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Chapter 4

Converting Low-grade Biomass to Produce Energy Using Bio-fuel Cells

Meng Tong¹, Zhuwei Du² and Tingyue Gu³

1 National Analysis and Testing Centre for Iron and Steel, China Iron & Steel Research Institute Group, Beijing 100081, People's Republic of China

2 National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, People's Republic of China

3 Department of Chemical and Biomolecular Engineering, Ohio University, Athens, Ohio 45701, USA

Abstract

Aerobic and anaerobic respiration of microorganisms involves redox reactions that provide energy for cell growth or maintenance. The energy comes from breaking up chemical bonds during the oxidation of organic carbons by the microorganisms. The electrons released from the oxidation are taken up by the reduction of an oxidant such oxygen, sulfate, nitrate, etc. If the oxidation and reduction reactions occur at the same place, for example, the cytoplasm of microbial cells, no electricity is produced. The energy produced will be used for cell growth or maintenance. The rest will be released as low-grade heat that cannot be harvested cost-effectively. Electrochemically, digestion of organic carbons can be split into anodic (organic carbon oxidation) and cathodic (proton reduction) reactions to produce an electric current that can be harvested when electrons from the oxidation reaction is donated to the anode and flow through an external circuit before be utilized by the reduction reaction at the cathode. Bio-fuel cells are classified into two different categories. One is the so-called microbial fuel cell (MFC) that relies on a microbial biofilm to provide enzyme catalysis to the anodic reaction while the other utilizes a cell-free enzyme system for catalysis. The recent energy crisis and concerns over global warming have reinvigorated interests in

bio-fuel cells because their potential applications in electricity generation and biohydrogen production from renewable sources that are often low-cost or zero-cost wastes. Currently, the bottleneck of real-world applications of bio-fuel cells in harnessing energy lies in their low power density and high costs, thus limiting their uses to powering small sensors or devices that require very little power. Numerous interesting and innovative approaches have been reported to increase the performances and to reduce reactor construction and operating costs of bio-fuel cells. Although significant hurdles remain ahead, new progresses are making bio-fuel cells closer to eventual practical applications utilizing low-grade biomass. This book chapter reviews various recent advances in bio-fuel cell research using various biomass feed stocks.

4.1 Introduction

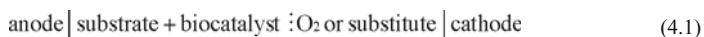
The conflict between the rapid growth in global energy consumption and the depletion of the traditional non-renewable fossil fuels has drawn much academic and industrial research efforts to find alternate renewable methods of providing power from biomass, wind and photovoltaic sources that is collectively known as green energy. Bio-fuel cells, as a suborder of fuel cells, have a special niche due to their efficiency and ability to sustain consistent power production by consumption of renewable biomass sources that are often considered worthless wastes. Microbial fuel cells (MFCs) and enzymatic fuel cells (EFCs) are two main types of bio-fuel cells reported in the literature^[1–3]. The former employ whole microorganisms as biocatalysts and the latter accomplish electrochemical energy conversion via enzymes isolated from them. The parallel uses of both types of catalysts are natural extensions of immobilized-cell and immobilized enzyme catalysis in non-electrochemical processes. The development of bio-fuel cells started in 1910 with an English botanist Potter who obtained a small current (0.2 mA) from *Escherichia coli* and *Saccharomyces* cultures using platinum electrodes^[4]. In the early 1960s the initial boom in space research ushered in a general interest in bio-energy. EFCs were first developed by Kimble and coworkers in 1964. They constructed three different bio-fuel cells using glucose oxidase, amino acid oxidase, alcohol dehydrogenase enzymes, respectively in the anodic chamber and then compared the their performances^[5]. However, research of bio-fuel cells declined after 1965 as a result of Lewis and Austin's less-than-optimistic conclusion that up to that time no successful biochemical fuel cell applications had been demonstrated^[6]. In recent years, the interest in bio-fuel cells was rekindled by a growing awareness of a shortage in the world oil supplies and the need for energy diversification including expanded use of green energy. Researchers were

encouraged by the demonstrated ability of electron mediators in enhancing bio-fuel cell's performance in the late 1970s and the early 1980s^[6]. And breakthroughs in MFCs and EFCs were both made in the late 1990s when researchers observed that some microbes were able to transfer electrons directly to the anode^[7, 8] and an enzymatic cascade consisting of alcohol dehydrogenase, formaldehyde dehydrogenase, and formate dehydrogenase was used to convert methanol to carbon dioxide^[9]. Today, bio-fuel cell research has become a promising research area bridging several disciplines that may contribute to the overall energy supply technology strategy of the future.

The relative advantages and disadvantages of whole-cell catalysts vs. enzymes are continually debated. Whole organisms are very attractive as multi-enzyme reactors that are capable of completing oxidation of a wide variety of fuels. And the regeneration ability of the living biocatalysts makes MFCs stable over long period of operation^[10]. Continuous running of MFCs over five years has been reported^[11]. But the limitation on the use of whole microorganisms in bio-fuel cells still comes from the barrier to electron transport which is presented by the cell walls and membranes of microorganisms. Hence they have low power densities in W/cm^2 ^[5]. By contrast, Enzymatic fuel cells possess orders of magnitude higher power densities. However, limitations including incomplete oxidation of fuels and short lifetimes due to enzyme loss and inactivation plague these systems^[10]. Because only a limited number of enzymes are employed, a feed that contains a large array of organic carbons from simple volatile fatty acids such as acetate to complicated polysaccharides such as cellulose in wastewater cannot possibly be processed by an EFC. In the following sections, the recent progresses in bio-fuel cells research using various biomass sources are discussed.

4.2 Theoretical principles of bio-fuel cells

From the electrochemical point of view, an electrochemical reaction consists of two half reactions: an oxidation reaction at the anode and a reduction reaction at the cathode. This split is necessary to prevent the direct oxidation of the fuel substrates. Biocatalyst can be used to accelerate either the anodic or the cathodic reaction, or both. Accelerating anodic reaction has attracted greater attention, because degradation of biomass in the anodic chamber is thermodynamically favorable but kinetically retarded without biocatalysis. Because this work deals with bio-fuel cells with a bioanode, the bio-fuel cell may be formally represented as

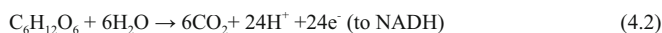


Bio-fuel cells can use biocatalysts (either enzymes or whole cell organisms) in two different ways^[6]: ① Indirect bio-fuel cells, in which the biocatalysts, by

biocatalytic transformation or a metabolic process, convert the fuel substrates to a secondary fuel utilized at the electrode. For example, *Clostridium butyricum* yields hydrogen and formic acid as the secondary fuel that can be oxidized to carbon dioxide on the electrode surface in the anodic compartment and oxygen is reduced on the electrode surface in the cathodic compartment^[12,13]. Other microorganisms such as *Desulfovibrio desulfuricans* were reported to yield sulfide ions that in turn were oxidized at the anode^[14, 15]. ② Direct bio-fuel cells^[7, 8], employ biocatalysts that are either microorganisms or redox-enzymes to facilitate the electron transfer chain between substrates and electrode surfaces.

EFCs are similar to chemical fuel cells especially polymer electrolyte fuel cells. Generally speaking, the system works with continuous recycling of the enzyme catalyst. If the enzyme acts with a cofactor, it is usually the reduced form of the cofactor that fuels the electrode reaction. Electrochemical coupling of the enzyme/co-enzyme system can be made more effective with the aid of an electron mediator.

In whole-cell microbial systems, i.e. MFCs, the cofactor resides in the cell cytoplasm. During normal bacterial metabolism, the substrate undergoes degradation via an oxidative process and supplies energy to drive biochemical reactions of assimilation or for cell maintenance. Electrons extracted from the substrate (or from secondary products) are transferred to cofactors such as FAD (flavin adenine dinucleotide), NAD(P)H (nicotinamide adenine dinucleotide, or its phosphorylated derivative), PQQ (pyrroloquinoline quinone)^[6], etc. Reaction (4.2) shows the case for metabolism of glucose.



Consumption of H^+ occurs in a reaction with oxygen at a terminal cytochrome site of the respiratory chain:



Reactions (4.2) and (4.3) together give the overall biochemical reaction for aerobic respiration (Reaction (4.4)) with ATP production, which stores energy within the microorganism.



It is generally acknowledged that the terminal reactions described by Reactions (4.2) and (4.3) in the process of biological energy generation take place at separate electron-donor and electron-acceptor sites in microorganisms. In MFCs the electrodes, locating in different compartments, are analogous to the electron-donor and electron-acceptor in microorganisms. Anode and cathode are at opposite ends of the respiratory chain of the microorganisms and reactions like Reactions (4.2)

and (4.3) occur at the electrode surfaces. Under normal conditions a respiring organism derives energy as electrical charge flows through the respiratory chain to eventually reduce oxygen at a cytochrome center, but in the anaerobic environment of an MFC's anodic chamber. The electrons released by the organic carbon oxidation reaction are donated to the anode. They flow through an external to drive a load before returning to the cathode to participate in the reduction reaction.

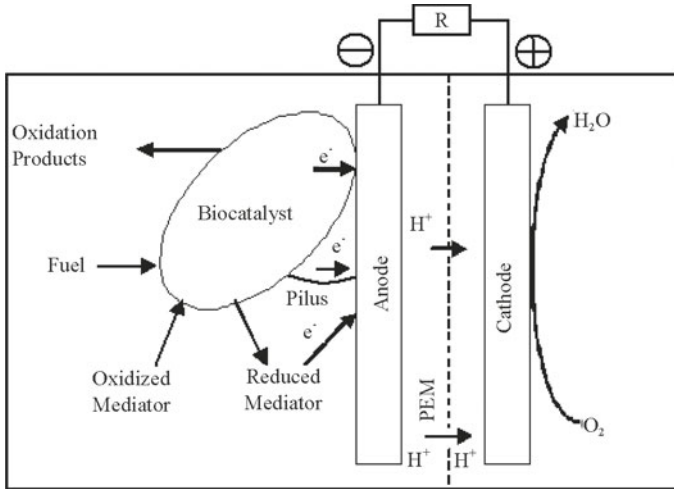


Figure 4.1 Schematic of a typical bio-fuel cell producing electricity

Figure 4.1 illustrates how a bio-fuel cell produces electricity from a substrate (fuel). Three electron transfer routes are depicted although only one or two may be present in an actual system. The anodic and cathodic chambers are partitioned by a proton exchange membrane (PEM) that allows proton diffusion to maintain electro-neutrality while preventing oxygen diffusion^[16, 17]. Biodegradation of organics is split into anodic (organic carbon oxidation) and cathodic (oxygen reduction) reactions to produce an electric current that can be harvested. Bio-catalyzed oxidation occurs at the anode and oxygen reduction at the cathode in a bio-fuel cell. As a renewable biomass that is derived originally from the ecosystem via photosynthesis approaches the anodic surface, it is electrochemically oxidized and this generates protons that enter the electrolyte and migrate toward the cathode, where oxygen is reduced to water. Inside a bio-fuel cell, the electricity flows from the anode to the cathode in the form of proton migration, while externally, the electricity flow is represented by the flow of electrons from anode to cathode. The essential feature of the bio-fuel cell is that the electron source of the bio-system is connected to the electrode via a suitable electron-transfer pathway, with

a current flow through the external circuit providing electric energy. Figure 4.1 shows direct electron transfer and mediator electron transfer. Pili grown by microbes are conductive nanowires that also transfer electrons between cells and an anode.

In addition, examples have been reported to exploit biocatalysis by micro-organismism for renewable H₂ production^[18,19]. Photoelectrocatalytic H₂ production and electroanalytic H₂ production are the two feasible routes, with the former successfully demonstrated in enzymatic fuel cells^[20, 21]. In EFCs, H⁺ reduction enzyme should be used at cathode in most cases. NAD⁺-dependent – glucose-dehydrogenase (anode) / NADH-dependent - hydrogenase (cathode) and NAD⁺-dependent-glucose-dehydrogenase / Mg-chlorophyll - a are examples of biocatalysis couples for H₂ production from glucose^[22]. Besides, the [FeFe]-hydrogenase from *Clostridium acetobutylicum* is a very good catalyst for H⁺ reduction^[23]. Microbial electrolysis cells (MECs) that are a modified version of MFCs can be used to produce high purity hydrogen gas in the cathodic chamber. Electrons donated by the anode from oxidizing an organic substrate in the anodic chamber flows through an external circuit to the cathode where they reduce the protons that diffused through a PEM from the anodic chamber to the cathodic chamber to produce hydrogen. An externally applied voltage is needed to make the reactions thermodynamically favorable. The hydrogen evolution reaction $2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$ in biological systems requires a voltage of -0.41V , while the microbial biofilm on the anodic surface degrades a organic substrate that typically has a reduction potential around -0.30V because the reduction potentials for $2\text{CO}_2/\text{acetate}$, NAD^+/NADH , FAD/FADH are -0.29 , -0.32 and -0.28V , respectively^[21, 24]. Thus, theoretically, an external voltage of roughly only 0.1V is needed to make renewable H₂ production thermodynamically feasible in a MEC. Compared to the 1.2V required for direct electrolysis of water at neutral pH, 0.1V is much lower because the anodic degradation of the organic substrate produces energy. Another advantage for hydrogen production using MECs is that MECs can reach a yield of about $8\sim 9$ mol H₂/mol glucose compared to the typical 4 mol H₂/mol glucose yield from conventional fermentation^[25]. Because the cathodic chamber is anaerobic during biohydrogen using MECs, oxygen leak to the anodic chamber is no longer an issue, and this enhances MEC efficiency. MECs produce higher purity hydrogen gas with reduced separation costs because the cathodic chamber does not involve complicated gas mixtures that are in the fermentation off gas. Hydrogen produced from MECs can be accumulated and then used to drive larger loads that cannot be driven by an online MFC due to its low power output. Some researchers foresee that biohydrogen produced from organic wastes from MECs can contribute to a future hydrogen economy^[26].

4.3 Designs for scaling up

Up to now, many miniature configurations for implantable applications or powering small sensors of devices have been reported^[27, 28]. The focus here is on the designs suitable for scale-up that are necessary for bio-fuel cells to become a practicable energy supply alternative. Two-compartment bio-fuel cells are more difficult and costly (e.g., high cost of the membrane) to scale up due to their complex designs. The simple one-compartment design of bio-fuel cells as shown in Figure 4.2 offers cost savings. The common point for one-compartment MFC design is to expose the cathode directly to the air. However, the PEM are still necessary to keep the anode anaerobic. Single chamber EFCs can follow the design as that of MFCs. In addition, if proper enzymes with good selectivity and stabilities are employed, the anode and the cathode of EFCs can be positioned in one chamber as in membrane-less EFCs^[29]. A membrane-less up-flow mode MFC is especially suitable for scaling up in wastewater treatment. Figure 4.3 shows an example. The continuous feed stream is fed from the bottom where the anode is. The stream flows upward and exits at the top where the cathode is^[30]. There are no separate anolyte and catholyte solutions. The distance between the anode and cathode presents a diffusion barrier that forms an oxygen (DO) gradient to avoid direct oxidation of organic carbon at the anode. Upward flow is better than horizontal or downward flow in prevent oxygen from entering the anode area. The convective flow from anode to cathode allows fast proton migration, thus greatly reduces internal resistance. Membrane-less is gaining popularity in MFC designs.

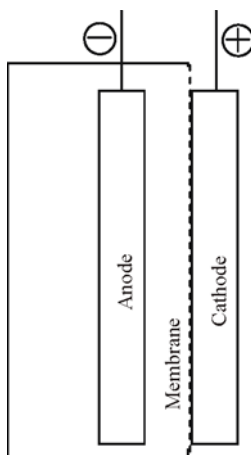


Figure 4.2 Schematic of a bio-fuel cell with the cathode directly exposed to the air

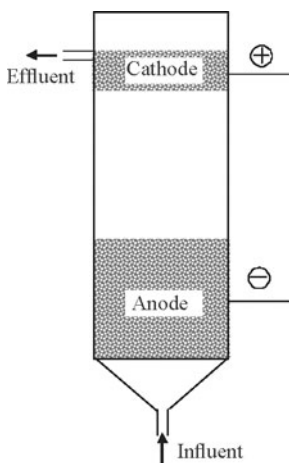


Figure 4.3 Schematic diagram of membrane-less up-flow mode MFC

4.4 Materials in bio-fuel cells

4.4.1 Fuels

Many organic chemicals can serve as both energy-sources and starting materials or intermediates for chemical syntheses. These fuels deserve special considerations because they are the forms in which energy can be stored economically for very long periods^[31] and some of them are present in organic wastes. The versatilities of the biocatalysts used in bio-fuel cells make it possible for bio-fuel cells to utilize a large variety of substrates from simple compounds to complicated mixtures of organic matters in wastewater. MFCs especially those using mixed culture biofilm consortia, compared with EFCs, have much a stronger capacity in utilizing complex low-grade biomass, offering the possibility of generating electricity from a wide range of substrates. Furthermore, substrates can be thoroughly oxidized to form CO_2 and H_2O in MFCs. It is difficult to compare substrate influence on bio-fuel cell performances, due to different cell configures, operating conditions, and different electrode materials and biocatalysts involved. Generally, low-grade biomass in wastewater has relatively low performance, but they are cheaper and often come without cost. Some popular substrates and their basic features are discussed below.

1. Pure compounds

Glucose is the most commonly used substrate for research in bio-fuel cells,

especially in EFCs^[32–34]. It is abundant in nature as a common source of carbon and energy source for microbial metabolism of many species. Its important metabolic intermediate is aldohexose carbohydrate involved in the glycolysis metabolic pathway, in which it is oxidized to pyruvate before entering the citric acid cycle. Glucose eventually is oxidized to form CO₂ and water after a series of biochemical transformations with a release of energy using either enzyme catalysis or biofilm catalysis^[10, 35]. Other sugars including monosaccharides such as fructose and xylose, disaccharides such as lactose, cellobiose, maltose and sucrose, polysaccharides such as cellulose and starch^[35–37] can also be digested by bio-fuel cells. However, only fructose, lactose and cellobiose are commonly reported as fuels in EFCs^[37].

Organic acids are another fuel choice often for electricity generation in MFCs. Acetate is the end product of fermentation and methanogenesis for higher order carbon sources. MFCs fueled by acetate often achieve higher Coulombic efficiency than other microbial conversions at room temperature^[38]. Other organic acids include fumarate, cysteine, glucuronic acid, lactate, propionate and pyruvate^[36]. Aliphatic alcohols are often used in EFCs. Methanol, ethanol and glycerol are renewable fuels from biomass. They can also be potentially substrates in EFCs used in future distributed power systems^[37]. In comparison with the three alcohols, sugar alcohols such as arabitol, mannitol, ribitol, sorbitol, xylitol, galactitol have more complex molecular structures and they have been used in MFCs^[39]. Even some industrial chemicals, such as phenol, 1,2-dichloroethane, usually toxic to microorganisms are also possible substrates in MFCs, but they have lower power output^[40, 41].

2. Mixtures

Low-grade biomass containing a huge variety of organic compounds can fuel MFCs. Municipal wastewater, food processing wastewater, lignocellulosic biomass, landfill leachate and marine sediments have been tested as substrates in MFCs^[35, 36]. MFCs are attractive for wastewater treatment because they can reduce solids by 50 to 90% while potentially halve the energy needed in a conventional treatment process for aeration^[26, 30]. Mixed-culture biofilms are especially suitable for energy recovery from low-grade biomass. Up to 80% removal of COD (chemical oxygen demand)^[42, 43] and a Coulombic efficiency as high as 80% have been reported in the literature^[44].

4.4.2 Biocatalysts

Theoretically, any biochemical process that involves oxidation and reduction

can be potentially used in a bio-fuel cell if the two reactions can be split up. Therefore many different biocatalysts are used in bio-fuel cells for electricity production including single enzyme (with or without co-factors), enzyme cascades and whole cells. The significance of microbes is that they contain molecular assemblies capable of catalyzing reactions under moderate conditions with efficiencies rarely matched by synthetic systems^[31]. Bio-fuel cells attempt to make use of these properties either by employing whole cells or their enzymes. If a biocatalyst is capable of transferring electrons between the electrode and the biocatalyst, the process is termed Direct Electron Transfer (DET)^[1]. Otherwise, mediators aiding electron transportation from an biocatalyst to the electrode are needed in the bio-fuel cells and the process is called Mediated Electron Transfer (MET)^[1].

Among over 1000 redox enzymes, less than 10% can carry out DET^[45]. Catalytic centers are usually buried fairly deeply in the proteins, and achieving efficient electron transfer at an electrode is a challenge for EFCs^[37]. MFCs have a similar situation. Though theoretically almost all bacteria can be used for electricity generation, not many of them can directly transfer electrons to the anode. Mediators can help those that are incapable of DET^[35]. In the cyclic process of MET, mediators should react readily with the electron source and be electrochemically active at the electrode surface in a bio-fuel cell. In order not to lose energy in the MET step, the formal redox potential of the mediator should be close to that of the redox couple providing the reducing action^[6]. Recently, with the progress in immobilization technology mediators might again draw the attention of the researchers. Worth noting is that mediators of low solubility which were considered not meeting the criteria for practical mediators in early studies have advantages for designs using a mediator-modified electrode. Mediators can be secreted by anodophiles in a biofilm or by synergistic microbes in the same biofilm consortium. This is probably one reason why mixed-culture biofilms tend to perform better.

4.4.3 Enzymes in EFCs

Typical biocatalysts employed at EFC anodes are glucose oxidases and dehydrogenases which differ in their cofactors and physiological electron acceptors. Most glucose-oxidizing enzymes target the C1 hydroxyl group of glucose forming gluconolactone which spontaneously hydrolyses to gluconate^[5]. Oxidases using O₂ as the electron acceptor can be isolated from many microorganisms. For example, fungal glucose oxidases come from *Aspergillus niger* and *Penicillium amagasakiense*^[20]. The glucose oxidase from *A. niger* is widely used. It has a mean molecular mass of 160 kDa, and

an isoelectric point of about 4.2^[46]. Its redox co-factor FAD is buried in the protein resulting in a very sluggish DET with an electrode^[37]. Dehydrogenases, also widely used in enzymatic fuel cell applications, have three cofactors for bioelectrocatalysis including quinone, flavin, or NAD^[5]. Glucose dehydrogenase from *Acinetobacter calcoaceticus* is a quinoprotein-dehydrogenase that is a dimer with identical subunits (ca. 50 kDa). It can oxidize a wide range of aldose sugars to the lactones with the hydride carrier PQQ as the catalytic center. Some classes of enzymes that catalyze oxidation of alcohol functionalities in sugars also oxidize simple primary alcohols such as methanol and ethanol^[47]. Alcohol oxidizing enzymes are usually suitable as anode catalysts in membrane-less EFCs as they are not damaged by O₂^[20]. Table 4.1 lists the selected enzymes for typical substrate employed in EFCs together with their co-factors.

Table 4.1 Typical substrates and enzymes used in enzymatic bio-fuel cells^[5, 20, 37]

Enzyme	Co-factor	Substrate	Anodic Reaction
Glucose oxidase	FAD	glucose	glucose → glucono-1,5-lactone + 2H ⁺ + 2e ⁻
Glucose dehydrogenase	PQQ	glucose	glucose → glucono-1,5-lactone + 2H ⁺ + 2e ⁻
Glucose dehydrogenase	NAD(P)	glucose	glucose → glucono-1,5-lactone + 2H ⁺ + 2e ⁻
Alcohol dehydrogenase	NAD(P)	ethanol	ethanol → acetaldehyde + 2H ⁺ + 2e ⁻
Alcohol dehydrogenase	PQQ	ethanol	ethanol → acetaldehyde + 2H ⁺ + 2e ⁻
Methanol dehydrogenase	PQQ	methanol	methanol → formaldehyde + 2H ⁺ + 2e ⁻
Fructose dehydrogenase	FAD	fructose	fructose → 5-dehydrofructose + 2H ⁺ + 2e ⁻
Glycerol dehydrogenase	NAD	glycerol	glycerol → dihydroxyacetone + 2H ⁺ + 2e ⁻
Glycerol dehydrogenase	PQQ	glycerol	glycerol → dihydroxyacetone + 2H ⁺ + 2e ⁻

In anodic systems using a single enzyme catalyzed partial oxidation of the fuel limits the number of electrons recovered. The biocatalyzed reaction likely breaks only one chemical bond. However, for EFCs using enzyme cascades, the complete oxidation of the fuels to CO₂ can be achieved to harvest more electrons to increase the current density^[10].

4.4.4 Microbes used in MFCs

Many microorganisms can be used in MFCs to generate electricity as seen in Table 4.2. Not all of them have the ability to transfer the electrons from substrate oxidation to the anode directly. MET to the anode is possible via electroactive metabolites, such as anthraquinone, the oxyanions of sulphur (sulphate and thiosulphate) or with the addition of non-natural (chemical) redox mediators such as dyes^[48, 49]. Cell surface structures in microorganisms vary widely, but the main features for bacteria are an out-layer containing peptidoglycan, a chain-like disaccharide heteropolymer glycan with peptide substituents, and the cytoplasmic membrane consisting primarily of phospholipids. Lipid-bilayer membranes generally contain about 50% proteins with varied and specific catalytic/carrier functions, but about 30% of the phospholipid component may be relatively pure. The fluid properties are affected by the aqueous content, and the electrical resistance of the bilayer is 102 ~ 105 ohms^[6]. For many bacteria, the non-conductive cell surface hinders the direct electron transfer to the anode^[50]. Mediators are required for those microbes that are incapable of DET. Mediators shuttle between the anode and the microbe to transport electrons. In the anodic chamber, they take up the electrons from substrate oxidation by microbes and then release them at the anodic surface. *Actinobacillus succinogenes*^[51], *D. desulfuricans*^[15, 49], *E. coli*^[52], *Proteus mirabilis*^[53], *Proteus vulgaris*^[54] need extraneous mediators, but some microorganisms secrete their own mediators. For example, pyocyanin molecules secreted by *Pseudomonas aeruginosa*^[55] are electron shuttles.

DET from microbes to a typical carbon-based anode via extracellular cytochromes or conductive microbial *pili* (electrically conductive nanowires) have also been observed^[7, 8]. Generally dissimilatory metal reducing microorganisms are anodophiles. They produce biologically useful energy from the dissimilatory reduction of metal oxides anaerobically in soils and sediments. The electrons are transferred to terminal electron acceptors such as Fe₂O₃ through DET between the mineral oxides and the metal reducing microorganisms^[56, 57]. In a mediator-less MFC, metal reducing bacteria such as those from the families of *Shewanella*, *Rhodospirillum rubrum*, and *Geobacter* use the anode as terminal electron acceptor in the dissimilatory respiratory metabolism of the biofilm as if they were metal oxides. In lab experiments, these microbes yielded high Coulombic efficiencies^[8, 58]. *Shewanella putrefaciens*^[59], *Geobacteraceae sulfurreducens*^[60], *Geobacter metallireducens*^[61] and *Rhodospirillum rubrum ferrireducens*^[8] have all been found to be operationally stable because these bio-electrochemically active microbes readily form a biofilm on the anode surface and DET is carried out by conductance through the cell membrane. These microorganisms have been isolated from fresh water, wastewater and

marine sediments, as well as activated sludge^[62]. So far almost all of mediator-less MFCs used dissimilatory metal reducing microorganisms with the exception of *Clostridium butyricum*^[63, 64]. Although mediator-less MFCs can run without mediators because they anodophilic biofilms, anodes containing electron mediators such as Mn⁴⁺ or neutral red have shown enhancement of the performance of MFCs using anodophile *Shewanella. putrefaciens*^[65].

Table 4.2 Microbes used in MFCs

<i>Microbes</i>	Electron Transfer Mechanisms
<i>Actinobacillus succinogenes</i>	MET/Neutral red or thionin as electron mediator ^[51, 66]
<i>Aeromonas hydrophila</i>	DET/anodophile ^[67]
<i>Alcaligenes faecalis</i> ,	MET/Self-mediate ^[55]
<i>Enterococcus gallinarum</i>	MET/Self-mediate ^[55]
<i>Desulfovibrio desulfuricans</i>	MET/Sulphate-sulphide as mediator ^[49, 15]
<i>Erwinia dissolven</i>	MET/Ferric chelate complex as mediators ^[34]
<i>Escherichia coli</i>	MET/methylene blue as mediator ^[49, 52]
<i>Geobacter metallireducens</i>	DET/anodophile ^[61]
<i>Geobacter sulfurreducens</i>	DET/anodophile ^[60, 68]
<i>Gluconobacter oxydans</i>	MET/HNQ, resazurin or thionine as mediator ^[69]
<i>Klebsiella pneumoniae</i>	MET/HNQ as mediator ^[70, 71]
<i>Lactobacillus plantarum</i>	MET/Ferric chelate complex as mediators ^[34]
<i>Proteus mirabilis</i>	MET/Thionin as mediator ^[53]
<i>Proteus vulgaris</i>	MET/Thionin as mediator ^[54]
<i>Pseudomonas aeruginosa</i>	MET/Self-mediate ^[55]
<i>Rhodoferrax ferrireducens</i>	DET/anodophile ^[8]
<i>Shewanella oneidensis</i>	MET/AQDS as mediator ^[72]
<i>Shewanella putrefaciens</i>	DET/anodophile ^[7, 65]
<i>Streptococcus lactis</i>	MET/Ferric chelate complex as mediators ^[34]

Apart from pure cultures, MFCs can also use mixed culture biofilms such as those enriched from marine sediments and anaerobic sludge. These biofilms tend to have better stability and also performances due to microbial synergy. One example of synergy in an anodic biofilm consortium is that one microbe secretes mediators for the other microbe both will benefit bioenergetically from the enhanced degradation of organic matters in the anodic chamber. Because there are many microbes in a single biofilm, a much wider range of substrates can be digested. Electrophiles and anodophiles in a anodic biofilm consortium with the help of locally secreted mediators can work together to digest the various organic substrates present in a wastewater stream.

4.4.5 Oxidants

Dissolved oxygen is a widely used oxidant for the cathodic reaction in bio-fuel cells. In two-compartment bio-fuel cells, pure oxygen (in lab tests) or air can

be sparged through the catholyte solution. Due to the low solubility of oxygen in water, power output of a bio-fuel cell can be strongly affected by the dissolved oxygen level because the driving force for mass transfer of oxygen from the bulk solution to the cytoplasm can be insufficient^[37]. Single-chamber bio-fuel cells with an air-cathode rely on oxygen in the air to dissolve in the web liquid film covering the cathode. Other oxidants used in MFCs include hydrogen peroxide and potassium ferricyanide. These highly reactive oxidants are toxic and their practical applications will be very limited or impossible^[73]. Biocathodes using anaerobic microbes can use oxidants that are already present in wastewater streams. They include sulfate, nitrate and nitrite that are common in agricultural runoffs. MFCs with bioanodes and biocathodes can process one wastewater stream in a membrane-less MFC or two different streams in an MFC with its two chambers partitioned by a membrane.

4.4.6 Electrode materials

Though bio-fuel cells possess some attractive features, they are still not widely employed at all. One major bottleneck is the slow electron transfer between catalysts and the electrodes. New electrode materials including composite or alloyed electrodes can help solve this problem. Because the redox units are buried in the 3-D enzyme protein structures shielded by a thick carbohydrate shell, many biofilms undergo a much lower metabolic rate in MFCs than that of their normal state^[74]. Therefore, modification of electrodes is a key issue. Many metals are toxic toward biocatalysts. Pt and some other precious metals are an exception under anaerobic condition, but they are cost-prohibitive for any large-scale applications.

The anode in bio-fuel cells must facilitate electron transfer. Mediators tend to be very helpful in achieving electrical communication. However, involving mediators in the system also brings the problems associated with mediator diffusion or leaching. Their cost can also be prohibitive for practical applications. Immobilization of enzymes and mediators is a possible solution to increasing current densities, enhancing enzyme stability, and improving electron transfer kinetics^[75]. Immobilization techniques can be classified into four well known categories: physical adsorption, covalent binding, cross-linking and entrapment in polymeric materials. Typical enzymatic electrodes include architectures with or without electron transfer mediators. Crosslinking is a common and simple way for immobilization. However, activity reduction of the enzymes in the process of crosslinking is not acceptable for enzymatic bio-fuel cells, because of the present low catalytic activity of the enzyme^[5]. Figure 4.4 shows four typical anode assemblies.

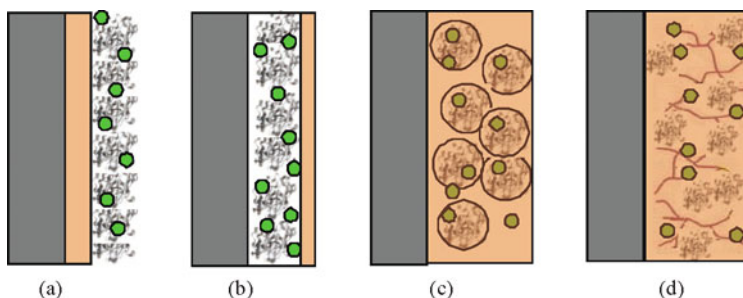


Figure 4.4 Typical anode assemblies (a) an initial matrix layer followed by enzyme and mediator adsorption or covalent. (b) an out-layer entrapping the enzyme. (c) the physical entrapment of an enzyme by microencapsulation. (d) immobilizing enzymes and mediators by physical entrapment or chemically binding in redox hydrogels (Figure redrawn after References [5] and [37] with modifications)

Compared with an enzymatic anode without a mediator, mediator based bioanodes are more commonly used in enzymatic fuel cells. For practical purposes, mediators should be immobilized to minimize mediator cost. Three-dimensional matrices like polymers, lipids and carbon nanotubes or their combinations are used to incorporate enzyme and mediators in various states^[76]. Adsorption and covalent binding are the two popular ways in fabrication of enzymatic electrode composites. Many systems have employed an initial matrix layer, followed by enzyme and mediator adsorption or covalent binding, but this simple method increases the distance from the enzyme catalytic site to the electrode surface. Entrapment by polymer coating, microencapsulation or gelation can entrap an enzyme in pores or matrices at the electrode surface^[5, 76]. Employing an out-layer to entrap the enzyme has the effect of adding an extra mass transfer barrier between fuel molecules and the catalytic sites of the enzyme in the system. Microencapsulation is the physical entrapment of an enzyme in pores or matrices of a membrane at the electrode surface. Modified Nafion without the destructive acidity and chitosan are suitable for this purpose^[5]. Immobilizing enzymes and mediators by physical entrapment or chemically binding in redox hydrogels has drawn much attention. The matrix provides a pathway for electrons to reach the redox centers attached to the polymer and can greatly enhance bio-fuel cell life expectancy and performance^[37]. Bio-fuel cells based on redox hydrogels have shown good characteristics for immobilization of GOx and other enzymes^[77, 78].

The first electrodes for MFCs were made from platinum^[4] but it was gradually substituted by other much less expensive materials. Nowadays, Graphite is commonly used for electrodes in MFC designs. There are also various types of relatively inexpensive carbon cloths and felts that have become commercially

available, and have proven very suitable for use in MFCs. They are cheaper and provide an electrode with a greater surface area. However, these materials are less electroactive than platinum, supporting lower exchange-current densities. Biofilm catalysis and mediators help to overcome this shortcoming. Large electrodes are feasible using such cheap materials. Non-porous graphite providing a large specific surface area as high as $1 \text{ m}^2/\text{g}$ has been reported^[6].

To improve electron transfer between the microorganisms and the electrode, electrode modification is actively being investigated to improve MFC performances. Graphite anode with immobilized mediators can greatly increase the current output compared to the graphite anode alone^[65, 79]. Anode modifications including immobilization of AQDS, NR or 1,4-naphthoquinone (NQ) and doping metal elements of Mn(II), Mn(IV), Ni(II), Fe(III) have shown improvements in electricity generation^[51, 66, 80]. The principle for their catalytic activity is the same as that of electron shuttles. Electrode modifiers of conductive polymers such as fluorinated polyanilines poly(2-fluoroaniline) and poly(2,3,5,6-tetrafluoroaniline) also serve as immobilized mediators thanks to their structural similarities to conventional redox mediators^[52, 81, 82]. From this point of view, proper electrode assemblies similar to enzymatic anodes may also be promising in anode design for MFCs. Cost will remain a factor in the success of any of the modifications.

4.4.7 Power output in bio-fuel cells

The electric power output from a bio-fuel cell depends on metabolic and electrochemical processes^[83]. The biological reactions in a bio-fuel cell are slower than the chemical reactions in a conventional chemical fuel cell. In a bio-fuel cell, the following factors influence the biological process: the amount of biocatalysts (microbial cell densities or concentrations of cell-free enzymes) and their bioactivities and reaction kinetics, the organic carbon loading rate and mass transfer^[83].

In the electrochemical process, a bio-fuel cell's output power P is the product of cell voltage V and the current I , i.e., $P=I \times V$. The open circuit potential that provides the maximum cell voltage (E_0) (usually between 750 to 800 mV) drops significantly when an external load is used to close the electrical loop. This is primarily because of overpotential, the potential losses owing to charge transfer resistance at the electrodes and internal resistance. A membrane-less bio-fuel cell has much smaller internal resistance, because convective flow of protons is possible. The cell configuration and operating conditions also impact the internal resistance significantly^[83–85]. The cell voltage V can be calculated using the following equation^[83]

$$V = E_0 - \eta_a - \eta_c - I \times R \quad (4.5)$$

in which η_a and η_c represent the overpotential losses at the anode and cathode, respectively, and R the overall internal cell resistance.

The output power densities of bio-fuel cells, especially MFCs, are much lower than that for chemical fuel cells. Chemical fuel cells can provide $10^6 \sim 10^7$ mW/m²[86–89]. The power densities of several chemical fuel cells and bio-fuel cells are listed in Table 4.3. Obviously, in order to use bio-fuel cells to generate electricity to power larger devices, more research is needed to improve both the chemical and electrochemical processes in bio-fuel cells. However, it is unrealistic and unfair to expect bio-fuel cells to become comparable with chemical fuel cells in terms of power densities simply because the fuels used by the former have much a lower energy density and biological reactions are slower than the chemical reactions in the latter. One distinct advantage for bio-fuel cells is obviously that they use low-grade energy sources, often wastes that are low cost or zero cost. In some cases, a source may even come with subsidy.

Table 4.3 Comparison of power densities of chemical fuel cells and bio-fuel cells

Fuel Cell Types	Power Density/ (mW/m ²)	References
Proton exchange membrane fuel cell (PEMFC)	2.5×10^7	[86]
Direct methanol fuel cell (DMFC)	2.1×10^6	[87]
Solid oxide fuel cell (SOFC)	8.8×10^6	[88]
Phosphoric acid fuel cell (PAFC)	5.6×10^6	[89]
Microbial fuel cell (MFC)	5.6×10^2	[90]
Microbial fuel cell (MFC)	2.63×10^2	[91]
Microbial fuel cell (MFC)	7.2×10^3	[92]
Microbial fuel cell (MFC)	4.31×10^3	[52]
Enzymatic bio-fuel cell (EFC)	9.3×10^3	[93]
Enzymatic bio-fuel cell (EFC)	1.32×10^4	[94]
Enzymatic bio-fuel cell (EFC)	4.6×10^3	[95]
Enzymatic bio-fuel cell (EFC)	2.5×10^3	[96]

4.5 Concluding remarks

In view of the progresses and the limited successes so far obtained for producing energy, bio-fuel cells appear to be competitive on the basis of Coulombic efficiency and the ability to use cheap low-grade wastes. However, the performances are still low on the basis of current or power density, especially for MFCs. Recent studies suggest that higher current and energy densities can be expected to accompany improvements in reactor design. Further improvements

in charge transfer and mass transfer are needed, so that the full reducing power of the biocatalysts can be delivered. “Super bugs” may be available in the future from wild-type selection, mutagenesis or even genetic engineering. Ideal super bugs can bind with an electrode tenaciously with a high surface density. They also exhibit hyperpilation that increases electron flow from a single cell to the anode and enable more than one layer of electron donating cells through massive networking by pili. Membrane-less bio-fuel cells are probably the only option for large-scale applications because of the high costs associated with membranes and their easy fouling.

Regarding the biocatalysts, a large variety of enzymes and microorganisms have been investigated by researchers, but a clear trend is to use enzyme cascades and mixed-culture microorganisms. EFCs yield higher power densities than MFCs. However, they are easily contaminated by microbes when they are fed complicated organic wastes such as wastewater because such wastes contain a large number of microbes. EFCs also lack the ability to digest the large array of organic carbons in a waste stream. Furthermore, EFCs cannot recover from the inevitable enzyme loss or attrition because it is not a living system. Regardless of these drawbacks, EFCs has an edge over MFCs in powering implanted medical devices, because EFCs can operate in a sterile environment. Because the demand for small-scale power sources and miniature devices will likely increase in the future, bio-fuel cells are promising in serving as distributed power systems for local uses and for powering miniature devices (especially remote sensors) for long term operations. Many underdeveloped regions in the world could make better use of readily available low-grade biomass. Bio-fuel cells offer alternatives to conventional routes of harnessing energy from various biomass. The potential for harnessing energy from bio-fuel cell systems should not be overlooked, but the full realization of this potential depends on socio-economic factors (such as energy costs and the push for green energy) as well as advances in scientific research and technological development. Relatively large-scale practical bio-fuel cells probably will first be practical in niche applications. One example is for wastewater treatment on remote US military forward operation bases such as those currently in Afghanistan, where there is a need for sustainable wastewater treatment processes with low energy input because the local fuel cost is 10 times than normal.

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