true fuel cells, however, as they are operated to produce hydrogen, not electricity. Through modifications of the MFC designs described above (to contain a second chamber for capturing the hydrogen gas), it should be possible to develop many new systems for hydrogen production.

Materials of Construction

Anode. Anodic materials must be conductive, biocompatible, and chemically stable in the reactor solution. Metal anodes consisting of noncorrosive stainless steel mesh can be utilized (*44*), but copper is not useful due to the toxicity of even trace copper ions to bacteria. The most versatile electrode material is carbon, available as compact graphite plates, rods, or granules, as fibrous material (felt, cloth, paper, fibers, foam), and as glassy carbon. There are numerous carbon suppliers worldwide, for example E_TEK and Electrosynthesis Co. Inc. (USA), GEE Graphite Limited, Dewsbury (UK), Morgan, Grimbergen (Belgium), and Alfa-Aesar (Germany).

The simplest materials for anode electrodes are graphite plates or rods as they are relatively inexpensive, easy to handle, and have a defined surface area. Much larger surface areas are achieved with graphite felt electrodes (*13, 45*) which can have high surface areas (0.47 m^2g^{-1}, GF series, GEE Graphite limited, Dewsbury, UK). However, not all the indicated surface area will necessarily be available to bacteria. Carbon fiber, paper, foam, and cloth (Toray) have been extensively used as electrodes. It has been shown that current increases with overall internal surface area in the order carbon felt > carbon foam > graphite (*46*). Substantially higher surface areas are achieved either by using a compact material like reticulated vitreous carbon (RVC; ERG Materials and Aerospace Corp., Oakland, CA) (*36, 47*) which is available with different pore sizes, or by using layers of packed carbon granules (Le Carbone, Grimbergen, Belgium) or beads (*35, 48*). In both cases maintaining high porosity is important to prevent clogging. The long term effect of biofilm growth or particles in the flow on any of the above surfaces has not been adequately examined.

To increase the anode performance, different chemical and physical strategies have been followed. Park et al. (*31*) incorporated Mn(IV) and Fe(III) and used covalently linked neutral red to mediate the electron transfer to the anode. Electrocatalytic materials such as polyanilins/Pt composites have also been shown to improve the current generation through assisting the direct oxidation of microbial metabolites (*49–51*).

Directing the water flow through the anode material can be used to increase power. Cheng et al. (*52*) found that flow directed through carbon cloth toward the anode, and decreasing electrode spacing from 2 to 1 cm, increased power densities (normalized to the cathode projected surface area) from 811 to 1540 mW/m^2 in an air-cathode MFC. The increase was thought to be due to restricted oxygen diffusion into the anode chamber, although the advective flow could have helped with proton transport toward the cathode as well. Increased power densities have been achieved using RVC in an upflow UASB type MFC (*36*) or in a granular anode reactor (*35*) with ferricyanide cathodes. Flow through an anode has also been used in reactors using exogenous mediators (*48*).

Cathode. Due to its good performance, ferricyanide ($K_3[Fe(CN)_6]$) is very popular as an experimental electron acceptor in microbial fuel cells (*31*). The greatest advantage of ferricyanide is the low overpotential using a plain carbon cathode, resulting in a cathode working potential close to its open circuit potential. The greatest disadvantage, however, is the insufficient reoxidation by oxygen, which requires the catholyte to be regularly replaced (*35*). In addition, the long term performance of the system can be affected by diffusion of ferricyanide across the CEM and into the anode chamber.

Oxygen is the most suitable electron acceptor for an MFC due to its high oxidation potential, availability, low cost (it is free), sustainability, and the lack of a chemical waste product (water is formed as the only endproduct). The choice of the cathode material greatly affects performance, and is varied based on application. For sediment fuel cells, plain graphite disk electrodes immersed in the seawater above the sediment have been used (*38*). Due to the very slow kinetics of the oxygen reduction at plain carbon, and the resulting large overpotential, the use of such cathodes restricts the use of this noncatalyzed material to systems that can tolerate low performance. In seawater, oxygen reduction on carbon cathodes has been shown to be microbially supported (*19, 20*). Such microbially assisted reduction has also been observed for stainless steel cathodes which rapidly reduces oxygen when aided by a bacterial biofilm (*53*).

To increase the rate of oxygen reduction, Pt catalysts are usually used for dissolved oxygen (*37*) or open-air (gas diffusion) cathodes (*34, 48*). To decrease the costs for the MFC the Pt load can be kept as low as 0.1 mg cm^{-2} (*54*). The long term stability of Pt needs to be more fully investigated, and there remains a need for new types of inexpensive catalysts. Recently, noble-metal free catalysts that use pyrolyzed iron(II) phthalocyanine or CoTMPP have been proposed as MFC cathodes (*54, 55*).

Membrane. The majority of MFC designs require the separation of the anode and the cathode compartments by a CEM. Exceptions are naturally separated systems such as sediment MFCs (*37*) or specially designed single-compartment MFCs (*30, 32*). The most commonly used CEM is Nafion (Dupont Co., USA), which is available from numerous suppliers (e.g., Aldrich and Ion Power, Inc.). Alternatives to Nafion, such as Ultrex CMI-7000 (Membranes International Incorp., Glen Rock, NJ) also are well suited for MFC applications (*6*) and are considerably more cost-effective than Nafion. When a CEM is used in an MFC, it is important to recognize that it may be permeable to chemicals such as oxygen, ferricyanide, other ions, or organic matter used as the substrate. The market for ion exchange membranes is constantly growing, and more systematic studies are necessary to evaluate the effect of the membrane on performance and long-term stability (*56*).

Fundamentals of Voltage Generation in MFCs

Thermodynamics and the Electromotive Force. Electricity is generated in an MFC only if the overall reaction is thermodynamically favorable. The reaction can be evaluated in terms of Gibbs free energy expressed in units of Joules (J), which is a measure of the maximal work that can be derived from the reaction (*57, 58*), calculated as

$$\Delta G_r = \Delta G_r^0 + RT\ln(\Pi) \qquad (1)$$

where ΔG_r (J) is the Gibbs free energy for the specific conditions, ΔG_r^0 (J) is the Gibbs free energy under standard conditions usually defined as 298.15 K, 1 bar pressure, and 1 M concentration for all species, R (8.31447 J mol^{-1} K^{-1}) is the universal gas constant, T(K) is the absolute temperature, and Π (unitless) is the reaction quotient calculated as the activities of the products divided by those of the reactants. The standard reaction Gibbs free energy is calculated from tabulated energies of formation for organic compounds in water, available from many sources (*59–61*).

For MFC calculations, it is more convenient to evaluate the reaction in terms of the overall cell electromotive force (emf), E_{emf} (V), defined as the potential difference between the cathode and anode. This is related to the work, W (J), produced by the cell, or

$$W = E_{emf}Q = -\Delta G_r \qquad (2)$$

TABLE 1. Standard Potentials E_0 and Theoretical Potentials for Typical Conditions in MFCs E_{MFC} (E_{MFC} Was Calculated Using Eq 5 and Half Cell Values from Ref *57*; All Potentials Are Shown against NHE)

electrode	reaction	E_0 (V)	conditions	E_{MFC} (V)
anode	$2\ HCO_3^- + 9\ H^+ + 8\ e^- \rightarrow CH_3COO^- + 4\ H_2O$	0.187[a]	HCO_3^-=5 mM, CH_3COO^-=5 mM, pH=7	−0.296[b]
cathode	$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	1.229	pO_2=0.2, pH=7	0.805[b]
	$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	1.229	pO_2=0.2, pH=10	0.627
	$MnO_2(s) + 4\ H^+ + 2\ e^- \rightarrow Mn^{2+} + 2\ H_2O$	1.23	$[Mn^{2+}]$=5 mM, pH=7	0.470
	$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$	0.695	pO_2=0.2, $[H_2O_2]$ = 5 mM, pH=7	0.328
	$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$	0.361	$[Fe(CN)_6^{3-}]$=[$Fe(CN)_6^{4-}$]	0.361

[a] Calculated from Gibbs free energy data tabulated in ref *61*. [b] Note that an MFC with an acetate oxidizing anode (HCO_3^- = 5 mM, CH_3COO^- = 5 mM, pH = 7) and an oxygen reducing cathode (pO_2 = 0.2, pH = 7) has a cell emf of 0.805 − 0.296= 1.101 V.

where $Q = nF$ is the charge transferred in the reaction, expressed in Coulomb (C), which is determined by the number of electrons exchanged in the reaction, n is the number of electrons per reaction mol, and F is Faraday's constant (9.64853 × 10^4 C/mol). Combining these two equations, we have

$$E_{emf} = -\frac{\Delta G_r}{nF} \qquad (3)$$

If all reactions are evaluated at standard conditions, $\Pi = 1$, then

$$E^0_{emf} = -\frac{\Delta G^0_r}{nF} \qquad (4)$$

where E^0_{emf} (V) is the standard cell electromotive force. We can therefore use the above equations to express the overall reaction in terms of the potentials as

$$E_{emf} = E^0_{emf} - \frac{RT}{nF}\ln(\Pi) \qquad (5)$$

The advantage of eq 5 is that it is positive for a favorable reaction, and directly produces a value of the emf for the reaction. This calculated emf provides an upper limit for the cell voltage; the actual potential derived from the MFC will be lower due to various potential losses (see below).

Standard Electrode Potentials. The reactions occurring in the MFC can be analyzed in terms of the half cell reactions, or the separate reactions occurring at the anode and the cathode. According to the IUPAC convention, standard potentials (at 298 K, 1 bar, 1 M) are reported as a reduction potential, i.e., the reaction is written as consuming electrons (*57*). For example, if acetate is oxidized by bacteria at the anode we write the reaction as

$$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O \qquad (6)$$

The standard potentials are reported relative to the normal hydrogen electrode (NHE), which has a potential of zero at standard conditions (298 K, pH_2 = 1 bar, $[H^+]$ = 1 M). To obtain the theoretical anode potential, E_{An}, under specific conditions, we use eq 5, with the activities of the different species assumed to be equal to their concentrations. For acetate oxidation (Table 1), we therefore have

$$E_{An} = E^0_{An} - \frac{RT}{8F}\ln\left(\frac{[CH_3COO^-]}{[HCO_3^-]^2[H^+]^9}\right) \qquad (7)$$

For the theoretical cathode potential, E_{cat}, if we consider the case where oxygen is used as the electron acceptor for the reaction, we can write

$$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O \qquad (8)$$

$$E_{cat} = E^0_{cat} - \frac{RT}{4F}\ln\left(\frac{1}{pO_2[H^+]^4}\right) \qquad (9)$$

A variety of catholytes has been used, and for each of these the cell voltage varies. For example, manganese oxide and ferricyanide have been used as alternatives to oxygen. The pH of the cathode solution can also vary, affecting the overall cathode potential. Using eq 9 and tabulated standard potentials available for inorganic compounds (*57*) for several different conditions, it can be seen that the theoretical cathode potential for these different catholytes range from 0.361 to 0.805 V.

The cell emf is calculated as

$$E_{emf} = E_{cat} - E_{an} \qquad (10)$$

where the minus sign is a result of the definition of the anode potential as reduction reaction (although an oxidation reaction is occurring). Note that the result using eq 10 equals that of eq 3 and eq 5 only if the pH at the anode and the cathode are equal. Equation 10 demonstrates that using the same anode in a system with different cathode conditions as listed in Table 1 would produce significantly different cell voltages, and thus different levels of power output. The power produced by an MFC therefore depends on the choice of the cathode, and this should be taken into account when comparing power densities achieved by different MFCs.

Open Circuit Voltage (OCV). The cell emf is a thermodynamic value that does not take into account internal losses. The open circuit voltage (OCV) is the cell voltage that can be measured after some time in the absence of current. Theoretically, the OCV should approach the cell emf. In practice, however, the OCV is substantially lower than the cell emf, due to various potential losses. For example, a typical measured potential of a cathode using oxygen at pH 7 is about 0.2 V. This is clearly lower than the expected value of 0.805 V, indicating the large energy loss occurring at the cathode. This energy loss is often referred to as overpotential, or the difference between the potential under equilibrium conditions and the actual potential, which for this case is 0.605 V (0.805 V − 0.2 V). This illustrates that the main application of thermodynamic calculations is to identify the size and nature of energy losses.

Identifying Factors that Decrease Cell Voltage

The maximum attainable MFC voltage (emf) is theoretically on the order of 1.1 V (see above). However, the measured MFC voltage is considerably lower due to a number of losses. In an open circuit, when no current is flowing, the maximum MFC voltage achieved thus far is 0.80 V (*62*). During current generation, voltages achieved up to now remain below 0.62

V (35). In general, the difference between the measured cell voltage and the cell emf is referred to as overvoltage and is the sum of the overpotentials of the anode and the cathode, and the ohmic loss of the system

$$E_{cell} = E_{emf} - (\Sigma\eta_a + |\Sigma\eta_c| + IR_\Omega) \qquad (11)$$

where $\Sigma\eta_a$ and $|\Sigma\eta_c|$ are the overpotentials of the anode and the cathode respectively, and IR_Ω is the sum of all ohmic losses which are proportional to the generated current (I) and ohmic resistance of the system (R_Ω). The overpotentials of the electrodes are generally current dependent and in an MFC they can roughly be categorized as follows: (*i*) activation losses; (*ii*) bacterial metabolic losses; and (*iii*) mass transport or concentration losses (see below).

In MFCs the measured cell voltage is usually a linear function of the current (see discussion of the polarization curve below), and can be described simply as

$$E_{cell} = OCV - IR_{int} \qquad (12)$$

where IR_{int} is the sum of all internal losses of the MFC, which are proportional to the generated current (I) and internal resistance of the system (R_{int}). A comparison of eqs 11 and 12 shows that the overpotentials of the anode and the cathode that occur under open circuit conditions are included in the value of OCV in eq 12, while the current dependent overpotentials of the electrodes and ohmic losses of the system are captured in IR_{int}. MFC systems that are well described by eq 12 show a maximum power output when the internal resistance, R_{int}, is equal to external resistance, R_{ext} (52). Although R_{int} includes more than just ohmic resistance (R_Ω), both terms are often used interchangeably but MFC researchers should be aware of the differences in these two terms. MFC performance can be assessed in terms of both overpotentials and ohmic losses or in terms of OCV and internal losses, based on various techniques discussed below.

Ohmic Losses. The ohmic losses (or ohmic polarization) in an MFC include both the resistance to the flow of electrons through the electrodes and interconnections, and the resistance to the flow of ions through the CEM (if present) and the anodic and cathodic electrolytes (63, 64). Ohmic losses can be reduced by minimizing the electrode spacing, using a membrane with a low resistivity, checking thoroughly all contacts, and (if practical) increasing solution conductivity to the maximum tolerated by the bacteria.

Activation Losses. Due to the activation energy needed for an oxidation/reduction reaction, activation losses (or activation polarization) occur during the transfer of electrons from or to a compound reacting at the electrode surface. This compound can be present at the bacterial surface, as a mediator in the solution (Figure 4), or as the final electron acceptor reacting at the cathode. Activation losses often show a strong increase at low currents and steadily increase when current density increases. Low activation losses can be achieved by increasing the electrode surface area, improving electrode catalysis, increasing the operating temperature, and through the establishment of an enriched biofilm on the electrode(s).

Bacterial Metabolic Losses. To generate metabolic energy, bacteria transport electrons from a substrate at a low potential (e.g., Table 1: acetate −0.296 V) through the electron transport chain to the final electron acceptor (such as oxygen or nitrate) at a higher potential. In an MFC, the anode is the final electron acceptor and its potential determines the energy gain for the bacteria. The higher the difference between the redox potential of the substrate and the anode potential, the higher the possible metabolic energy gain for the bacteria, but the lower the maximum attainable MFC voltage. To maximize the MFC voltage, therefore, the potential of the anode should be kept as low (negative) as possible. However, if the anode potential becomes too low, electron transport will be inhibited and fermentation of the substrate (if possible) may provide greater energy for the microorganisms. The impact of a low anode potential, and its possible impact on the stability of power generation, should be addressed in future studies.

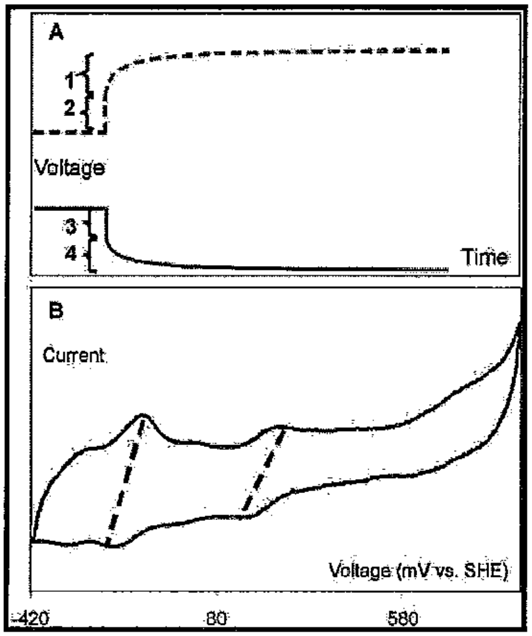

FIGURE 4. Electrochemical analysis of microbial fuel cells. (A) Anodic (blue solid line) and cathodic (red dashed line) voltage profiles over time when applying the current interrupt method for determination of the ohmic resistance of an MFC. Sections 2 and 3 indicate the voltage differences related to the ohmic resistance; sections 1 and 4 indicate voltage losses caused by the activation overpotentials. (B) Cyclic voltammogram (solid line) of an electrochemically active mixed microbial community. The dashed lines connect the oxidation and reduction peaks of redox active compounds (6).

Concentration Losses. Concentration losses (or concentration polarization) occur when the rate of mass transport of a species to or from the electrode limits current production (63, 64). Concentration losses occur mainly at high current densities due to limited mass transfer of chemical species by diffusion to the electrode surface. At the anode concentration losses are caused by either a limited discharge of oxidized species from the electrode surface or a limited supply of reduced species toward the electrode. This increases the ratio between the oxidized and the reduced species at the electrode surface which can produce an increase in the electrode potential. At the cathode side the reverse may occur, causing a drop in cathode potential. In poorly mixed systems diffusional gradients may also arise in the bulk liquid. Mass transport limitations in the bulk fluid can limit the substrate flux to the biofilm, which is a separate type of concentration loss. By recording polarization curves, the onset of concentration losses can be determined as described below.

Instruments for Measurement

In addition to conventional instruments used for chemical measurements in microbial systems (e.g., for determining substrate concentrations and degradation products), MFC experiments can require specialized electrochemical instrumentation (6, 30). In most cases, cell voltages and electrode potentials are adequately measured with commonly available