic chamber. A few advantages of using CuO are high specific surface area, high catalytic activity and easy synthesis, environmentally friendly, and good redox potential (Yadav et al. 2020b). On the other hand, they have a weak adsorption property that is overcome by heat treatment by immobilizing CuO particles on the electrode surface (Li et al. 2020b). Promising results were obtained with CuO as an electrocatalyst in removing caffeine waste and electricity generation. Results revealed that the CuO/C cathode achieved the highest caffeine removal (97.67%) and maximum power density (28.75 mW/m²) under aerated conditions. The maximum power density and current density increased up to 51.79% and 36.84%, respectively, thus proving its economic performance (Yap et al. 2020). A consortium of microbial communities from various habitats is becoming a choice in replacing expensive platinum as a cathode catalyst in MFCs. Because of their low cost, environmental friendliness, and long-term sustainability, microbial biocathodes are gaining popularity. A comparative study for treating waste-activated sludge and power generation using MFC was elucidated in the anodic microbial consortium. The MFCs were supplied with two feed sludge matrices of freezing/thawing (F/T) liquid versus fermentation liquor for exploring cooperative interactions in anodic microbial consortia of MFCs. The F/T liquid cultivated main genera of Azospira, Povalibacter, Thauera, Terrimonas, Alicycliphilus, Dokdonella and Simplicispira; the fermented liquor was enriched with Phenylobacterium, Cellulomonas, Edaphobacter, Burkholderia, Clostridium, Sphingomonas, Leifsonia, and Microbacterium in anodic biofilm. The study showed anodic fermentative bacteria in synergy with exoelectrogens microbial diversity, and larger functional genes played a collective role in more power generation through MFCs. The optimal power density of 0.152 and 0.182 mW/m² were produced from sludge F/T liquid and fermentation liquor (Xin and Qiu 2020).

9.4.2 Anode Electrode

The efficacy of electricity generation at the anode electrode depends on the material used as an electrode. Anode primarily serves as a current collector while providing a surface for biofilm development (Sarathi and Nahm 2013). Carbonaceous materials, stainless steel, copper, nickel, silver, gold, and titanium have been used as anode electrodes because they are highly stable. While the drawback of these metals is that they suffer from less electro-catalytic activity toward the redox reaction, and the metal ions could be poisonous to microbes, thus hindering the performance of MFC. This in turn reduces the degradation competence of the MFC (Suresh et al. 2022). The commonly used anode material is carbon in its various forms and configurations such as carbon-brush, felt, fiber, granule, mesh, nanotube, paper, plate, rod, graphite

embedded stainless steel frame, and titanium plates coated with mixed metal oxide. The implementation of anode surface modification by nanostructured materials has been extensively studied. The nanocatalyst has shown significant performance in the transfer of electrons to the electrode, enhancing the surface area to enrich bacteria adhesion and greater resistance against fouling (Li et al. 2019). For power generation, nanocatalysts such as iron oxide (FeO), iron (II) molybdate (FeMoO₄), transition metal oxides or carbides such as ruthenium oxide (RuO₂), manganese oxide (MnO₂), and molybdenum have been used as electrodes (Yamashita and Yokoyama 2018). Scientist have also tried dual metal organic frameworks (D-MOFs), TiO₂ @ZIF-67/ZIF-8 composite (Zeolitic imidazolate frameworks). The maximal power density of TiO₂@ZIF-67/ZIF-8 microbial fuel cell (MFC) was 341.506 mW/m² and continuous output voltage was 413.43 mV. The power density was 1.30 times higher ZIF-67/ZIF-8-MFC and 2.07 times of ZIF-67-MFC (164.836 mW/m²). The framework was able to maintain stable voltage output for 8.3 days (Yang et al. 2022). A novel anode electrocatalysts iron (II) molybdate coated on the graphite plate showed a fivefold reduction in resistance and a threefold increase in redox current. The power density (106.2 mW/m^2) achieved was 1.4-folds higher than control electrodes. Considering the economy and high-performance FeMoO₄ it can be successfully developed for enhancing bioelectricity generation in the MFC (Mohamed et al. 2020a). Graphene is used as both anode and cathode materials. As an anode, it improves the deficiency of electron transfer and bacterial attachment. When used as a cathode material, it supports the oxygen reduction reaction (Olabi et al. 2020). Chemically reduced graphene oxide (CGO) prepared using L-cysteine is considered the best choice as an anode electrode because of its high electrical conductivity, high surface area, great flexibility, and excellent mechanical properties (Pareek et al. 2019). Likewise, electrophoretic deposition of graphene oxide on the surface of carbon brush as anode significantly increased power density from 33 to 381 mW/m², thus enhancing the performance and Coulombic efficiency of the MFC. Studies by Yaqoob et al. (2022) have shown anode electrodes consisting of graphene oxide (GO) and GO-polymer-metal oxide (GO-PANI-Ag) high productivity of 1.022 mW/m^2 and GO-PANI-Ag at 2.09 mW/m². The biomass for this study was provided with oil palm trunk sap as organic substrate. The MFC was able to remove heavy metals such as Cd(II) (80.25%) and Pb(II) (78.10%). Polyaniline functionalized activated carbon (PANi-FAC) composite as a capacitive anode coated with stainless steel mesh improved the maximum power density to 322 mW/m² (Yellappa et al. 2020). The NiFe2O4-MXene@CF (Carbon felt) anode was considered preferable because of its low charge transfer resistance, high conductivity, and a large number of catalytically active sites. The power density was improved to 1385 mW/m² (Tahir et al. 2020). Similarly, polymerized nanofiber polyaniline (PANI) for carbon felt (CF) electrodes aimed at increasing the conductivity of the anodic electrode facilitated the adherence of exoelectrogenic yeast cells of *Cystobasidium slooffiae* JSUX1. This further improved bioelectricity generation in MFCs from using xylose as the substrate (Soni et al. 2020). An increased surface area of nanofiber PANI boosted the conductivity of the PANI/CF anode for a robust attachment of C. slooffiae JSUX1 to form a dense biofilm. The authors reported with

PANI/CF it was possible to achieve a derived power output about 2.2 times $(119.35 \pm 3.27 \text{ mW/m}^2)$ that of CF only $(50.41 \times 6.9 \text{ mW/m}^2)$. The maximum hydrogen yield was 25.83 mL (Moradian et al. 2022). Bioanode electrode synthesized using graphene oxide deposited on the surface of the carbon brush showed enhanced electron transfer rate and the bioactive surface area. The maximum power and current densities increased more than 10 and 6 times, and the columbic efficiency increased by 12 times when operated with waste water (Sayed et al. 2021).

9.4.3 Membranes

The use of membranes has its own merits and demerits. In addition to the high cost of membranes, MFC performance can be compromised by biofilm formation, fouling on the membrane surface, and increased oxygen permeability (Logan 2008; Choi et al. 2013). A variety of membranes are garnering renewed attention for use in MFC to facilitate the transport of protons from the anode to the cathode. Irrespective of the membrane material, they should have some key features such as (1) preventing direct electrical interaction between anodes and cathodes; (2) reducing the undesired crossover of oxygen and other substances; (3) maintaining effective transport of proton mass through the separator; (4) low internal resistance; (5) low mass transfer between oxygen-containing water of cathode and anaerobic anode; (6) high proton conductivity; (7) high energy recovery; (8) high ionic conductivity; (9) and longterm stability (Daud et al. 2015; Yang et al. 2019). Although the elimination of membrane has its advantage, the relatively broad electrode spacing leads to high internal resistance and restricts the electrode surface area and power density ratio. Therefore, further reduction in electrode spacing is required (Cheng et al. 2006). Membranes are classified based on their porous/nonporous nature. Nonporous membranes are subdivided into a cation exchange membrane (CEM), anion exchange membrane (AEM), and bipolar membrane (BPM). Porous membranes are categorized into UFM, MFM, and CMs (not within the scope of discussion).

Ion exchange membranes (IEMs) are a class of polymeric membranes containing highly swollen gels carrying fixed positive or negative charges. Ion-exchange membranes are permeable to ions of opposite charge (counter ions), but repel ions of the same charge (co-ions). The only exception is the protons (Luo et al. 2018). IEM has better selectivity, lower electrical resistance, and improved thermal, chemical, and mechanical properties. IEMs are categorized as cation exchange membranes (CEM) or PEM and anion exchange membranes (AEM) where the protons can permeate freely (Daud et al. 2015).

9.4.3.1 Cation Exchange Membrane (CEM)

CEM is designed to allow the transfer of protons and cations through a membrane resulting in a net negative charge (Harnisch and Schröder 2010). Flat plate type MFC with Nafion PEM and anode assembly provides a larger surface area for the membrane and cathode (Kumar et al. 2017). Nafion, a perfluorosulfonic acid polymer, is an excellent choice as a proton exchange membrane because it has good

proton conductivity and chemical stability. The oxygen permeability through these membranes can reduce the Coulombic efficiency of the MFC (des Roches and Omiya 2014). Proton conducting membrane devices such as PEMFCs (Polymer electrolyte membrane fuel cells) and DMFCs (Direct methanol fuel cells) work better with the Nafion-based operation at low temperatures (<80 °C). Whereas at higher temperatures (120–200 $^{\circ}$ C), high-temperature hydrocarbon polymers poly (phenoxyphosphazene) (POP), sulfonated naphthalic polyimide, polybenzimidazole (PBI), alkyl sulfonated polybenzimidazole (PBI-AS), sulfonated poly (arylene ether ether ketone) (PEEK-SO3H), and sulfonated poly (arylene sulfone) (PSU-SO3H) are used (Shi 2014). Nation-based PEM suffers from extreme biofouling; hence it is being replaced by non-fluorinate sulfonated membranes, which come at lower cost and higher energy recovery (Shabani et al. 2020). Fabricated ceramic separators like clayware ceramic pots used to treat rice-mill wastewater produced a power density of 2.3 W/m³ (Behera and Ghangrekar 2011), and earthen CEM produced a maximum power output of 16.8 W/m³ (Bhaduri and Behera 2023). Similarly, the terracotta flowerpot generated a maximum volumetric power density of 14.59 W/m³, which was 46% higher than Nafion as a PEM (Jana et al. 2012). Taskan (2020) obtained a maximum power density 26,680 mW/m² and oxygen pressure of 10 psi with a sandwich-type microbial fuel cell having three chambers (2 anodes and 1 cathode) with a hollow fiber gas transfer membrane aerated cathode. Branched polyethyleneimine membrane (BPEI) has been shown to increase membrane permeability, improve mediator access to electron carriers and biofilm formation at the anode in the presence of E. coli, and neutral red as the mediator, the power densities generated were 2.6 mW/m² (Soh et al. 2020). Nanocomposite membranes are the other alternative for PEM. PEM used in fuel cells should possess the following properties: high proton conductivity, good mechanical strength, excellent chemical resistance, and good durability (Zakaria et al. 2016).

9.4.3.2 Anion Exchange Membrane (AEM)

In an AEM, hydroxide ions are transferred from the cathode to the anode through the anion conducting polymer electrolyte, where it combines with hydrogen to form water during electrochemical oxygen reduction at the cathode to produce OH⁻. In polymeric AEM, there is no liquid phase the positive charges, such as phosphate or carbonate, attached to the membranes facilitate the proton transfer by applying proton carriers (pH buffers) (Fan et al. 2007b). The ideal polymer for AEMs must have excellent OH[•] conductivity, chemical and thermal stability, strength, flexibility, low gas permeability, low water drag, low cost, and good availability. The possible fuels used in AEM are hydrogen, methanol, ethanol, propanol, ethylene glycol, and sodium borohydride. AEMs suffer from poor solubility in low boiling solvents, chemical instability and low ionic conductivity. The synthesis of AEM is complex as it involves chloromethylation, which is a potent carcinogen (Hren et al. 2021).

9.4.3.3 Bipolar Membranes (BPM)

A bipolar membrane is a double-layer structure comprising a cation exchange membrane and an anion exchange membrane, directly attached to one another. It also has an interfacial layer where water dissociation occurs. The double layer enables the transport of protons and hydroxyl ion, and block co-ions. The innovation of these membranes is the separation of mono- and divalent ions, anti-deposition, anti-fouling, and water dissociation. However, in the use of such membranes, a pH gradient is the main concern (Kim et al. 2017). The bipolar membrane provides physical support for the embedded electrode. It minimizes electrode thickness, thereby reducing the distance between the structure which supplies protons and the electrode, thus minimizing ohmic losses (Mayerhöfer et al. 2020).

9.5 Factors Responsible That Affect Performance of Microbial Fuel Cell

9.5.1 Effect of pH, Ionic Strength, and Temperature on Power Generation

By adding NaCl, Liu et al. (2015) noticed an increasing ionic strength of the solution from 100 to 400 mM, and the power output increased from 720 to 1330 mW/m². This was perhaps because of the increased fluid access in the chamber with holes on both sides of the anode electrode and higher Pt content on the cathode (0.5 mg Pt/cm^2) . Shewanella marisflavi strain EP1 could generate a power density of 9.6 mW/m² when ionic strength was increased to 1146 mM (8% NaCl). Due to a reduction in internal resistance, increasing the ionic strength of the electrolyte significantly enhanced power output (Huang et al. 2010). Miyahara et al. (2015) observed the abundance of Geobacter bacteria increased when the NaCl concentration increased from 0 to 0.1 M but markedly reduced when the NaCl concentration was increased to 0.3 M due to intolerance. This indicated a strong correlation between the bacteria, ionic strength, and power output. Most reviewed studies reported that power density and temperature were exponential rather than linear. The influence of temperature had only a negligible effect in most of the studies suggesting the maximum power output drops at lower temperatures (10 °C) or higher temperatures of (55 °C) (Li et al. 2013). This is because MFCs cannot operate at extremely low temperatures because microbial reactions are sluggish at low temperatures or denatured at higher temperatures.

Nevertheless, a short-side-chain Hyflon[®] perfluorinated ionomer-based membrane produced a power density of 300 mW/cm² at 140 °C in the presence of 1 M methanol and air fed (Baglio et al. 2006). Wastewater-fed reactors were less susceptible to temperature than acetate-fed reactors (Heidrich et al. 2018). Although there is a contradiction with respect to the ideal operating pH in MFC, the most frequently mentioned is neutral pH (Borole et al. 2008). Low pH (<6) showed an adverse effect on the electrochemically active bacterial population resulting in a drastic fall in power output. Also, proton production is mainly related to the electrochemical oxidation of the organic fuels at the anode (Zhang et al. 2013).

9.5.2 Microbes as Biocatalyst Used in MFC

Inoculum selection, enrichment, operating conditions, and cell architecture impact the MFC reactor's start-up phase (Kumar et al. 2018). Bacteria generate electrical energy by the oxidation of organic matter and transfer the electrons to an electron acceptor outside of their cells; hence they are termed as "Exoelectrogens." These microbes can transfer the electrons directly from the cytoplasmic membrane to electron acceptors such as insoluble and soluble metals, flavins, or electrodes (Wu et al. 2013). The electrogenic bacteria only prefer non-fermentable substrate acetate and are capable of completely oxidizing acetate, whereas the fermentative bacteria convert carbohydrates into short-chain fatty acids and acetate (Yang et al. 2015). Proteobsludacteria (α -proteobacteria, β -proteobacteria, γ -proteobacteria and δ -proteobacteria) have the ability to directly transfer electrons to the electrode and represent the largest category of electricigens. Other bacteria used in MFC are archaea, cyanobacteria, firmicutes, yeast, and eukaryotic algae, which can oxidize organic compounds and transfer electrons to the anode (Cao et al. 2019). Primitive prokaryotes (Archaebacteria) that can survive extreme conditions have been tested as possible sources for electricigens when complex compounds have to be degraded. Two halophilic archaea, Haloferaxvolcanii and Natrialbamagadii, used as a biocatalyst at the anode, were evaluated for electricity generation. Maximum power densities of 50.98 and 5.39 µW/cm² were obtained, which was higher when compared to mediator-less MFCs (Abrevaya et al. 2011). The exoelectrogenic bacteria preferably used in MFCs are dissimilatory metal-reducing bacteria such as Geobacter and Shewanella (Proteobacteria) referred to as metal-reducing microbes since they reduce the solid metal oxides (Cao et al. 2019). According to Bond and Lovley (2005), Geobacter sulfurreducens and Rhodoferax ferrireducens could produce electricity by forming a monolayer directly on the anode electrode surface and use this as their end terminal electron acceptor in anaerobic respiration; hence, these also called anodophiles. Sulfur-reducers, microorganisms are especially Desulfuromonas and Desulfovibrio, could convert sulfate to sulfide, which is then oxidized to elemental sulfur and can be reduced again to sulfide. Geobacter species have several possible advantages over Shewanella species. Shewanella species incompletely oxidize a limited range of organic acids to acetate, inefficient since most of the electrons present in the initial fuel remain as acetate. Shewanella species appear to transfer electrons to anodes by releasing a soluble molecule that acts as an electron shuttle. On the other hand, Geobacter species can completely oxidize organic compounds to carbon dioxide with the recovery of >90% of the electrons available in the fuels as electricity (Bond et al. 2002; Kumar et al. 2019). Geothrix fermentans are iron-reducing acid bacteria capable of producing electron mediators that facilitate reduction reactions in graphite electrodes (Bond and Lovley 2005). A new model for nitrogen removal and power production was developed using MFCs with nitrite as an electron acceptor in the cathode (Jin et al. 2018). A novel denitrifying exoelectrogenic Mycobacterium sp. EB-1 revealed the strain was capable of producing electricity by direct electron transfer. Mutant strains of S. oneidensis and S. putrefaciens have shown improved performance and good bacterial adhesion to the electrode, enhancing power generation. *S. oneidensis* MR-1 was constructed using the *yde* H gene from *E. coli* under the control of an IPTG-inducible promoter, and the strain *yde* H itself was under the control of a constitutive promoter. The recombinant Shewanella strains showed significant enhancement in biofilm formation and bioelectricity generation, which was about 2.8-fold of the original strain (Liu et al. 2015).

Mixed cultures were demonstrated to be beneficial compared to pure cultures due to the presence of different kinds of bacteria along with electricigens providing a high power density. Mixed culture minimizes the effects of oxygen diffusion into the anode chamber by scavenging any dissolved oxygen and maintaining anaerobic conditions in the anode chamber (Rabaey et al. 2004). Any oxygen diffusion into the system will result in substrate loss and reduced Coulombic efficiencies (Min et al. 2005). Activated sludge, anaerobic sludge, and domestic wastewater are excellent examples of mixed inoculum. including fermentative or methanogenic microorganisms carrying initial metabolism (Rout et al. 2020). A recent study also proved mixed, or co-culture of Escherichia coli and Pseudomonas aeruginosa generated a maximum power density of 190.44 mW m⁻², which was comparatively higher when the organism was used individually. The study further proved co-cultures when coupled with Chlorella vulgaris a synergistic effect was observed that improved mean power density from 248 mW/m², a 41.7% rise (Aiyer 2021). Khan et al. (2022) observed live diatoms (Nitzschia palea) in the anodic chamber could replace bacterial cell in generating electricity. Photosynthetic diatom microbial fuel cell (PDMFC) was supplied with f/2 media rich in nitrates, phosphates, metasilicates, trace metals, and vitamins as the anolyte. The maximum derived power output was 12.62 mW/m² and coulombic efficiency of 22.95%. Besides the diatom cells showed about 64.28% increase in lipid production on 15th day compared to the 1st day. This was accompanied by formation of complex fatty acid methyl esters and carotenoids. Table 9.1 provides the list of biocatalysts, substrates, and electrodes involved in bioelectricity generation.

9.5.3 Organic Waste as Microbial Substrate

A great variety of substrates have experimented with high current production in MFC. Owing to the poor conversion of nutrients, the use of solid organic waste for electricity generation has drawbacks. Therefore, the nutrients need to be converted into monomers before being fed to the microbial cells. Enzymatic hydrolysis has been used to overcome the problem mentioned above (Ma et al. 2016). Increasing the substrate concentrations from 100 to 850 mg/L boosted the power output from 0.2 to 1.2 W/m³; however, concentrations higher than the above-mentioned were not beneficial (Jiang and Li 2009). Depending on the particular application for which an MFC is used, the metabolic substrate needed for electrogenic bacteria should be carefully selected, as not all electrogenic bacteria can completely oxidize multiple substrates. The substrate used includes carbohydrate (glucose, sucrose, maltose, galactose, fructose, sucrose, xylose, trehalose, rhamnose, cellulose, dextran), organic

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		Current				
Organism	Type	density	Power density	Substrate	Cathode/anode electrode	References
Acidiphilium cryptum	Proteobacteria (α)	1	12.7 mW/m ²	Glucose	Anode: graphite (2.5 \times 7.5 \times 0.63 cm) felt connected to graphite rod. Cathode: platinum (5 \times 5 cm) deposited carbon cloth	Borole et al. (2008)
<i>Acidiphilium</i> sp. strain 3.2 Sup 5	Proteobacteria (α)	3 A/m ²	1	Glucose	Anode and cathode: graphite felt	Malki et al. (2008)
Actinobacillus succinogenes	Proteobacteria (y)	2.7 mA	348.6 mW	Glycerol	Anode and cathode: graphite	Zheng et al. (2020)
Aeromonas hydrophila	Proteobacteria (γ)	1.8 mA	I	Acetate	1	Pham et al. (2003)
Aeromonas hydrophila	Proteobacteria (γ)	8.77 mA/ cm ²	I	Chitin	Anode and cathode: carbon felt $(3 \text{ cm} \times 3 \text{ cm} \times 3 \text{ mm})$	Park et al. (2008)
<i>Aeromonas</i> sp. strain ISO2-3	1	1	800 mW/m ²	Glucose	Graphite	Chung and Okabe (2009)
Alcaligenes faecalis	Proteobacteria	1	90 W/m ³	Acetate and glucose	Anode and cathode:	Rabaey et al.
Enterococcus gallinarum Pseudomonas aeruginosa					granmar grapmic maunx	(2004)
Anaerobic and facultative microbes	Mixed culture	1	644 mV	Dairy waste	Anode and cathode: copper electrode	Sanjay and Udayashankara (2020)
Anaerobic sludge	Mixed culture		29.96 mW/m ²	Bakery waste	Two-stage bioprocess method	Han et al. (2020)
Anaerobic sludge	Mixed culture	150 mA/m ²	I	Solid potato waste	Anode and cathode: graphite electrode	Du et al. (2020)
Anaerobic microbes	Mixed culture	I	0.040-0.044 W/ m ²	Kitchen waste	Anode; stainless steel mesh Cathode graphite plate	Dhulipala et al. (2020)

Table 9.1 List of microorganism substrates and electrodes used for bioelectricity generation in MFC

(continued)

Table 9.1 (continued)						
		Current				
Organism	Type	density	Power density	Substrate	Cathode/anode electrode	References
Arcobacter butzleri strain ED-1	Proteobacteria (ε)	1	296 mW/L	Acetate	Anode and cathode: graphite felt with semidry cathodes	Fedorovich et al. (2009)
Azoarcus sp. and Desulfuromonas sp.	Proteobacteria (δ)	1	488 mW/m ²	Ethanol	Anode: plain porous carbon Cathode: carbon paper incorporating Pt catalyst	Kim et al. (2017)
Bacillus subtilis	Firmicutes	1	1.05 mW/cm ²	Glucose	Anode: carbon cloth Cathode: platinum	Nimje et al. (2009), Kashyap et al. 2019
Bacillus, Klebsiella, and Enterobacter species	Firmicutes	1	0.0744 mW/m ²	Yam (Dioscorea alata) waste	1	Fadzli et al. (2021)
Bacteroidetes, and Proteobacteria	Firmicutes	1	610 mW/m ²	Citric acid waste	Dual-chamber MFC	Zhang et al. (2021)
Citrobacter sp. SX-1	Proteobacteria (γ)	58 mA/m ²	88.1 mW/m ²	Citrate, glycerol, sucrose	Anode and cathode: carbon cloth	Xu and Liu (2011)
Clostridium beijerinckii Clostridium butyricum	Firmicutes	1.3 mA/ cm ²	1	Starch, glucose	Anode and cathode: woven graphite fuel cell cathode and anode	Niessen et al. (2004)
Clostridium beijerinckii SR1	Firmicutes	I	61.5 mW/ m ²	Sago hampas	Anode: carbon cloth (1.5 × 1.5 cm) cathode: 20% platinum on Vulcan carbon cloth	Jenol et al. (2020)
Clostridium butyricum EG3	Firmicutes	0.22 mA		Starch and glucose	Anode and cathode: graphite	Park et al. (2001)

246

Comamonas denitrificans	Proteobacteria (β)	1	35 mW/m ²	Acetate	Anode: carbon paper or a graphite fiber brush	Xing et al. (2010)
					Cathode: Pt with PTFE diffusion layers on 30 wt% wet-proofed carbon cloth	1
Cupriavidus basilensis	Proteobacteria (β)	902 mA/m ²	44 mW/m ²	Acetate	Anode: graphite rod	Friman et al. (2013)
					Cathode: carbon cloth	
Desulfobulbus propionicus	Proteobacteria (δ)	28.35 mA/ m ²	I	Fumarate, lactic, pyruvic, propionic acid	Anode and cathode: graphite	Holmes et al. (2004)
Desulfovibrio desulfuricans	Proteobacteria (δ)	233 mA/m ²	I	I	Anode and cathode: graphite electrodes	Kang et al. (2014)
Dysgonomonas oryzarvi	Bacteroidetes	50 μA/cm ²		Acetate, lactate	Cassette-electrode MFC	Kodama et al. (2012)
Enterobacter cloacae	Proteobacteria (γ)	493.8 mA/ m ²	4.9 mW/m ²	Cellulose, sucrose, glycerol	Anode and cathode: carbon cloth	Rezaei et al. (2009)
Enterobacter ludwigii	Proteobacteria (γ)	440 mA/m ²	I	Citrate, acetate and cellulose	Anode and cathode: carbon cloth	Feng et al. (2014)
Escherichia coli	Proteobacteria (y)	1	600 mW/m ²	Glucose	Anode: carbon/PTFE composite. Cathode: Nafione+/Pt/+C gas diffusion layer	Zhang et al. (2006)
Escherichia coli	Proteobacteria (γ)	1	120–140 mW/ m ²	Green bean sprouts	1	Mulyono (2020)
Escherichia coli K12	Proteobacteria (γ)	1.45 mA/ cm ²	6000 mW/m ²	Glucose, sucrose	Anode: graphite cloth (30 × 25 mm) Cathode: graphite	Schröder et al. (2003)
						(continued)

Table 9.1 (continued)						
Organism	Type	Current density	Power density	Substrate	Cathode/anode electrode	References
Escherichia coli	Proteobacteria (y)	1750 mA/ m ²	788 mW/m ²	Glucose	Anode: Mn ⁴⁺ -graphite	Park and Zeikus (2003)
		325 mA/m^2	91 mW/m ²	-	Cathode: Fe ³⁺ -graphite	1
Geobacter	Proteobacteria δ	1	40 mW/m ²	Acetate	Anode: carbon paper	Min et al.
metallireducens					Cathode: Pt catalyst	(2005)
Geobacter spp.	Proteobacteria δ	262 mA/m ²	106 mW/m ²	Sugar wastewater	Anode and cathode: graphite	Mohamed et al. (2020a)
Geobacter sulfurreducens	Proteobacteria δ	456 mA/m ²	188 mW/m ²	Acetate	Anode and cathode: graphite	Nevin et al. (2008)
Geobacter sulfurreducens	Proteobacteria δ	11,143 mA/ m ²	15 mW/m ²	Acetate	Graphite	Bretschger et al. (2007)
Geobacteria sulfurreducens and Shewanella oneidensis	Proteobacteria δ	3.74 mA/ m ²	45.50 μW	Sucrose, acetate	Generation II fuel cell	Ieropoulos et al. (2005)
Geobacteria sulfurreducens PCA	Proteobacteria δ	0.40 A	13 mW/m ²	Acetate	Anode and cathode: graphite	Bond and Lovley (2003)
Geopsychrobacter	Proteobacteria δ	121.43 mA/	1	Fumarate	Anode and cathode:	Holmes et al.
electrodiphilus		m²		Acetate, malic, fumaric and citric acid	graphite	(2004)
Geothrix fermentans	Acidobacteria	0.6 mA	I	Acetate	Anode and cathode: graphite electrode	Bond et al. (2002)
Geothrix fermentans	Acidobacteria	50 mA/m ²	I	Acetate, propionate, malate, lactate, or succinate	Anode and cathode: graphite electrode	Bond and Lovley (2005)
Gluconobacter oxydans	Proteobacteria (α)	I	7.23 mW	Glucose	Anode and cathode: cylinder graphite electrodes	Reshetilov et al. (2006)

248

Haloferax volcanii	Archaeabacteria	49.67 μA/ cm ²	11.87 μW/cm ²	Acetate	Anode and cathode: plain carbon paper TGP-H-030 (Toray [®] , Tacoma, WA)	Abrevaya et al. (2011)
Klebsiella pneumoniae L17	Proteobacteria (γ)	0.08 mA	218.51 mW/m ²	Glucose, starch, lactic acid, lactate, fructose, sucrose, lactose, and maltose	Anode and cathode electrodes: carbon felt $(4.5 \times 4.0 \text{ cm})$	Zhang et al. (2008)
Lactobacillus plantarum	Firmicutes	1	0.22 MW	Glucose	Anode: carbon cloth cathode: electrode platinum	Vega and Fernández (1987)
Lactobacillus, Clostridiumsensu stricto and Bacteroides	Firmicutes	1	1	Watermelon rind	Membraneless biocathode microbial fuel cell (MB-MFC)	Yang et al. (2021a)
Lactococcus lactis	Firmicutes	3 A/m ³	1	Lactate, acetate	Anode: glass cylinder Cathode: Pt wire	Freguia et al. (2009)
Lysinibacillus sphaericus	Firmicutes	≈270 mA/ m ²	85 mW/m ²	Protein components	Anode and cathode: graphite felt	Nandy et al. (2013)
Ochrobactrum anthropi YZ-1	Proteobacteria (α)	1027 mA/ m ²	89 mW/m ²	Acetate	Anode: ammonia gas pre-treated plain carbon cloth Cathode: graphite fibers	Zuo et al. (2008)
Mixed microbial consortia	1	168.05 mA/ m ²	2.01 W/m ³	Pharmaceutical waste	The paraboloid shape MFC Anode and cathode: graphite	Rashid et al. (2021)
Marine sediment sludge	1	1	2.08 mW	Orange peel waste	Multiple single solid phase MFC	Hariti et al. (2021)
						(continued)

Table 9.1 (continued)						
Organism	Type	Current density	Power density	Substrate	Cathode/anode electrode	References
Mixed culture Ochrobactrum (53%),	1	1	369 mW/m ²	Aquaculture wastewater	Saline anode microbial fuel cell (SA-MFC)	Pugazhendi et al. (2021)
Marinobacter (22%) and Rhodococcus (15%). Bacillus,					Anode and cathode: carbon felt separated by nafion	
Stenotrophomonas, Xanthobacter,						
Sphingomonas, Pseudomonas and Sedimentibacter						
Propionibacterium freudenreichii ET-3	Actinobacteria			Glucose	Anode and cathode: carbon Felt	Wang et al. (2008)
Proteus mirabilis	Proteobacteria	6 mA	1	Glucose	Anode: reticulated vitreous carbon $(35 \times 50 \times 7 \text{ mm})$	Thurston et al.
					Cathode: bright platinum foil (10 × 40 mm)	
Proteus vulgaris	Proteobacteria (γ)	0.4 mA		Galactose	Anode: reticulated vitreous carbon	Kim et al. (2000)
					Cathode: platinum $(40 \times 40 \times 1 \text{ mm})$	
Rhodobacter sphaeroides	Proteobacteria	1	790 mW/m ²	Sistrom's minimal	Anode and cathode:	Cho et al.
	(α)			medium (nitrogen, succinate)	platinum-coated carbon paper	(2008)
Rhodococcus pvridinivorans HR-1	Proteobacteria (α)	2.309 A/m ²	0.336 W/m ²	Acetate	Anode: unilaminar carbon cloth cathode: Pt/C	Cheng et al. (2020)
Rhodoferax ferrireducens	Proteobacteria	74 mA/m ²	33 mW/m ²	Glucose, fructose,	Anode and cathode:	Chaudhuri and
,	(α)			sucrose	graphite felt/rod/porous	Lovley (2003)

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knoaopseuaomonas	Proteobacteria	0.99 mA/	7/80 mw/m	volatile acids, yeast	Anode: carbon paper	Aing et al.
palustris DX-1	(α)	cm ²		extract, and thiosulfate	Cathodes Pt and carbon cloth	(2008)
Rhodospirillum rubrum	Proteobacteria	1	1.25 W/m ²	Light	Anode: carbon-based	Gomez et al.
	(α)				Cathode: chamber-stainless	(2014)
Shewanella algae (MTCC-10608)	Proteobacteria (y)	141 mA/m ²	50 mW/m ²	Dairy wastewater	Anode and cathode: acrylic (single chamber MFC)	Choudhury et al. (2021)
Shewanella marisflavi BBL25	Proteobacteria (y)	6.850 mA/ cm ²	52.80 mW/cm ²	Barley straw Miscanthus, Pine	Anode: carbon felt	Gurav et al. (2020)
		6.661 mA/ cm ²	40.95 mW/cm^2	hydrolysate (Lignocellulose,	Cathode platinum-coated carbon felt	
			<i>c</i> <u>-</u>	olinose)		
		6.294 mA/ cm ²	34.05 mW/cm ²	gurcosc)		
Shewanella oneidensi	Proteobacteria	100 mA/m^2	24 mW/m^2	Lactate	Anode and cathode: glassy	El-Naggar et al.
DSP10	(λ)				carbon	(2008)
Shewanella oneidensis	Proteobacteria	1100 mA/	167.6 mW/m^2	Lactate	Anode and cathode: carbon	Liu et al. (2015)
MR-1	(λ)	m ²			cloth (2.5 cm \times 2.5 cm)	
Shewanella putrefaciens	Proteobacteria	0.031 mA	0.19 mW/m ²	Lactate	Anode: woven graphite	Kim et al.
	(λ)					(1999)
Shewanella putrefaciens	Proteobacteria	312.5 mA/	10.2 mW/m^2	Lactate, pyruvate,	Anode and cathode:	Park and Zeikus
	(λ)	m ²		acetate, glucose	graphite	(2003)
Spirulina platensis	Proteobacteria	400 mA/m^2	98 mW/m ²	Cafeteria waste	Anode and cathode:	Christwardana
	(λ)				graphite rod	et al. (2020)
Synechococcus sp. and	Cyanobacteria	260 mA/m^2	41.5 mW/m^2	Kitchen waste	Anode and cathode graphite	Mohamed et al.
Chlorococcum sp.					electrode	(2020b)
		534 mA/m ²	30.2 mW/m^2			
						(continued)

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able	

Table 9.1 (continued)						
Organism	Type	Current density	Power density	Substrate	Cathode/anode electrode	References
Thauera, Nitrosomonas Desulfomicrobium Thiobacillus	Denitrifying bacteria	0.48 mA/ cm ²	1250 mW /m ²	Acetate	Anode: carbon felts (3.0 cm × 3.0 cm). Cathode: manganese-based catalyzed carbon E4 air Cathode	Yang et al. (2019)
Thermincola potens strain JR	Firmicutes	50 mA/m ²		Acetate	Anode and cathode: graphite blocks or graphite carbon fiber	Wrighton et al. (2011)
Thiobacillus, Afipia, Devosia Ignavi bacterium and Anaerolineaceae	Denitrifying bacteria and Proteobacteria	18–19 A/ m ³	0.518-0.594 W/ m ³	Acetate	Anode and cathode: graphite electrode	Zhao et al. (2016)
Xanthomonas translucens in synergistic with Staphylococcus saprophyticus ICBB 9554	Proteobacteria	1	0.33 mW/m ²	Rice straw (cellulose)	Anode and cathode: carbon fiber	Khoirunnisa et al. (2020)

acids (acetate, butyrate, lactate, propionate, malate, succinate) (Bond and Lovley 2005), amino acids (serine, glycine, asparagine, aspartic acid, alanine, lysine, histidine, arginine), alcohols (methanol, glycerol, ethanol), inorganic compounds (sulfate, dye), and complex substrates (peptone, pectin, chitin, yeast extract, molasses) (Hu 2008; Lee et al. 2008; Chae et al. 2009) from waste waters, food waste, green waste, wood waste, brewery wastewater, industrial waste, sewage sludge, animal manure, slaughter houses, agriculture biomass, seafood biomass, food processing waste (Pant et al. 2010; Palanisamy et al. 2019; Hosur et al. 2020). The synergy between fermentative and electrogenic bacteria becomes a priority when a complex substrate is fed to MFCs. Using more complex substrates in combination resulted in a lower utilization rate and efficiency (Xiao and He 2014). The majority of the analyzed studies used acetate as a substrate to fuel the MFC, and the response was mixed. The power generation was higher in acetate-fed systems than in those produced with butyrate, propionate, and glucose, probably because of high degree of oxidation and energy efficiency in acetate (Yang et al. 2015) Bacteria in MFCs oxidize organic substrates, such as acetate, glucose, lignocellulose, and other sugars to produce electrons. The oxidation reaction is carried out by the anode, whereas the reduction process is carried out by the cathode (Eq. 9.3).

The overall biological reaction of acetate can be written as follows:

$$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O + electricity + biomass$$
 (9.3)

Another popular substrate for MFC is glucose, the overall biochemical reaction is written as in Eq. (9.4).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + electricity + biomass$$
 (9.4)

A comparative study of fermentable (glucose, glycerol) and non-fermentable (acetate, lactate) substrates showed glycerol performed more efficiently than acetate since fermentable substrate could augment the biodiversity and growth of biocathodic organism (Vicari et al. 2018). The electric current generation was significantly higher in Glucose-Fe(III) than with only glucose, suggesting the role of Fe(III) in electric current production (Gurav et al. 2020). Du et al. (2020) observed that there was a good relation between dissolved organic matter (DOM) coupled with electricity generation and total and viable bacteria. Their results demonstrated that mixing waste-activated sludge into solid potato enhanced the presence of the tyrosine-like aromatic amino acids and aromatic protein-like substances that promoted hydrolysis and humification of the solid potato. Studies have shown power output, and current density could be maximized by addition of antibiotics. Wen et al. (2011) have demonstrated that glucose-penicillin can be degraded to produce electricity in a single chamber MFC with an air-cathode. The maximum power density for glucose + penicillin (101.2 W/m^3) was sixfold higher than the sum of glucose (14.7 W/m³) and penicillin (2.1 W/m³) as the sole fuel. The maximum current density of penicillin (10.73 A/m²) was 3.5-fold compared with that without penicillin (3.03 A/m^2). In the presence of the anode biocatalyst *Rhodococcus*

pyridinivorans, a remarkable increase in power production (1.64-fold) and current density (1.28-fold) was observed by applying livestock antibiotic salinomycin to sewage waste. Salinomycin, a cationic binding agent was able to transfer the cation to the cell membrane through protein transport, thus improving the power production (Cheng et al. 2020). Although lignocellulosic compounds derived from residues of agriculture are favorable for low-cost electricity generation, microorganisms in MFC cannot directly digest lignocellulosic biomass for energy production. It must be degraded into monosaccharides or other reduced matters (Yadav et al. 2020a; Yaqoob et al. 2021).

9.6 Future Outlook and Conclusion

In reality, the success of an experiment is in scaling up from the lab to the field level. Strategy should be adapted to enhance the overall efficiency of oxygen reduction and increase in microbial fuel cell output. Deeper understanding of genetically engineered organisms and hybrid systems using recombinant technology can be used for strain improvement that can efficiently transfer electrons to anode. Using nanoparticles can increase the electron transfer mechanisms (Kumar et al. 2018). MFC technology in combination with other application should be focused such as bioremediation, proton generation, and biosensors for toxicity detection. Lately, microbial fuel cell(MFC)-based biosensors have been extensively developed as a novel alternative for water pollutant detection such as ammonia, styrene, nickel, and copper. The novel gene circuit engineered in E. coli Rosetta (sentinel Rosetta) was constructed by expressing ribB (riboflavin synthesis gene) and OprF (porin synthesis gene) with the promoters P_{cusC} and P_{t7}, enabled sensing Cu²⁺and generating electricity (Zhou et al. 2021). Ammonium-based MFC biosensors have proven to indicate the presence of excess ammonium in waste water. Excess ammonium inhibits the activity of electrogenic bacteria in the anode chamber and subsequently affecting electricity production (Do et al. 2021). MFCs are successfully used to achieve efficient treatment of styrene-contaminated wastewater by using activated sludge as an inoculum with maximum power density of 13.6 mW m^{-2} and styrene removal was 100% (Oveisi et al. 2021).

Before commercialization of the technology, the reactor designs, operating conditions, data collection, interpretation, and kinetic models should be thoroughly investigated. Commercialization of the technology depends on cost-effectiveness, eco-friendliness, and safety. The surface area of the electrodes should be increased so that power generated within cells can be used to run other parts of a fuel cell (Rahimnejad et al. 2015). Long-term operation of the MFC must be carried out instead of short periods of time; this could be achieved by optimizing various parameters from laboratory scale to outdoor scale and can be made possible for power generation in outdoor scale (Pandit and Das 2015). One of the best examples of commercialization of MFC is in wastewater treatment in association with electricity production, reducing the biological oxygen demand (BOD) and chemical oxygen demand (COD) of effluents.

Although the MFC technology is convoluted, it is still gaining popularity as a promising future technology that can be used without polluting the environment for the simultaneous generation of energy and reduction of organic waste. The constant hunt for novel electrode materials for enhancing the power generation of MFCs has opened up new directions for fabricating novel electrodes. Biotechnology involving metabolic engineering can be applied to increase the rate of bacterial metabolism, which can lead to enhanced cell potential. The chapter focuses on physical and chemical parameters that influence better bioelectricity generation by careful monitoring of substrate, which can promote an electrochemically active microbial community to utilize waste. Careful reactor design, choice of compatible electrodes and membranes can have a dramatic influence on power and current density.

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