4.11 Biological and Microbial Fuel Cells

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4.11.1 Introduction

The demand for energy is growing rapidly worldwide and with the increasing requirement to limit and control carbon emissions a major emphasis is being placed on providing sustainable sources of energy and more efficient use of that energy. Faced with this challenge, major efforts are being put into technologies based on renewables and in producing hydrogen as a fuel. Consequently, systems are under development that use, for example, wind or solar power to produce hydrogen by electrolysis [1–5]; hydrogen can also be produced by solar thermochemical processes [6]. The debate is still open on whether or not this is a viable means of storing energy (as hydrogen) or whether the new battery technology is more appropriate. Fermentation, photobiological methods, and use of algae [7] are alternative ways of producing hydrogen (or methane) from plant and biomass. As yet, none of these technologies can compete costwise with the generation of hydrogen from fossil fuels. Many of these processes have limitations in efficiency, for example, converting sugars to hydrogen, and it is unlikely that any single technology will solely satisfy the potential requirements for hydrogen (or electrical) energy. Thus, more efficient alternative methods are needed to develop and operate in parallel with other energy supply routes.

In parallel with research and technology development (R&TD) to produce hydrogen, there has been a significant growth in fuel cell R&TD due to the potential of fuel cells to provide a continuous supply of clean and efficient power from hydrogen. This research and development, while potentially very useful, fails to tackle the growing needs for sustainable energy generation because fuel cells mainly use hydrogen produced from hydrocarbon sources. However, the Earth has an abundant resource of 'renewable' carbon-based potential fuels that are both occurring naturally and produced via industrial processes in the form of wastes or by-products. While research is underway to indirectly use fuel cells to capitalize on some of these potential fuel sources, for example, through purification (and reforming) of biogas, many carbon sources are not immediate, viable fuels for current fuel cell technology. Most of these carbon materials are currently disposed of as waste. In comparison, biofuel cells (BioFC) have the potential to directly use a wide range of carbon sources, for example, urea, waste, and sludge, at low cost.

The fact that biofuel cells can convert readily available substrates (fuel type) from sustainable sources into hydrogen or electrical energy, presents an opportunity to make a major contribution to energy requirements. Such a process would also provide a means

of simultaneously reducing the waste treatment costs currently associated with many of the waste carbon sources, which are the potential fuels for the biofuel cells, and their use would not likely to be affected by the cost, storage, and distribution of the fuel substrate, unlike conventional hydrogen fuel cells. However, biofuel cells are at an early stage of development compared to other fuel cell types and significant research and development is still needed to approach technology readiness.

4.11.2 Fuel Cells and Biological Fuel Cells

4.11.2.1 Conventional Fuel Cells

Fuel cells are electrochemical devices that convert the intrinsic chemical energy in fuels into electrical energy directly. The fuel cell was first demonstrated by William Grove in 1839 [8] using electrochemically generated hydrogen and oxygen in an acid electrolyte with platinum electrodes. The hydrogen and oxygen produced were then used to generate a small current (and voltage).

One simple way of considering how a fuel cell works is to say that the fuel is being combusted in a simple reaction without generation of heat. As the intermediate steps of producing heat and mechanical work, typical of most conventional power generation methods, are avoided, fuel cells are not limited by the thermodynamic limitations of conventional heat engines, defined by the Carnot efficiency [9]. As such, fuel cells promise power generation at high efficiency and low environmental impact. In addition, because combustion is avoided, fuel cells produce power with minimal pollutants. However, unlike batteries, the reductant (hydrogen) and oxidant (oxygen) in fuel cells must be continuously replenished to allow continuous operation. This is a significant attraction for the use of fuel cells – extended operation limited only by the storage capacity of the fuel tank. A schematic representation of a classical H_2/O_2 fuel cell is presented in **Figure 1**.

Fuel cells can, in principle, process a wide variety of fuels and oxidants, although of most interest today are common fuels, such as natural gas (and derivatives) or hydrogen, and using air as the oxidant.

In a typical fuel cell, fuel is fed continuously to the anode (negative electrode) and an oxidant (often oxygen in air) is fed continuously to the cathode (positive electrode). The electrochemical reactions take place at the electrodes to produce an electric current through the electrolyte, while driving a complementary electric current that performs work on the load. At the anode of say an acid electrolyte fuel cell using hydrogen fuel, the hydrogen gas ionizes (reaction [1]), releasing electrons, and creating H^+ ion (protons), thereby releasing energy [8, 9].

$$2\,H_2 \rightarrow 4\,H^+ + 4\,e^- \quad E_a^0 = 0\,V \qquad [1]$$

At the cathode oxygen reacts with the protons that have migrated internally from the anode to cathode of the fuel cell, and electrons (reaction [2]) delivered from the anode via the external electrical circuit to form water [8, 9]

$$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O \quad E_a^0 = 1.229\,V \qquad [2]$$

For the reaction to proceed continuously, the electrons produced at the anode must pass through an external circuit and the H^+ ions must pass through the electrolyte. An acid is a fluid with free protons and thus serves as a good electrolyte for proton transfer. Proton conductivity [9] can also be achieved using solid electrolytes such as polymers and ceramics. Importantly, the electrolyte should only allow proton transfer and not electron transfer. Otherwise the electrons would not pass around the external circuit and thus would 'short-circuit' the cell and the function of the fuel cell would be lost.

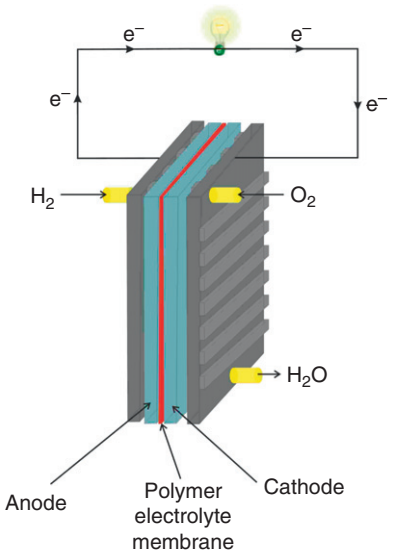

Figure 1 A hydrogen–oxygen fuel cell.

In theory, any substance capable of chemical oxidation (the reductant) that can be supplied continuously can be burned 'galvanically' as a fuel at the anode of a fuel cell. Similarly, the oxidant can be any fluid that can be reduced at a sufficient rate. For practical reasons, the most common oxidant is gaseous oxygen, which is readily available from air. Moreover, because of kinetic limitations in catalysts for fuel oxidation [9], the fuels typically used are ones with simple molecules such as hydrogen, methane, and methanol. It is the kinetic limitation in classic chemical fuel cells that has helped to stimulate greater interest in biological fuel cells to utilize a wider range of fuel feedstuffs.

4.11.2.2 Biological Fuel Cells

Biological fuel cells use biocatalysts for the conversion of chemical energy to electrical energy. Biological fuel cells work, in principle, in the same way as a chemical fuel cell: there is a constant supply of fuel into the anode and a constant supply of oxidant into the cathode however typically the fuel is a hydrocarbon compound. At the anode a fuel is oxidized, for example, glucose

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad E^0 = 0.014\,V \qquad [3]$$

and at the cathode the oxidant is reduced, for example, oxygen

$$24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O \quad E^0 = 1.2\,V \qquad [4]$$

The resultant electrochemical reaction creates a current as a flow of electrons through the external electrical circuit, and protons internally within the cell are produced from the oxidation of the fuel. The theoretical cell potentials, quoted in reactions [3] and [4] for such reactions, are similar to those of conventional fuel cells, as can be seen in reactions [1] and [2]. The distinguishing feature, central to a biological fuel cell, is the use of biocatalysts.

There are two types of biological fuel cells, namely 'microbial' fuel cells and 'enzymatic' fuel cells, depending on the biocatalysts used. Microbial fuel cells (MFCs) use whole living organisms and enzymatic biofuel cells use isolated and purified enzymes as specific catalysts [10–16].

Biofuel cells function in one of two ways, using biocatalysts,

1. The biocatalyst generates the fuel substrate for the cell via a biocatalytic transformation or metabolic process.
   The biocatalysts in this type of fuel cell are not directly involved in energy generation, which is actually produced by a conventional fuel cell. For example, convert carbohydrate to hydrogen via a fermentation process using a multienzyme system and hydrogen-producing bacteria, then use a conventional H_2/O_2 fuel cell using metal catalysts, such as Pt [17], to connect to the bioreactor, and generate electricity from the biohydrogen. In this type of enzyme fuel cells, enzymes do not involved in direct energy generation, and the energy generation is realized by a conversional fuel cell. Enzymes generate the fuel substrate for fuel cell by a biocatalytic transformation or metabolic process. There have been several studies demonstrated using hydrogenase to produce hydrogen from glucose for conventional hydrogen–oxygen fuel cells [18, 19]. This type of biofuel cell is less common in enzymatic fuel cells.
2. The biocatalyst participates directly in the electron transfer reactions between the fuel and the anode.
   In this type of biofuel cells, biocatalysts are directly involved in the bioreactions for energy production. At the anode, microorganisms or enzymes oxidize organic matter and produce electrons, and on the cathode, either living organisms (microbes) or enzymes act as catalysts for oxidant reduction and accept electrons, the same principle as the conventional fuel cells. The performance of this type of biofuel cell is mainly dependent on the activity of the biocatalyst.

Compared with traditional chemical fuel cells, biological fuel cells are considered as potentially more 'environmental friendly'. Unlike conventional fuel cells, which typically use hydrogen as fuel and usually require extreme conditions of pH or high temperature, biological fuel cells use organic products produced by metabolic processes or use organic electron donors utilized in the growth processes as fuels for power generation. Biological fuel cells operate at ambient/room temperature and at neutral pH. In addition, microbes offer major advantages over enzymes; they can catalyze a greater extent of substrate oxidation of many fuels and can be less susceptible to poisoning and loss of activity under normal operating conditions.

4.11.2.3 Enzymatic Fuel Cells

Enzymes are known for their highly specific catalytic activities for bioreactions. The interest in developing enzyme-based bioelectronics, for example, for fuel cells and sensors, has arisen due to the increasing number of implantable medical devices for health care applications within the last decade. Many applications of the technology are proposed as biosensors for monitoring the changes in physiological substances, such as glucose sensing for diabetes patients [20, 21], and employing *in vivo* biofuel cells as the power sources for these implantable devices [22–24]. **Figure 2** shows a schematic diagram of a biofuel cell working in a blood vessel using glucose and dissolved oxygen as fuel and oxidant, respectively. Electrochemical glucose sensors are the most successful commercial biosensor devices for point-of-care and personal use because of the simplicity, flexibility, and low cost of electrochemical transduction instrumentation. Enzymes have also been used on environmental sensors to monitor some specific pollutants [25–27]. Portable electronic devices, such as laptops, mobile phones, and mp3 players, are new areas to explore the use of

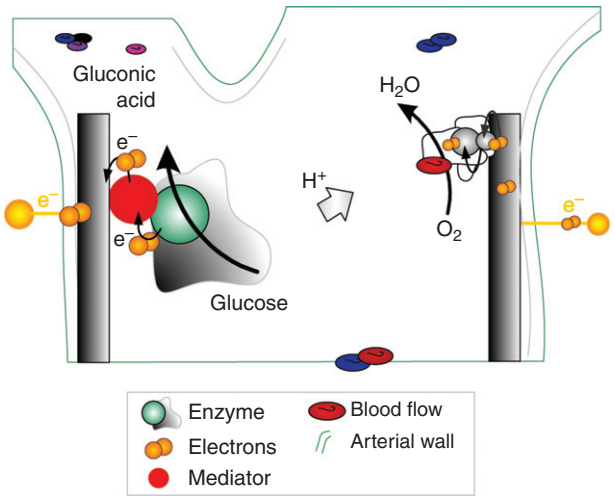

Figure 2 Schematic diagram of an enzymatic biofuel cell working in blood.

enzymatic biofuel cells [10–12], for example, Sony has developed a biofuel cell using sugar as the fuel and enzymes as catalysts to power a Walkman [28].

Enzyme-based fuel cells have been reported since the 1960s [29]. However, the development of enzymatic biofuel cells is still in its infancy, compared to conventional fuel cells, due to the low stability and low power outputs achieved. Electrodes biocatalytically modified with enzymes are the key for enhancing the performance of biofuel cells. Research in the development of enzyme electrodes for biofuel cell and biosensor applications has been carried out extensively in recent years. Studies on understanding the reaction mechanisms of enzyme catalytic reactions [30, 31] and developing new biomaterials [32–36] on enzyme modification [37–43], enzyme immobilization methods [44–50], and enzyme electrode structures [51] have been reported in the literature with the effort to improve the performance of enzyme electrodes.

4.11.2.4 Types of Biofuel Cells and Enzymes

4.11.2.4.1 Types of enzymes based on electron transfer methods

Redox enzymes can be divided into three groups (see **Figure 3**) based on the location of the enzyme active centers and methods of establishing electron transfer between enzymes and electrodes [52, 53].

1. Enzymes with nicotinamide adenine dinucleotide (NADH/NAD^+) or nicotinamide adenine dinucleotide phosphate (NADPH/$NADP^+$) redox centers, which are often weakly bound to the protein of the enzyme. Glucose dehydrogenase (GDH) and alcohol dehydrogenase belong to this group.
2. Enzymes where at least part of the redox center is conveniently located at, or near, the periphery of the protein shell, for example, peroxidases, laccase, and other multicopper enzymes fall into this category. Peroxidases, such as horseradish peroxidises and cytochrome c peroxidise, have been commonly used in enzyme reactions and immunoassay.

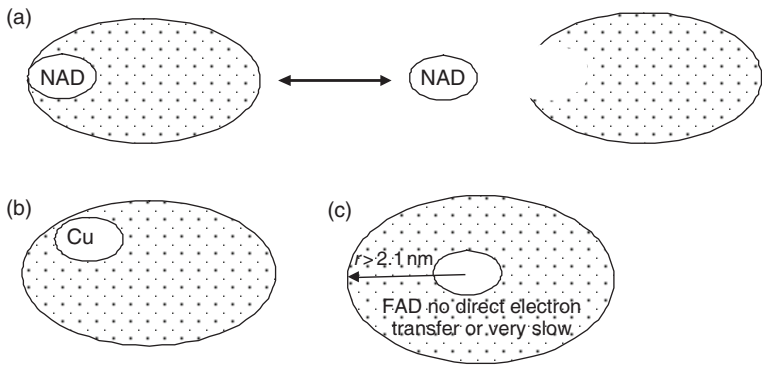

Figure 3 Three groups of enzymes based on location of enzyme active center. (a) Diffusive active center, (b) active center located on the periphery of the enzyme, and (c) strongly bound and deep-buried redox centers. Yu EH and Sundmacher K (2007) Enzyme electrodes for glucose oxidation prepared by electropolymerization of pyrrole. *Process Safety and Environmental Protection* 85(5): 489–493 [38]; Willner I, Blonder R, Katz E, *et al.* (1996) Reconstitution of apo-glucose oxidase with a nitrospiropyran-modified FAD cofactor yields a photoswitchable biocatalyst for amperometric transduction of recorded optical signals. *Journal of the American Chemical Society* 118(22): 5310–5311 [39].

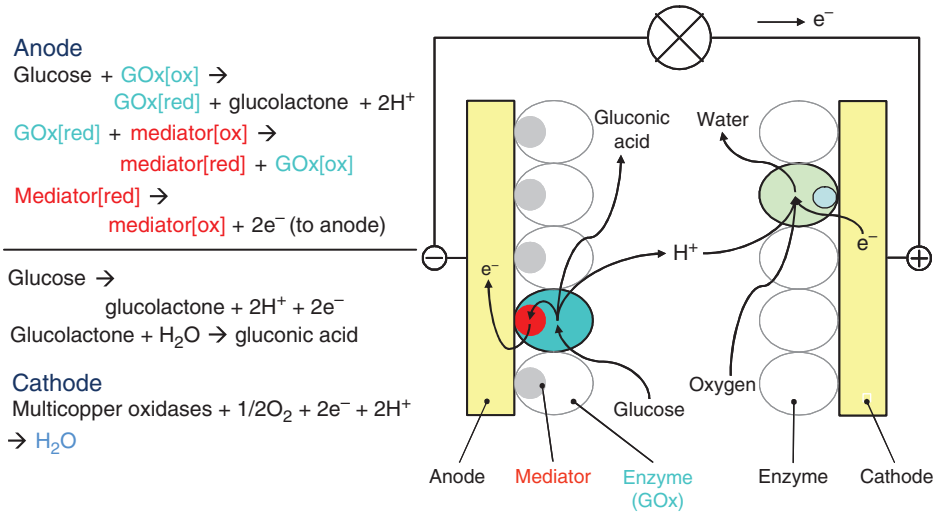

Figure 4 Schematic diagram of work principle for mediated electron transfer in enzymatic biofuel cells.

3. Enzymes with a strongly bound redox center deeply bound in a protein or glycoprotein shell. Glucose oxidase is the most studied enzyme, example for this type of applications particularly on glucose sensors and biofuel cells [53].

The first two groups are able to carry out direct electron transfer (DET) between the enzyme active centers and the electrode surface. For the second group, the orientation of the enzyme on the electrode surface is the key factor affecting the activity of the enzyme. Enzymes in the third group are not able to have DET between the active centers and electrodes due to the large distance, >21 Å, between the enzyme active centers and the electrode surface [54]. In this case, for enzymes with the active center deeply buried inside the protein shell, direct electrical communication with electrodes can be established by using electron transfer mediators. These artificial electron donor or acceptor molecules (in case of reductive or oxidative enzymes, respectively) can be accepted by many redox enzymes in place of their natural oxidants or reductants. These enzymes have a varied range of structures and hence properties, including a range of redox potentials. **Figure 4** demonstrates the working principle of mediated electron transfer (MET) in enzymatic biofuel cells. It is clear that the performance of an enzymatic biofuel cell largely depends on the properties and activities of both the enzyme and mediator molecules.

Mediators that act as the electron transfer relay are based on a diffusional mechanism. Diffusional penetration of the oxidized or reduced relay into the protein can shorten the electron transfer distance between the enzyme active center and electrode [55]. Ferrocene derivatives are one of the most commonly used mediators for glucose oxidase. 'Wired' enzymes, which have a covalently binding mediator molecule to the enzyme to establish electron transfer, were first developed by Degani and Heller [56]. Benzoquinone [57, 58], hydroquinone [59], and pyrroloquinoline quinone (PQQ) [60, 61] have also been reported as mediator for glucose oxidase.

4.11.2.4.2 Enzyme electrodes

The proper functioning of an enzyme-based electrode relies on both the chemical and physical properties of the immobilized enzyme layer. Methods for immobilization of enzymes can be divided into physical and chemical methods.

Physical methods include

1. Gel entrapment – Here the enzymes were entrapped in a gel matrix, such as gelatine and polyacrylamide, as well as dialysis tubing [62].
2. Adsorption – Adsorption of the enzyme to the electrode surface is simple and no additional reagents are required, as there is only weak bonding involved between the enzymes and electrode surface. Enzyme electrodes using Ni-Fe hydrogenase and laccase for use in a biofuel cell were prepared by adsorption of enzymes to a graphite surface by Vincent *et al.* [63]. Rapid electrocatalytic oxidation of hydrogen by the hydrogenase, which was completely unaffected by carbon monoxide, was obtained. The reaction was only partially inhibited by oxygen.

Chemical methods are the main methods used for fabricating enzyme electrodes for biofuel cell applications. The methods include covalent immobilization and immobilizing enzymes in polymer matrices.

4.11.2.4.2(i) Enzyme electrodes with layered structures

Covalent immobilization is the most irreversible and stable immobilization technique, with the most commonly used materials being noble metals and carbon. The enzyme electrodes typically have a layered structure based on covalent bindings, with the

enzymes immobilized on the electrode surface either in self-assembled monolayers (SAMs) or in layer-by-layer structures binding mediators to transfer electrons from the site of fuel oxidation at the enzyme to the electrode surface.

Katz and Willner developed a method to establish DET between the active center of glucose oxidase and the electrode surface through a defined structured path by reconstitution of the enzyme with nitrospiropyran-modified and 2-aminoethyl-modified flavin adenine dinucleotide (FAD), cofactor [39, 40, 64–67]. They produced a fuel cell using enzymes on both anode and cathode where the electrode substrate was gold. The anodic reactions, defined reactions [5]–[7], were glucose oxidation using reconstituted glucose oxidase connecting with a monolayer of PQQ as the mediator, and the cathodic reaction was reduction of hydrogen peroxide by microperoxidase-11 (MP-11) [64]. The open-circuit voltage of the cell was ~310 mV, and the maximum power density was around 160 $\mu W\,cm^{-2}$.

$$\text{Electrode} - \text{PQQ} - \text{FAD} - \text{GOx} + \text{Glucose} \rightarrow \text{Electrode} - \text{PQQ} - \text{FADH2} - \text{GOx} + \text{Gluconic acid} \quad [5]$$

$$\text{Electrode} - \text{PQQ} - \text{FADH2} - \text{GOx} \rightarrow \text{Electrode} - \text{PQQH2} - \text{FAD} - \text{GOx} \quad [6]$$

$$\text{Electrode} - \text{PQQH2} - \text{FAD} - \text{GOx} \rightarrow \text{Electrode} - \text{PQQ} - \text{FAD} - \text{GOx} + 2\text{H} + \quad + \quad 2\text{e}^- \quad [7]$$

On the enzyme anode, glucose was first oxidized by the reconstitutioned glucose oxidase and produced gluconic acid and two electrons. The FAD cofactor in GOx accepts $2e^-$ and simultaneously is reduced to $FADH_2$. These processes are described by reaction [5].

In reaction [6], $FADH_2$ was oxidized by PQQ, released $2e^-$ and hydrogen, and recovered to oxidation form GOx. PQQ accepted $2e^-$ and hydrogen, and was reduced to $PQQH_2$ in the mean time.

In the further reaction [7], the $PQQH_2$ was oxidized on the electrode and released the $2e^-$ and hydrogen in the form of proton. Through a series of redox reaction from glucose, GOx (FAD) layer and PQQ mediator layer, the electrons produced from glucose oxidation were able to reach the electrode surface.

SAM enzymatic electrodes were fabricated using thio- [68–70] groups attaching to the gold electrode surface SAMs having biospecific affinity for lactate dehydrogenase for the electroenzymatic oxidation of lactate [71]. Gooding *et al.* [49], Sato and Mizutani [72], and Dong and Li [73] have covalently immobilized redox proteins, enzymes, and phospholipids to the SAMs of 3-mercaptopropionic acid on a gold electrode surface. The electrochemical characteristics of self-assembled octadecanethiol monolayers on polycrystalline gold electrodes were studied by means of cyclic voltammetry and by measuring the monolayer transient total capacitance, as well as the differential capacitance changes during the CV scan, in the presence of various redox probes placed in the bulk of the supporting electrolyte [74]. The results showed that the capacitance measurements are very sensitive to the changes in the structure of a monolayer in the course of the redox reaction.

Enzyme electrodes with multilayer structures have been studied with mono- and bienzymes for molecular recognition and generation of electrical signals [75–78]. Calvo *et al.* established enzyme electrodes using layer-by-layer supramolecular structures composed of alternate layers of negatively charged enzymes and cationic redox polyelectrolyte. Glucose oxidase (GOx), lactate oxidase (LOx), and soybean peroxidase (SBP) have been electrically wired to the underlying electrode by means of poly(allylamine) with $Os(bpy)_2ClPyCOH+$ covalently attached (PAA-Os) in organized structures having high spatial resolution. The concentration of redox mediator integrated into the multilayers, obtained from the voltammetric charge and an estimation of the layer thickness, exceeds by 100-fold the amount of deposited enzyme assessed by quartz crystal microbalance [79]. An electrode was fabricated by alternate layer-by-layer deposition of periodate-oxidized glucose oxidase (GOx) and poly(allylamine) (PAA) by Zhang *et al.* [48]. The covalent attachment process was followed and confirmed using electrochemical impedance spectroscopy (EIS). The gold electrodes modified with the GOx/PAA multilayers showed excellent electrocatalytical response to the oxidation of glucose with ferrocenemethanol as the mediator. From the analysis of the voltammetric signals, the coverage of active enzyme on the electrode surface had a linear relationship with the number of GOx/PAA bilayers suggesting that the analytical performance can be tunable by controlling the number of attached bilayers.

4.11.2.4.2(ii) Enzyme electrodes with polymer matrix

Although enzyme electrodes with layered structures have shown efficient electron transfer in various applications, there are some limitations. First, the amount of enzymes immobilized on the electrode is limited by the electrode surface due to a monolayer covalent binding scheme. Second, the more molecular layers immobilized on the electrode surface, the more electric resistance would be introduced to the electrode, which in turn will affect the electronic response of the electrode. Also, the electrode activity will be influenced by the orientation of the enzymes and mediator molecules.

Conducting redox polymers can be a solution to overcome these limitations. Conducting polymers, such as polypyrrole (PPy) and polyaniline (PANI), are very commonly used to immobilize enzymes and fabricating enzyme electrodes. PPy is one of the most extensively used conducting polymers in design of bioanalytical sensors and has some unique properties that prevent some undesirable electrochemical interactions and facilitation of electron transfer from some redox enzymes [80]. Enzyme electrodes with PPy are fabricated by electropolymerization and enzymes are entrapped in the polymer as a dopant during the polymerization process [38, 81–86]. PPy can also be functionalized by adding cationic pendant groups, such as the tris(bipyridyl)ruthenium (II) complex to the polymer films to introduce an electron relay [87]. A two-step method consisting of the adsorption of an aqueous amphiphilic pyrrole monomer-enzyme mixture on an electrode surface followed by the electropolymerization of the adsorbed monomers was developed by Cosnier [88]. A new biotin derivative functionalized by an electropolymerizable pyrrole group has been synthesized, and the electrooxidation of this biotin pyrrole has allowed the formation of biotinylated conducting

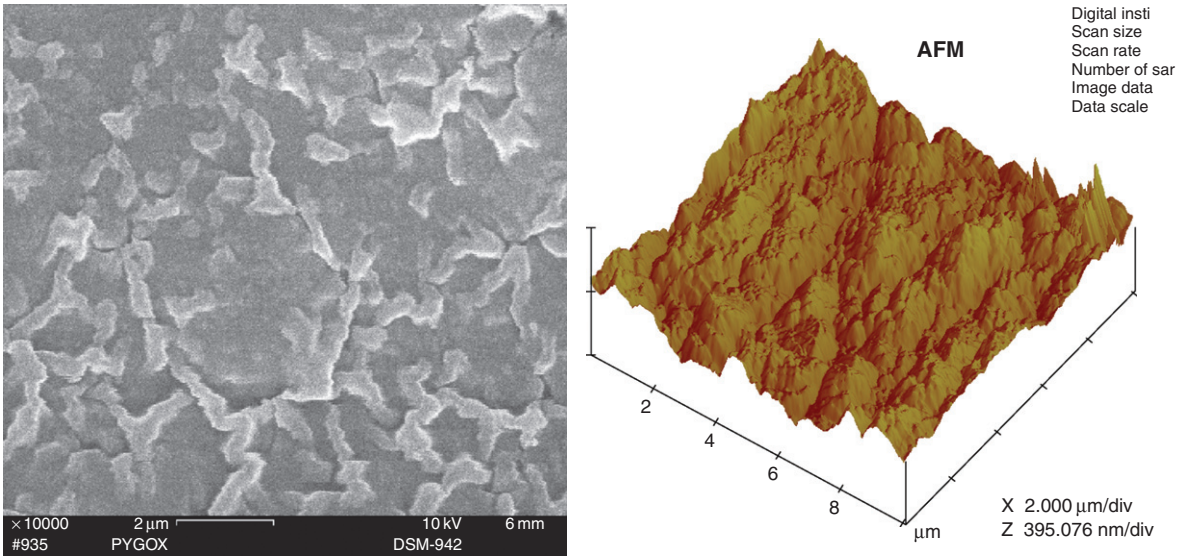

Figure 5 Au electrode coated with polypyrrole-FeFcGOx layer. Reproduced with permission from Larossa-Guerrero A, Scott K, Head IM, *et al.* (2010) Effect of temperature on the performance of microbial fuel cells. *Fuel* 89(12): 3985–3994. Copyright (2010) Elsevier [168].

PPy films in an organic electrolyte, which revealed an avidin-biotin-specific binding at the interface of the polymer solution. This provided a simple electrochemical approach to allow reagentless immobilization of enzymes on electrode surfaces [89, 90]. **Figure 5** shows the scanning electron microscopy (SEM) and atomic force microscope (AFM) images of the PPy film entrapped with ferrocene-modified glucose oxidase [38].

PANI is another extensively used polymer for enzyme immobilization. An enzyme-mediator-conducting polymer model using benzoquinone (Q)-PANI system was established by Cooper and Hall [72], which can produce enhanced current densities [91]. Raitman *et al.* integrated PANI/poly(acrylic acid) films and redox enzymes for the study of the bioelectrocatalyzed oxidation of glucose or lactate [92]. Improved selectivity and stability of a glucose biosensor was obtained based on *in situ* electropolymerized PANI-polyacrylonitrile composite film [93]. A novel method was developed by Willner's group to generate an integrated electrically contacted GDH electrode by the surface reconstitution of the apo-enzyme on a PQQ-modified PANI [94]. The same group also developed an integrated enzyme-electrode where the glucose oxidase reveals direct electrical contact with the electrode using poly (aniline–aniline boronic acid) wires generated on ds-DNA templates [95].

In order to establish electron transfer between the enzyme active centers and the electrode surface and provide the structure for enzyme immobilization, polymer mediators have been developed and applied to the enzyme electrodes. Osmium-based polymers are the most studied polymer. Current commercially available continuous glucose sensors have been using osmium-based polymers to fabricate enzyme electrodes. The advantages of these polymers include wide redox potential windows from different derivatives for various redox reactions, fast electron transfer rate, and good chemical stabilities [21, 96–105].

In 1991, Heller's group developed a redox epoxy, which was designed for use in enzyme electrodes and was formed by reacting two water-soluble components (a poly(vinylpyridine) complex of $Os(bpy)_2Cl$ and a diepoxide) under near-physiological conditions. The binding simultaneously immobilizes the enzyme, glucose oxidase, and connects it electrically with the electrode. The catalytic 'reaction layer' in this case extends through the entire film [106, 107]. Since then they have developed various Os polymer derivatives used for enzymatic oxidation and oxygen reduction reactions, as well as biofuel cells with these enzyme electrodes [21, 96–105]. Micro enzyme electrodes were developed with 7 µm diameter carbon fibers using poly(vinylpyridine) $Os(bipyridine)_2Cl$ derivative-based redox hydrogels to immobilize glucose oxidase [108]. A miniaturized biofuel cell with this carbon fiber electrode configuration was developed [109]. The power density of this device was 5 times greater than the previous best biofuel cells, which at 37 °C, a power output of 600 nW was obtained, which was enough to power small microelectronics.

For implantable applications, there is concern over possible leach out of Os compounds over the long term, due to their toxicity. Biocompatibility is another issue for implantable devices. Biopolymers based on phospholipid polymer mimicking the cell membrane were developed and these polymers have good biocompatibility and inhibit the adhesion and activation of blood cells, thus minimizing blood coagulation that could inhibit the device operation when it contacts blood [33, 35, 110]. The feasibility of introducing redox properties to phospholipid polymers was investigated and through modification of the polymer side chain, it is possible to use the biopolymers for enzyme electrodes for implantable applications [111]. A hydrophilic copolymer, poly (vinylferrocene-*co*-2-hydroxyethyl methacrylate) (poly(VFc-*co*-HEMA)), also a biopolymer, was prepared as a polymeric, electron transfer mediator for producing amperometric biosensors. The poly(VFc-*co*-HEMA) membrane is useful as an enzyme-immobilizing carrier matrix for fabricating glucose sensors as well as a polymeric, electron transfer mediator [112].

4.11.2.4.3 Performance of enzymatic biofuel cells

One of the first enzymatic biofuel cells reported by Willner and Katz used a PQQ monolayer-functionalized Au electrode as the anode and a microperoxidase-11 (MP-11)-modified Au electrode as the cathode [64]. In this system, H_2O_2 was the cathodic oxidizer, whereas the anodic fuel-substrate is 1,4-dihydronicotinamide adenine dinucleotide, NADH. The biofuel cell generates an open-circuit voltage of ~320 mV and a short-circuit current density of ~30 μA cm^{-2}. The maximum electrical power extracted from the cell was 8 μW at an external load of 3 kΩ.

Another biofuel cell developed by Willner and Katz was a novel glucose/O_2 biofuel cell without compartmentalization between anode and cathode. The anode consisted of a surface reconstituted glucose oxidase monolayer, whereas the cathode was the reconstituted cytochrome c/cytochrome oxidase couple. The biofuel cell was assembled by the engineering of layered bioelectrocatalytic electrodes. DET between enzyme and mediator, as well as mediator and the electrode surface was established. The enzyme active center, cofactor, was first removed to form apoenzyme. The mediator bound on the electrode surface was covalently bound to artificial active center before reconstitution of enzyme with artificial active center to establish an electron transfer pathway [113]. An open-circuit cell voltage of 0.11 V and peak power output of 4 μW were achieved. This system paves the way to tailoring implantable biofuel cells for generating electrical power [113].

Katz and Willner applied the property of conductivity change for oxidation and reduction status of Cu-poly(acrylic acid) polymer and developed an electroswitchable and tunable biofuel cell based on the biocatalyzed oxidation of glucose. By the cyclic electrochemical reduction and oxidation of the polymer films associated with the anode and cathode between the Cu-0-poly(acrylic acid) and Cu^{2+}poly(acrylic acid) states, the biofuel cell performance is reversibly switched between 'ON' and 'OFF' states, respectively. The open-circuit voltage of the cell was 120 mV and the short-circuit current density reached 550 μA cm^{-2}. The maximum extracted power from the cell was 4.3 μW with an external load resistance of 1 kΩ. The slow reduction of the Cu^{2+} polymer films allows for the control of the content of conductive domains in the films and the tuning of the output power of the biofuel cell [114].

An enzyme-based biofuel cell with a pH-switchable oxygen electrode, controlled by enzyme logic operations processing *in situ* biochemical input signals, was developed recently [115]. Two Boolean logic gates (AND/OR) were assembled from enzyme systems to process biochemical signals and to convert them logically into pH changes of the solution, as shown in **Figure 6**. The electrochemical activity of the modified electrode was switchable by alteration of the solution pH value. The electrode was electrochemically mute at pH > 5.5 and was activated for the bioelectrocatalytic oxygen reduction at pH < 4.5. The sharp transition between the inactive and active states was used to control the electrode activity by external enzymatic systems operating as logic switches in the system. When the biofuel cell was activated (through activating the biocatalytic cathodic process), an open-circuit voltage (V_{oc}) of 380 mV and short-circuit current density (I_{sc}) of 3 μA cm^{-2} were obtained. The maximum power density was 700 nW cm^{-2} [116].

The latest development from Mano *et al.* for a miniature, membraneless glucose-O_2 biofuel cell built with Os derivative polymer mediators for glucose and bilirubin oxidase on the anode and cathode, respectively was reported with a power density of 4.8 μW mm^{-2} produced at a voltage of 0.60 V in a physiological buffer containing phosphate buffer saline at p. 7.0 at 37.5 °C [100].

Fruit juices, such as orange juice, grape juice, and banana juice, have all been studied as potential fuels for a membraneless biofuel cell. The cell was prepared based on glucose oxidase and laccase as anodic and cathodic catalyst, respectively, by using 1,1′-dicarboxyferrocene as the mediators on both anode and cathode. This research demonstrated the possibility of using easy access fruit juice to power portable electronics [10]. By adopting grape or banana juice instead of glucose as fuels in the biofuel cell, the V_{oc} (0.191 V) and I_{sc} (60 μA, current density ~146.3 μA cm^{-2}) for grape juice and V_{oc} (0.202 V) and I_{sc} (72 μA, current density ~175.6 μA cm^{-2}) for banana juice were achieved, which are similar to glucose. The V_{oc} and I_{sc} of the fuel cell by using the orange juice as fuels are approximately twofold and threefold higher than glucose. The maximum power density of 11.66 μW (power density ~28.4 μW cm^{-2}) at 0.216 V was achieved with orange juice [10].

For implantable medical devices, nontoxic mediators for enzyme electrodes are essential. In Kyoto University, a biofuel cell was developed using Vitamin K-3-modified poly-L-lysine (PLL-VK3) as the electron transfer mediator during catalytic oxidation of NADH by diaphorase (Dp) at the anode of the biofuel cell. PLL-VK3 and Dp were co-immobilized on an electrode and then coated with NAD(+)-dependent GDH. An oxidation current of ~2 mA cm^{-2} was observed when the electrochemical cell contained a stirred

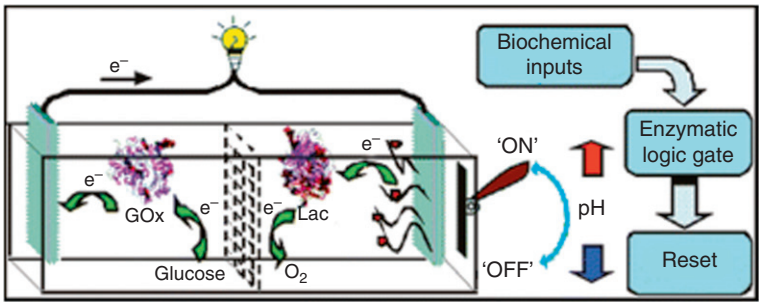

Figure 6 Schematic diagram of a biofuel cell with a pH-switchable oxygen electrode, controlled by enzyme logic operations processing *in situ* biochemical input signals. Reprinted with permission from Amir L, Tam TK, Pita M, *et al.* (2008) Biofuel cell controlled by enzyme logic systems. *Journal of the American Chemical Society* 131(2): 826–832 [115]. Copyright (2009) American Chemical Society.

30 mM glucose, 1.0 mM NAD(+), p. 7.0 phosphate-buffered electrolyte solution. The open-circuit voltage of a glucose/O_2 biofuel cell with the polydimethylsiloxane (PDMS)-coated Pt cathode was 0.55 V and its maximum power density was 32 μW cm^{-2} at 0.29 V when a p. 7.0 buffered fuel containing 5.0 mM glucose and 1.0 mM NAD(+) was introduced into the cell at a flow rate of 1.0 ml min^{-1}. The cell's output current density declined by ~50% during 18 h of operation [117].

Apart from glucose, other organic fuels such as alcohol and glycerol have also been used in enzymatic biofuel cells. An enzymatic biofuel cell using ethanol and operated at ambient temperature has been developed. The anode of this biofuel cell was based on immobilized quinohemoprotein alcohol dehydrogenase (QH-ADH), while the cathode was based on co-immobilized alcohol oxidase (AOx) and microperoxidase (MP-8). The enzymes are able to have DET to the electrode surfaces. The maximal open-circuit potential of the biofuel cell was 240 mV and maximal power for completed biofuel cell was 1.5 μW cm^{-2} [118].

Glycerol has attracted increasing interest because it is a by-product from biodiesel production. An enzymatic biofuel cell was developed by using glycerol as the fuel and employing a three-enzyme cascade on the anode that can accomplish the complete oxidation of glycerol [119]. The bioanode that was developed contained PQQ-dependent alcohol dehydrogenase (PQQ-ADH), PQQ-dependent aldehyde dehydrogenase (PQQ-AldDH), and oxalate oxidase immobilized within a tetrabutylammonium-modified Nafion membrane. This glycerol/air biofuel cell yielded power densities of up to 1.32 mW cm^{-2} and has the ability to operate at 100 mM glycerol.

Nanocarbon materials, such as carbon fiber and carbon nanotubes, have also been applied in enzymatic biofuel cells because of their excellent electronic properties. A passive-type biofuel cell, which generated a power of over 100 mW with a cell volume of 80 cm^3, operated at a pH of 7, gave a maximum power density of ~1.45 ± 0.24 mW cm^{-2} at 0.3 V. This performance was achieved by densely packed enzymes and mediator on carbon-fiber electrodes with the enzymatic activity retained. These cell units, with a multistacked structure, successfully operated a radio-controlled car and a memory-type walkman for more than 2 h [120].

Membraneless and mediator-free DET enzymatic biofuel cells with bioelectrodes comprised single-wall carbon nanotubes (SWCNTs) deposited on porous silicon substrates were reported. Anodic glucose oxidase (GOx) and cathodic laccase (Lac) were immobilized on the porous silicon/SWNT substrates used in the fuel cell, in a p. 7 phosphate buffer solution (PBS). A peak power density of 1.38 μW cm^2 (with a lifetime of 24 h) down to 0.3 μW cm^2 was obtained using a 4 mM glucose solution as fuel and air as an oxidant [121].

4.11.3 Microbial Fuel Cells

4.11.3.1 Development of MFC

A large amount of energy exists within various waste streams and can be degraded by microbes. At the turn of the nineteenth century, the idea of using microbial cells to produce electricity was first envisaged by Potter [13], who tried to generate electricity with *E. coli*. In 1931, Cohen created a number of microbial half fuel cells connected in series [122]. DelDuca *et al*. succeed in operating a hydrogen and air fuel cell using hydrogen production from fermentation of glucose by *Clostridium butyricum* at the anode [14]. Rohrback *et al.* [15] designed a biological fuel cell in which *C. butyricum* was also used to generate hydrogen by glucose fermentation. In 1969, Yao *et al.* showed that glucose could be used as a fuel in the presence of a platinum-black anode [16]. In the early 1980s, Bennetto studied MFCs in more detail and designed a fuel cell as a possible method for the generation of electricity for third world countries [123]. Bennetto showed that mediators could enhance the efficiency of electron- transfer and the reaction rate. Since then a large amount of research has examined various aspects of MFCs from materials, to bioelectrochemistry to microorganisms.

Tanisho *et al*. [124] studied an MFC with *Enterobacter aerogenes* and a stainless-steel net anode plated with platinum black. The main anode reactant for Tanisho was hydrogen, which was biochemically produced from glucose by the bacteria. An alternative strategy was direct conversion of the sugars to electrical power. Existing transition metal-catalyzed fuel cells cannot be effectively used to generate electric power from carbohydrates [12]; however biofuel cells, in which whole cells or isolated redox enzymes catalyze the oxidation of the sugar, have been developed [28–35].

4.11.3.2 Electricity Generation Mechanism in MFC

The mechanism by which electricity can be produced directly from the degradation of organic matter in an MFC is still not completely understood. Heterotrophic bacteria liberate energy from the oxidation of organic matter, the process called as catabolism. MFCs make use of the catabolic activity of living cells, that is, bacteria (biocatalysts), to convert chemical energy into electricity. When bacteria oxidize a chemical, they capture the electrons and transfer them to a series of respiratory enzymes used to store energy in the form of adenosine triphosphate (ATP) within the cell. Electrons are then released to an electron acceptor such as iron, nitrate, sulphate, or oxygen. The same bacteria that respire using iron have recently been found to be able to transfer electrons to an anode [125].

When microorganisms consume a substrate (e.g., glucose) in the presence of oxygen, they produce carbon dioxide and water through an oxidative metabolism, as defined in reaction [8]:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad [8]$$

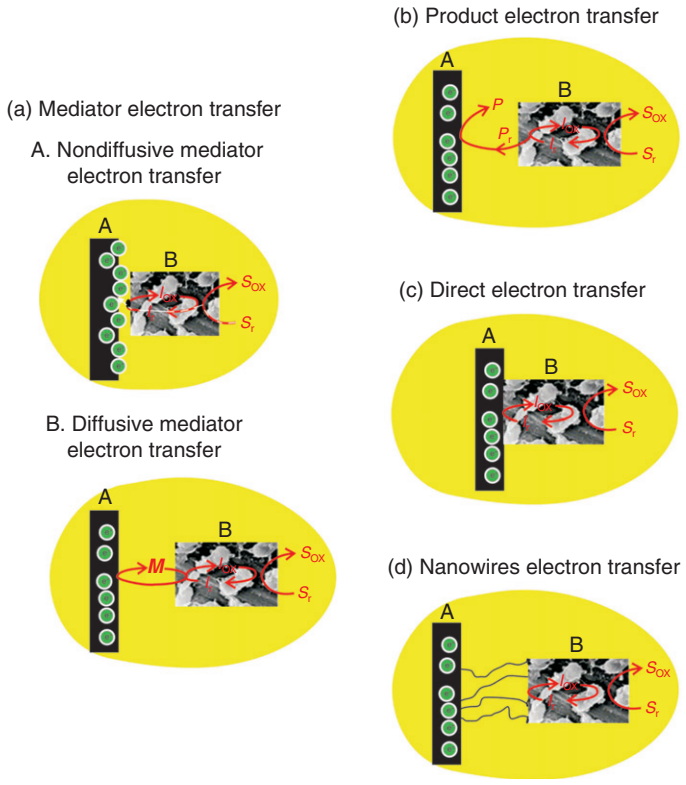

Figure 7 Mode of electron transfer mechanisms in an MFC. M, mediator; A, anode; B, bacteria; C, cytochromes; D, nanowires; S_{ox}, substrate oxidation; S_{red}, substrate reduction; I_{ox}, intermediate oxidation; I_{red}, intermediate reduction; P_{ox}, production oxidation; P_{red}, production reduction; and e^-, electron.

However, when oxygen is not present, in an MFC, they produce carbon dioxide, protons, and electrons according to reactions [3] and [4]. Various mechanisms have been proposed by which electron transfer occurs in MFC between bacteria and the anode (**Figure 7**). A mechanism has been proposed describing DET, in which some outer-membrane bound proteins, such as cytochromes [126, 127], play the role of transferring electrons to the electrode.

$$\text{Substrate} \xrightarrow{\text{Microbial metabolism}} \text{Wastes} + (\text{membrane cytochrome})- (\text{membrane cytochrome})- \xrightarrow{\text{anode}} (\text{membrane cytochrome}) + \text{e}- \quad [9]$$

Another mechanism concerns the use of external or self-produced mediators:

$$\text{Substrate} + \text{Mediator} \xrightarrow{\text{Organism}} \text{Product} + \text{Mediator Mediator} \xrightarrow{\text{Anode}} \text{Mediator} + \text{e}- \quad [10]$$

It has also been suggested that bacteria are able to form 'nanowires' contacting the electrode, through which electrons are transferred [128].

4.11.3.3 Working Principles of MFC

The operation principle of the MFC is shown in **Figure 8**. An anode and cathode are placed in aqueous solutions in two chambers separated by a proton exchange membrane (PEM). The generation of current is due to the nature of microorganisms; they transfer electrons from a reduced electron donor to an electron acceptor at a higher electrochemical potential. Bacteria in an anode biofilm carry out oxidation of organic matter, producing electrons and protons: one proton for every electron, and dependent on fuel source, carbon dioxide may eventually be produced as an oxidation product. Electrons are transferred to the cathode through the external circuit, thereby powering an external electrical load, and protons are transferred through the membrane. Electrons and protons react on the cathode, reducing the oxidant (generally oxygen) to water, and generating electricity.

Unless the species in the anode chamber are anodophiles, the bacteria having ability to reduce inert electron acceptor, the microbes are incapable of transferring electrons directly to the anode [130]. Hence, to enhance power output of the device electron mediators (e.g., neutral red [131], methylene blue, thionine [132], and Fe(III)EDTA [133, 134]) can be used in the MFCs to accelerate the electron transfer. Mediators in an oxidized state are reduced by accepting electrons. These electrons

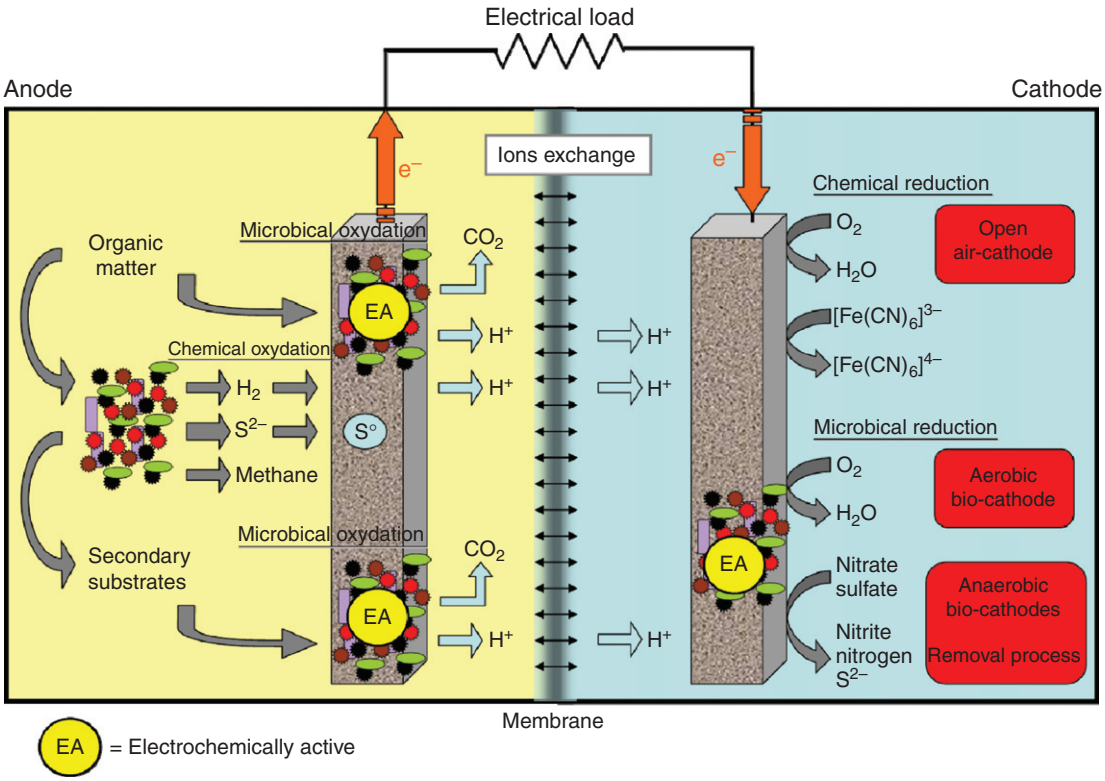

Figure 8 Schematic representation of microbial fuel cell operation. Reprinted with permission from Duteanu NM, Ghangrekar MM, Erable B, and Scott K (2010) *Microbial fuel cells – An option for wastewater treatment. Environmental Engineering and Management Journal* 9(8): 1069–1087. [129]. Copyright (2010) Environmental Engineering and Management Journal.

are released to the anode and mediators are oxidized again in the bulk solution in the anode chamber. This cyclic process can accelerate electron transfer and enhance power output of the MFC. However, the use of mediators causes several problems for practical devices and technology development is focused on mediatorless MFCs, that is, cells in which external chemical mediators are not used.

4.11.3.4 Mediatorless MFC

Recent studies [135, 136] showed that complex microbial communities in wastewater-fed MFCs produce soluble redox mediators, for example, pyocyanin [137]. It has been shown that certain metal-reducing bacteria, belonging primarily to the family *Geobacteraceae* can directly transfer electrons to electrodes using electrochemically active redox enzymes, such as cytochromes, on their outer membrane [136]. Furthermore, *Geobacter sulfurreducens* is known to transfer electrons beyond cell surfaces to electrodes through membrane proteins [138, 139] or nanowires [128]. The electron transfer between the electrode and *E. coli* cells is reported to be carried out by soluble compounds in the culture [140]. *E. coli* cells evolved under electrochemical tension in an MFC pose direct electrochemical behavior due to excretion of hydroquinone derivatives through a highly permeable outer membrane [141]. In addition to these species, metabolites produced by *Pseudomonas* species enable Gram-positive bacteria that can also achieve extracellular electron transfer [142]. Several other anodophilic bacteria have been identified in recent research, those are described in detail in Section 4.11.3.6. MFCs containing such electrochemically active bacteria (EAB) do not need mediators for electron transfer to electrodes and are called mediatorless MFCs.

4.11.3.5 Organic Matter Removal in MFC

Compared with the other fuel cells including enzymatic biofuel cells, MFCs may use a wider range of fuel sources (e.g., complex organic matter in wastewater), although the level of power achieved, as yet, is not high. Highest power per unit volume of 2.15 kW m^{-3} is reported using *G. sulfurreducens* in the anode [143]. Differences in power production and bacteria present on the anode suggest that substrate composition influences bacterial enrichment on the anode and in turn the current production efficiency. Extensive research on developing reliable MFCs has focused mostly on selecting suitable organic and inorganic substances that can be used as sources of energy. It now seems that electricity can be generated from any biodegradable material,

ranging from pure compounds, such as acetate and glucose, to complex mixtures and wastes, such as glucose, acetate, butyrate [144], cysteine [145], proteins [146], and lignocellulose [147]. MFCs have generated electricity directly from complex organic mixtures in food processing [148, 149], brewery [149], domestic [150–153], chemical [154, 155], starch [156] wastewaters, swine manure slurry [157, 158], manure waste [159], landfill leachate [160], and meatpacking wastewater [146].

Various studies have demonstrated that the treatment of wastewater is one of the most promising applications of MFCs. Under different operating conditions and with various reactor types used, chemical oxygen demand (COD) removal ranging from 60% to 90% is reported in the literature [129]. Most of the MFC configurations are reported to be capable of giving COD removal efficiencies ranging from 80% to 95% while treating different wastewaters; demonstrating the utility of MFC as a wastewater treatment system. This efficiency is comparable with existing popularly used anaerobic processes, such as the upflow anaerobic sludge blanket (UASB) reactor [161]. Synthetic wastewater generally gives higher organic matter removal and Coulombic efficiency (CE) compared to actual wastewaters [153]. The CE of the MFC is defined as the ratio, expressed in percentage, of amount of Coulombs that is actually harvested by the MFC to the total theoretical Coulombs that can be generated from the substrate supplied. Lower CE while treating actual wastewaters is due to a more complex nature of the organic matter in actual wastewater than synthetic wastewater, where usually a single carbon source is used by the researchers.

Apart from the treatment of soluble organic matter, it is interesting that MFCs can be used for the treatment of cellulose-containing wastewater to generate electricity. Unlike typical soluble substrates that have been used as electron donors in MFC, cellulose is unique because it requires a microbial consortium that can metabolize both an insoluble electron donor (cellulose) and an electron acceptor (electrode). Successful electricity generation from cellulose-fed MFC was reported using a defined coculture of *Clostridium cellulolyticum* and *G. sulfurreducens* [162]. The coculture achieved a maximum power density of 143 (anode area) and 59.2 $mW\,m^{-2}$ from 1 $g\,l^{-1}$ carboxymethyl cellulose and MN301 cellulose, respectively [162]. A pure culture alone could not produce any electricity from these substrates.

Coulombic efficiencies for MFCs vary but, in general, increase with power density because there is less time for substrate to be lost through competing physical and biological processes. The maximum power density produced appears to be related to the 'complexity' of the substrate (i.e., a single compound versus many compounds). This trend of reduced power production has been observed in studies using the same system that power output was only 146 $mW\,m^{-2}$ using domestic wastewater versus 494 $mW\,m^{-2}$ using glucose [163]. Min and Logan [150] found in a flat-plate MFC that power output was 86% less when dextran was used instead of glucose in the feed. Thus, it appears that the effect of multiple substrates or polymers in the organic solution can reduce the maximum power output. Also nonfermentable substrates, such as acetate and butyrate, yielded Coulombic efficiencies of 50–65%, while fermentable substrates, such as glucose, dextran, and starch, produced Coulombic efficiencies of only 14–21%.

4.11.3.6 MFC Operating Conditions and Material Aspects

MFC performance is affected by several factors, which includes the inoculum, that is, the source of bacterial culture and bacterial strain(s) used at the anode, the fuel substrate and concentration, pH, conductivity, temperature and conditions of operation of the MFC, including hydraulic loading rate, as well as the reactor design and cell materials for anode, cathode, and anode-to-cathode separator.

4.11.3.6.1 Operating temperature

Temperature is one of the most important parameters in anaerobic digestion and methane production is strongly dependent on it. Most anaerobic digesters operate at the mesophilic range and the characteristics of this process have been widely studied and documented [164]. Most of the studies report a marked decrease in methane production as temperature decreased, with an optimum temperature for mesophilic bacteria known to be around 35–40 °C [165]. When the reactor temperature is lower, the mesophilic bacterial consortia goes through a long selection and adaptation process during which their activity slows down drastically and results in developing a group of mesophilic psychrotrophic bacteria. There is also a group of bacteria called psychrophilic bacteria that naturally prefer low-temperature environments [166]; they have more recently become the object of study [167, 168].

Similar to other biological wastewater treatment processes, performance of the anode in an MFC is affected by the temperature. However, just as in chemical fuel cells, increasing temperature also improves the kinetics of oxygen reduction and reduces the internal resistance of the cell, which can lead to greater current densities and greater CE; for example, 43% at 30 °C compared to 8% at 22 °C [169].

With an increase in temperature, the biochemical reaction rate can also increase and hence results in an increase of biomass growth rate due to increase in the substrate utilization rate. Higher growth rate would also result in faster microbial attachment on the electrode.

An operating temperature of 35 °C was reported to be optimum [170], although this is clearly dependent upon the bacterial strain used. Reductions in power density (70–43 $mW\,m^{-2}$), CE, and COD removal efficiency were reported with a reduction in temperature from 30 °C to 20/22 °C [171]. Conversely, thermophilic operation of an MFC at 55 °C for over 100 days was reported to produce a power density of 37 $mW\,m^{-2}$ at a CE of 89% [172]. In MFC generating electricity from marine sediments, cell operation at 60 °C was reported to produce 10 times more power as compared to operation at 22 °C [173]. Successful current production is also reported in MFCs operated at 50 °C [174].

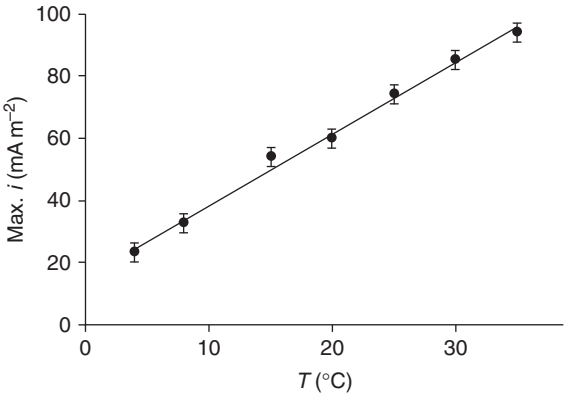

Figure 9 Variation of maximum current density single-chamber MFCs with carbon cloth cathodes, under 1 kΩ working at temperatures between 4 °C and 35 °C. Reproduced with permission from Larossa-Guerrero A, Scott K, Head IM, *et al.* (2010) Effect of temperature on the performance of microbial fuel cells. *Fuel* 89(12): 3985–3994. Copyright (2010) Elsevier [168].

Research in MFCs at different temperatures ranging from 4 °C to 35 °C has been performed with single-chamber MFCs (SCMFCs) and two-chamber MFCs. In one report, the reactor feed was brewery wastewater diluted in domestic wastewater [168]. These data showed that an increase in temperature increased COD removal, current densities, and cell voltages (**Figure 9**). Further, the power density increased by an order of magnitude over the temperature range studied, with results ranging from 58% final COD removal and maximum power of 15.1 mW m^{-3} reactor at 4 °C to 94% final COD removal and maximum power of 174.0 mW m^{-3} reactor at 35 °C for SCMFCs with carbon cloth-based cathodes. Bioelectrochemical processes in these MFCs were found to have a temperature coefficient, Q_{10}, of 1.6. Temperature coefficient in chemistry and biochemistry represents a measure of the processes rate of change of the system when the temperature is increasing with 10 °C. Thus, the temperature coefficient Q_{10} is defined as [168]

$$Q_{10} = (R_1/R_2)^{10/(T_2-T_1)}$$

where R is the rate and T is the temperature expressed in Celsius or Kelvin degrees.

4.11.3.6.2 Operating pH

MFCs are typically operated at pH values between 6 and 8 in the anode chamber and neutral (p. 7), or a little higher, in the cathode chamber (**Figure 10**). This is because the anodic microbial process performs well around neutral pH and microbial activity decreases at higher or lower pH: an anodic chamber pH between 7 and 8 is reported to produce maximum CE and current [135]. Such pH values are often inherent in the feed/waste stream being processed; however, reductions in current and CE were reported at p. 6 and above p. 9.0 [176]. Data presented so far suggest that a pH between 6 and 7 may give optimum power production from the MFC, although operation of MFC at feed pH up to 10 is possible. Higher pH in the anode chamber favors higher COD removal but reduces power, and a higher pH difference between the anode and cathode can improve power output of the MFC due to improvements in the kinetics of oxygen reduction [129, 135, 176].

4.11.3.6.3 Organic loading rates and hydraulic retention time

Organic loading rates (OLRs) and retention time (residence time) generally influence MFC performance, which is particularly dependent on the substrate being used as a fuel. Nonfermentable substrates, such as acetate, give higher power densities and energy conversion efficiencies as compared to fermentable substrates, such as glucose [177]. When using humic acid (HA) as a mediator in two-chamber MFCs, acetate produced higher power due to a simpler metabolism than glucose and xylose [178]. In the presence of HA, the power increased by 84% and 30% for glucose and xylose, respectively, due to the mediating effect of HA. No specific effect of HA addition was reported for acetate. External mediator addition increases power output during fermentable substrate degradation indicating limited electron transfer ability of the microbes developed in the cell.

Generally, there is an optimum range of OLR to obtain maximum COD removal efficiency and maximum power that depends on the configuration of the MFC used and the wastewater being treated. The OLRs used in MFCs are comparable with those used in activated sludge processes. However, these are only comparable with the OLRs adopted for sewage treatment in high-rate anaerobic processes, such as UASB reactor and anaerobic filters, and far less than the OLRs used in case of industrial wastewater treatment in UASB reactors.

The applied OLRs will have a marked influence on both power yield and substrate degradation rate in the MFC. Typically OLRs in the range of 0.05–2.0 kg COD m^{-3} day^{-1} are used by researchers to achieve maximum power from the MFC. It is reported that in treating wastewater a maximum power yield (274 mW g^{-1} COD) was obtained at an OLR of 0.574 kg COD m^{-3} day^{-1} [179]. Operation of the MFC at the higher OLR is reported to reduce the CE. While treating leachate, increasing OLR from 0.65 to 5.2 kg COD m^{-3} day^{-1} resulted in a decrease of overall CE from 14.4% to 1.2% [180].

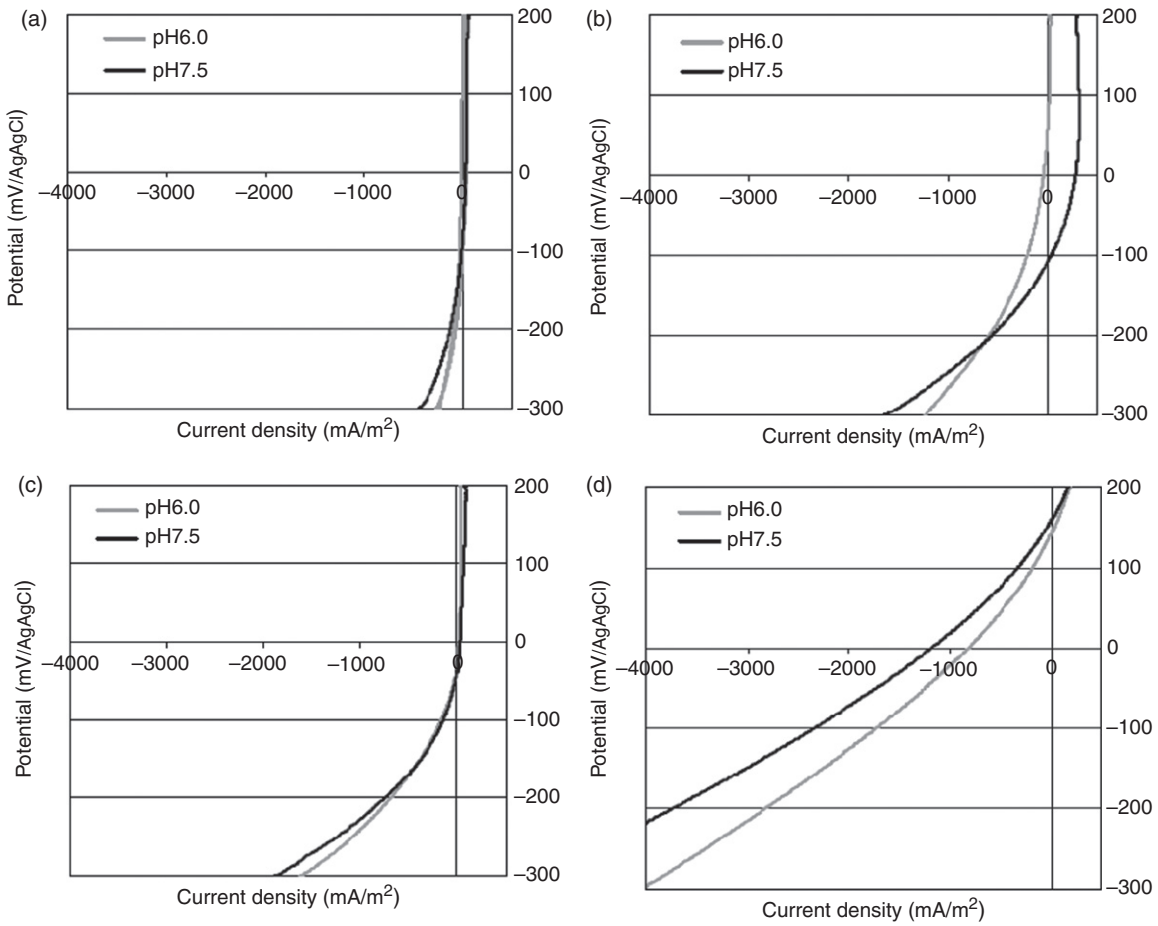

Figure 10 Effect of pH on noncatalyzed oxygen reduction – linear sweep of oxygen reduction with air-breathing cathode prepared from (a) untreated carbon Vulcan XC-72R, (b) H_2O_2-treated carbon Vulcan XC-72R, (c) HNO3-treated carbon Vulcan XC-72R, and (d) platinum supported on carbon Vulcan XC-72R. Reprinted with permission from Duteanu N, Erable B, Senthil Kumar SM, *et al.* (2010) Effect of chemically modified Vulcan XC-72R on the performance of air breathing cathode in a single chamber microbial fuel cell. *Bioresource Technology* 101: 5250–5255 [175]. Copyright (2010) Bioresource Technology – Elsevier.

The hydraulic retention time (HRT) affects the contact between the substrates and microorganisms. It is evident that higher HRT in the anode chamber favors higher treatment efficiency and higher power production [181]. The optimum HRT depends on the type of organic matter being treated, the reactor geometry, and the strength of the wastewater. The favorable HRTs reported in the literature are a little higher than the HRTs generally adopted for established wastewater treatment systems such as UASB reactor. Hence, to make the size of MFCs competitive with other already established treatment processes, it is required to modify the configuration of MFC to process higher OLR at lower HRTs.

SCMFC, with a high surface packed bed of irregular graphite granules as the anode, in batch and continuous mode operation, have been used to treat wastewater [182]. CEs varied from 30% to 74%, depending upon feed COD (**Table 1**). In continuous operation, the

Table 1 Effect of the COD loading rate in batch mode

Inlet COD (ppm)	*Max current (mA)*	*Max volumetric power density ($W m^{-3}$)*	*Maximum power density ($mW m^{-2}$)*	*COD removal (%)*	*Coulombic efficiency (%)*	*COD removed by non Faradaic reaction (%)*
1000	0.450 ± 0.009	8.1	81	69 ± 1	30 ± 2	48
500	0.354 ± 0.007	4.9	49	62 ± 3	57 ± 5	27
200	0.331 ± 0.005	4.4	44	60 ± 5	76 ± 2	14
100	0.312 ± 0.009	3.8	38	68 ± 4	74 ± 1	18

Graphite granule bed depth: 3 cm. Fuel: AW. R_{ext}: 500 Ω

Table 2 Effect of the hydraulic retention time (HRT) on the SCMFC performance

Flow rate ($cm^3\ min^{-1}$)	*HRT (min)*	*Current output at the steady state (mA)*	*Power output at the steady state (mW)*	*Coulombic efficiency (%)*	*COD removal (%)*
0.028	446	0.413 ± 0.007	0.085 ± 0.004	63 ± 4.5	69 ± 5.2
0.1	125	0.478 ± 0.008	0.114 ± 0.005	44.3 ± 7	31.3 ± 4.6
1	12.5	0.55 ± 0.004	0.151 ± 0.003	7.4 ± 1.9	19.9 ± 4.1

Graphite granule bed depth: 3 cm. Fuel: AW with 200 ppm of COD. R_{ext}: 500 Ω

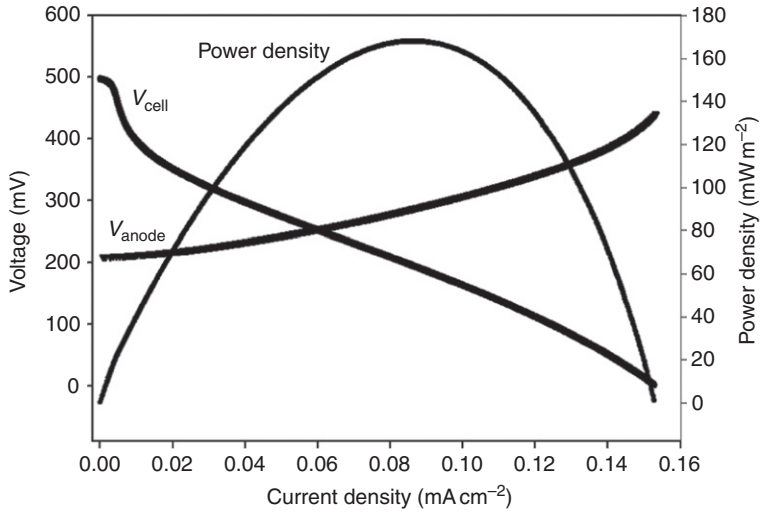

Figure 11 Polarization and power density curves for an MFC. Anode: graphite granules (3 cm layer). Power and current density refer to the anode area: 12.5 cm^2. The cells were fed with AW containing 1000 ppm as COD. Reproduced with permission from Yu FH and Sundmacher K (2007) Enzyme electrodes for glucose oxidation prepared by electropolymerization of pyrrole. *Process Safety and Environmental Protection* 85(5): 489–493. Copyright (2007) Elsevier [38].

COD removal of 89% and CE of 68% was reported with a feed COD of 1000 ppm and at a flow rate of 0.0028 $cm^3\ min^{-1}$. Power performance was a volumetric power density of 1.3 $W\ m^{-3}$, with respect to the net anodic volume (12.5 cm^3) (**Figure 11**).

Saturation-type relationships between substrate concentration and power or voltage generated are typically observed in MFCs at sufficiently high concentrations [157]. For example, when the effect of influent COD concentration in the wastewater, ranged from 129 to 1124 $mg\ l^{-1}$ was studied, the maximum power density was 164 $mW\ m^{-2}$, with a half-saturation concentration of 259 $mg\ l^{-1}$ [181]. At low COD concentration, electricity generation is limited by the anode due to kinetic limitations.

Conductivity of the wastewater in the anode chamber also affects the power output by reducing the internal resistance at higher conductivity and hence increasing the power [163]. An MFC used to treat paper recycling plant wastewater was reported to be limited by conductivity [183]. When only wastewater (conductivity 0.8 $mS\ cm^{-1}$) was used as a feed, a power density of 144 $mW\ m^{-2}$ was produced with total COD, soluble COD, and cellulose removals of approximately 29%, 51%, and 16%, respectively. When a 50 mM PBS (5.9 $mS\ cm^{-1}$) was added to the wastewater, power densities reached 501 $mW\ m^{-2}$ (CE of 16%), with removal of soluble COD of 73% and total COD removal of 76%. Cellulose was removed at levels up to 96% during treatment.

Nutrient requirement is also a factor that will influence MFC performance. Certain ratio of carbon source supplied as substrate to nitrogen and phosphorous is necessary to support the bacterial growth by avoiding nutrient limitation. The COD/N ratio required for the MFC-type wastewater treatment system is reported to be lower than the conventional treatment [184]. Nitrogen can be supplemented in the form of urea, ammonium sulfate, and ammonium nitrate producing equivalent power [185]. However, removal of nitrogen from the feed is reported to adversely affect power production. The effect of phosphorous concentration in the feed on performance of MFC is not available so far. In general, it appears that the 'P' requirement similar to anaerobic process satisfies the requirement of electrogenic, that is, anodophilic bacteria. The exact nutrient requirement of the MFC will depend on the type of microorganisms used in the anode for specific organic matter removal. Studies are required to establish exact macronutrient and micronutrient requirements of the electrogens to sustain their growth in the MFC.

4.11.3.6.4 MFC design

The geometrical design of the MFC, its dimensions and positioning of the electrode with respect to membrane, and the arrangement of influent and effluent for proper distribution of substrate to the anode chamber are among the parameters that will play an important role in MFC performance [186].

Oxygen in air is the obvious choice of oxidant for MFCs. Although other chemicals such as hydrogen peroxide, hexacyanoferrate $[Fe(CN)_6]^{3-}$, and permanganate can also be used as effective cathodic electron acceptors and give higher power density, they are not considered as sustainable because they still require continuous replacement. An alternative to oxygen is ferric ions which can be reduced to ferrous ions (Fe^{2+}) at the cathode. MFC with ferric iron reduction at the cathode and simultaneous biological ferrous iron oxidation of the catholyte was demonstrated using a bipolar membrane separating the anode and cathode [187]. The immobilized microorganism *Acidithiobacillus ferrooxidans* oxidized ferrous iron to ferric iron at a rate high enough to ensure an MFC power output of $1.2\,W\,m^{-2}$ and a current of $4.4\,A\,m^{-2}$.

In general, using air as an oxidant in MFCs is reliant on a suitable choice of catalyst material. A near neutral pH is not a preferred condition for good oxygen reduction kinetics [129]. Using dissolved oxygen in aqueous catholyte solutions will limit the cell voltage and power capabilities; hence air cathodes are frequently used to enhance the performance of MFCs. An air cathode MFC is an efficient configuration not requiring active aeration or addition of chemicals for cathodic reaction (**Figure 12**).

An alternative to oxygen reduction in MFCs is to use protons to form hydrogen gas. Such hydrogen can then be used as a fuel to generate energy by other power devices. However, such microbial electrolysis does not produce power in conjunction with hydrogen but requires a power input (some several hundred millivolts) to realize reasonable production rates. This is due to the difference of ~1.2 V in the standard potential for oxygen reduction and proton reduction.

The ideal material selected for the cell electrodes should offer a higher surface area per unit volume to maximize opportunity for direct growth of microorganisms on the anode surface. Graphite granules, felt, and carbon brush or fibers can be suitable alternatives for use as the electrodes. An electrode material offering very high surface area and very fine pore size may not be suitable as it may lead to the formation of dead pockets (area not used for direct growth of microorganism) and reduction in the MFC power output [188]. Graphite fiber brush anodes that have high surface areas and a porous structure can produce high power densities ($1430\,mW\,m^{-2}$, $2.3\,W\,m^{-3}$) as compared to other carbon forms [189]. A power density of as high as $2.01\,W\,m^{-2}$ has been reported for an MFC using a carbon brush anode [190]. Furthermore, it is required to explore the possibility of non-noble metal catalyst coating on the electrode surface to maximize power production with minimum cost for MFC construction.

The membrane used in MFCs to separate the anode from the cathode acts as an electrolyte and allows typically proton transfer. However, the use of membrane can limit the application of MFC for wastewater treatment. Proton transfer through the membrane may be a limiting factor especially due to membrane fouling expected due to suspended solids and soluble contaminants in large-scale wastewater treatment [191]. In addition, membranes are expensive and thus will limit acceptance of MFCs for large-scale wastewater treatment, due to higher production costs. Hence, to make MFC economically competitive, a low-cost alternative to the use of membranes or a design appraisal of the cell to eliminate the need for a membrane is needed. An issue with the membraneless design is to prevent large quantities of oxygen diffusing toward the anode chamber that would reduce the CE. A higher power density of $346\,mW\,m^{-2}$ was reported in mediatorless and membraneless MFC using plastic sieves rather than polymer electrolyte membrane (PEM) [170]. In another example, maximum power of $49\,W\,m^{-3}$ ($215\,A\,m^{-3}$) was reported for a membraneless MFC [192]. The use of porous fabrics such as J-Cloth, instead of PEM, was evaluated as a separator between the anode and the cathode. Due to the significant reduction of oxygen diffusion with two layers of J-Cloth, over a 100% increase in CE was demonstrated in comparison to cell without J-Cloth: power densities of $627\,W\,m^{-3}$ in fed-batch mode and $1010\,W\,m^{-3}$ in continuous-flow mode were reported [193]. Recently, Behera *et al.* [194] have demonstrated the performance of a low-cost MFC, with a relatively high volume of 400 ml, made from an earthen pot without using commercially available expensive membranes. This earthen pot MFC, with total production cost less than 1.0 US$, gave a maximum power output of $16.8\,W\,m^{-3}$, while treating synthetic wastewater, and demonstrated competitive performance compared to MFCs incorporating polymer membranes and expensive cathode catalysts.

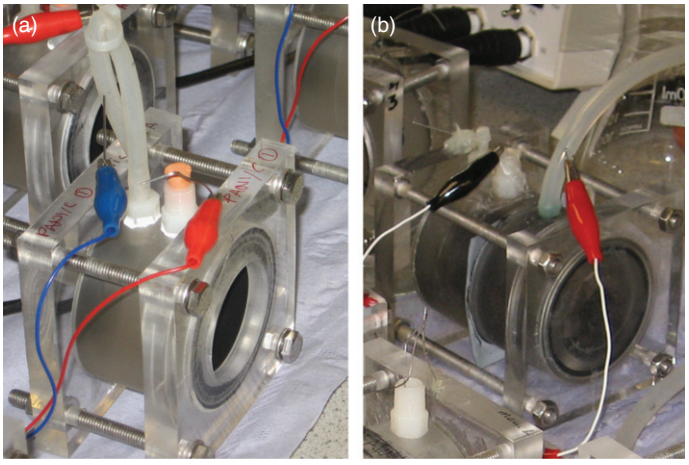

Figure 12 (a) Microbial fuel cell (MFC) with aqueous cathode, (b) MFC with aqueous cathode.

4.11.3.6.5 Inoculum in MFCs

Hydrolysis of complex polymers (transformation of complex polymers into substance that can be readily biodegraded by microbial consortium [195]) by hydrolytic organisms is the first and one of the most important steps in the bioconversion of organic waste. Despite the hydrolytic capabilities of many anaerobic bacteria by secretion of exocellular enzymes or attachment of the bacteria to the solid substrate, this step is considered to be the most rate-limiting in the fermentation of organic matter and is also usually yield-limiting in the biological conversion processes [196]. The lower efficiencies of anaerobic digestion are, in practice a result of the rigidly structured, slowly biodegradable compounds (e.g., plant waste lignocelluloses) in mixed waste streams. Efficiencies higher than 80% can be reached with high-quality biomass such as cellulolytic crops or carbohydrate-rich wastewaters from the food industry.

Pure cultures have frequently been used as inoculum of the MFC. Facultative anaerobic bacteria, *Aeromonas hydrophila* and *Enterobacter aerogenes*, were reported to be electrochemically active in the anode chamber of an MFC [124, 197]. The facultative anaerobe grows rapidly under aerobic conditions, consuming oxygen to oxidize organic substrates, and also grow under anaerobic conditions, degrading substrates into hydrogen and a residue. Due to the property of H_2 production and O_2 consumption, they are very suitable for an MFC [124]. *Ochrobactrum anthropi* YZ-1 has also demonstrated the ability to produce current using a wide range of substrates, including acetate, lactate, propionate, butyrate, glucose, sucrose, cellulose, glycerol, and ethanol [198]. A *Klebsiella pneumoniae* strain L17 biofilm also degraded starch and glucose to generate electricity [199] and *K. pneumoniae* biofilm cells showed DET from fuels to electrode.

Bacteria of the genus *Shewanella* are known for the diversity of terminal electron acceptors they can reduce and are one of the primary families of bacteria used in MFCs [200]. *Shewanella oneidensis* (originally known as *Shewanella putrefaciens*) is a nonfermenting, motile (self-propelled motion under appropriate circumstances), facultative anaerobic bacterium found in suboxic sediments (region where concentrations of oxygen is extremely low) [201]. *Shewanella oneidensis* MR-1 is the wild-type strain while *S. oneidensis* DSP10 is a spontaneous rifampin-resistant (a bactericidal antibiotic drug resistant) mutant more recently used in MFCs [202]. *S. oneidensis* grown with glucose in the presence of oxygen generates more power than under strictly anaerobic conditions, where the elimination of oxygen should typically increase the fuel cell efficiency and increase power output [203]. An increase in power with oxygen exposure is an indication that aerobic *S. oneidensis* can effectively utilize complex carbon sources as electron donors in MFCs.

G. sulfurreducens were reported to give higher power than when mixed anaerobic sludge is used as inoculum [143]. Phototrophic (the organisms that carry out photosynthesis to acquire energy) purple nonsulfur bacterium (*Rhodopseudomonas palustris* DX-1) can efficiently generate electricity by DET in MFCs using a wide range of substrates (volatile acids, yeast extract, and thiosulfate) making it another useful culture for high power generation (2720 $mW\,m^{-2}$) compared to mixed culture MFCs [204]. *Acidiphilium* sp. strain 3.2 Sup 5 cells, isolated from an extreme acidic environment, were reported to produce high currents, up to 3 $A\,m^{-2}$, by oxidizing glucose even with solution saturated in air and at very low pH [205]. Identification of such strains will be useful in the MFC for generation of higher current density as such strains are unaffected by the presence of oxygen, which will help in solving the problem of O_2 diffusion from the cathode and for developing MFC without membranes.

Metal-reducing bacteria *Rhodoferax ferrireducens* have been shown to play an important role in the anaerobic environment with sugars and that microbial electricity generation was attributed to the electrochemical and biological active cells attached to the electrode. Planktonic, that is, the cells grown in liquid suspension rather than attached to the electrode surface, cells showed limited/no ability to catalyze electricity generation [206, 207].

The use of mixed cultures can develop higher current in MFCs due to wide acceptance of different forms of organic matter present in the real wastewaters as a substrate. Recent studies have shown that the MFC inoculated with mixed anaerobic sludge can also generate current densities comparable with selected pure cultures [186]. Domestic wastewater can also be used as an inoculum [170]. Heat treatment was reported to be effective for pretreatment of the inoculum to enhance power production in MFC [191]. Mild ultrasonication pretreatment to the mixed anaerobic sludge, used as inoculum, is also reported to be effective in improving MFC performance, and the performance reported was 2.5 times higher than that obtained without any pretreatment of the mixed anaerobic sludge [208].

Pretreatment of sludge is particularly important for suppressing the methanogens, the group of bacteria responsible for production of methane, present in the mixed anaerobic culture. Electrogenic bacteria have the ability to outcompete methanogens when nonfermentable substrate is used. However, typically when a fermentable substrate is used in MFCs, that is, in the case of real wastewater treatment, methane formation is reported in the MFC during longer operation times [209, 210]. Hence, a strategy is needed to suppress the methanogens during inoculation and also intermittently during reactor operation.

4.11.3.7 Microbial Electrolysis

An important spin-off from MFC research has been hydrogen production by 'microbial electrolysis cells' (MEC), which is particularly interesting because of the considerable international effort directed toward hydrogen's use as an energy carrier. Microbial electrolysis is effectively a biological analogue to chemical electrolysis in the same way that an MFC is a biological analogue to a chemical fuel cell. In MECs an organic substrate is oxidized microbially to generate protons that transfer to the cathode to be reduced to hydrogen gas (see **Figure 13**). In a MEC, the evolution of hydrogen at the cathode is the same as for traditional water electrolysis:

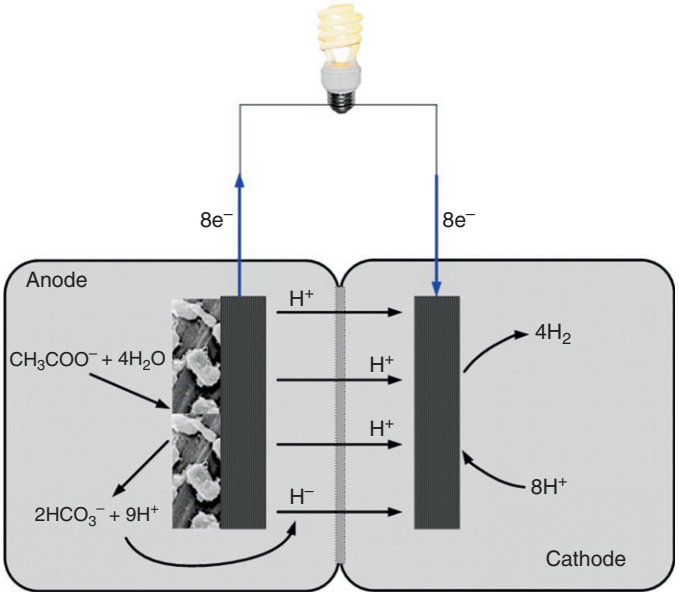

Figure 13 Schematic diagram of an MFC for wastewater treatment producing hydrogen.

$$2H^+ + 2e- \rightarrow H_2 \quad [11]$$

while, at the anode, the oxidation of water is replaced by the oxidation of organic compounds, for example, acetate, which is converted to bicarbonate:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e- \quad [12]$$

Comparing the thermodynamic equilibrium of this system, 0.236 V (at p. 7.0), to the 1.23 V required for water electrolysis indicates the promising potential of MEC technology. MEC technology has progressed rapidly in only a few years and production rates as high as 3.12 m^3 of H_2 m^{-3} day^{-1} have been reported [210, 211].

Overall the attractions of MECs are the very low energy requirements to produce hydrogen, for most substrates, and the ability to fully mineralize substrates to carbon dioxide, unlike most chemical electrolysis analogues. Furthermore, the selection of MECs over MFCs can be justified if hydrogen rather than electrical energy is required, but some technical reasons are equally compelling. Engineering cells for gas evolution is simpler and therefore less costly than for oxygen reduction; oxygen (air) reduction must overcome the mass transport limitations in gas diffusion electrodes as well as kinetic and catalytic limitations.

Two different functional MECs have been reported: first, using simple separators isolating anode and cathode solutions and second, a membraneless cell used to liberate hydrogen from a gas electrode [212, 213]. Using a substrate such as acetate is attractive as it offers a lower cost of operation in terms of energy and has considerable relevance to waste treatment. However, other more energetic substrates could deliver higher rates of hydrogen generation with associated lower capital cost per m^3 of hydrogen. Conventional electrolysis uses cell voltages of around 1.8 V and high current densities; orders of magnitude higher than in MECs, which dictates the energy cost. Thus, for MECs to compete with water electrolysis requires a major reduction in capital cost coupled to low-operating costs, which means operating at low cell voltages with low-cost cell design and materials. Thus, the vast majority of MEC research, which has demonstrated the concept, is not practical because of the use of high-cost materials such as Pt catalysts, gas diffusion electrodes, and Nafion membranes.

4.11.4 Conclusions

Rapid development on enzymatic biofuel cells has been achieved in the past decade. Much of the research has focused on establishing efficient electronic communications and interactions between enzyme and electrode using various approaches. With the demands for reliable power for medical devices for implantable applications, enzymatic biofuel cells have shown particular advantages over conventional energy devices because of the specific activity available from enzymes and the capability of miniaturization. In addition to medical applications, enzymatic biofuel cells can use renewable fuels with high energy density and safety for microelectronics.

However, there are great challenges for further advances in the technology. The most significant issues to achieve increased power output from biofuel cells include long-term stability of the enzyme electrodes; efficient electron transfer between enzymes and electrode surfaces; and improved enzyme biocatalytic activity. These are the main objectives for the next-generation enzyme

electrodes for biofuel cells. In order to meet the challenges and achieve these goals, following areas are essential for pushing forward the technology for practical applications and commercialization:

1. Protein engineering of native enzyme molecules with desired properties tailored for specific applications.
2. New immobilization methods and biomaterials to improve the stability of enzymes.
3. Nanomaterials integrated in the enzyme electrode structure to improve the electron transfer and enzyme catalytic activity.
4. Novel fuel cell design configurations to improve the cell voltage and power output.

The development of MFCs is still in its infancy with the need for considerable improvements in power output from accessible substrates. Thus, most MFCs still require their fuel to be of low molecular nature. Abundant energy, stored primarily in the form of carbohydrates, can be found in waste biomass from agricultural, municipal, and industrial sources as well as in dedicated energy crops, such as corn and other grains. If a glucose molecule were to be completely oxidized to CO_2, there are potentially 24 electrons available, but there is no direct simple chemical method to harvest this process. Thus, exploitation of microorganisms that contain a range of enzymes to facilitate this transformation is of importance.

MFCs represent a promising technology for renewable energy production; their most likely near-term applications are as a method of simultaneous wastewater treatment and electricity production. They will be useful in other specialized applications as well – for example, as power sources for environmental sensors and environmental bioremediation. With modifications, MFC technologies could find applications ranging from hydrogen production to renewable energy production from biomass. Around 2 billion people worldwide do not have adequate sanitation, and a treatment system based on MFCs provides an opportunity to develop the technology, because the substrate is 'free' and wastewater must be treated.

Successful development of biofuel cell technology relies on the joint efforts from different disciplines: biology to understand biomolecules; chemistry for knowledge on biochemical reactions and electron transfer mechanisms; material science to develop novel materials with high biocompatibility and maintain activity from biomolecues; and chemical engineering to design and establish the system. This innovative technology will encourage energy production from renewable sources and will have major impacts and benefits for medical science, clinical research, and health care management. The cost of materials used to construct MFCs will be a key factor for the successful application of the technology at large scales.

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