voltage meters, multimeters, and data acquisition systems connected in parallel with the circuit. Cell voltages can be determined directly from the voltage difference between the anode and cathode; electrode potentials can only be determined against a reference electrode that needs to be included in the electrode compartment (*65*). Current is calculated using Ohm's law ($I = E_{cell}/R$) using the measured voltage.

A more detailed understanding of the (bio-)electrochemical system can be obtained using a potentiostat (e.g., Ecochemie, The Netherlands; Princeton Applied Research, USA; Gamry Scientific, USA). With a potentiostat either the potential or the current of an electrode can be controlled in order to study the electrochemical response of the electrode at that specific condition. The potentiostat is typically operated in a three-electrode-setup consisting of a working electrode (anode or cathode), a reference electrode, and a counter electrode (*65*). In MFC experiments, the potentiostatic mode of this instrument is often used for voltammetry tests in which the potential of the working electrode (anode or cathode) is varied at a certain scan rate (expressed in V s^{-1}). In the case where a scan only goes in one direction the method is referred to as linear sweep voltammetry (LSV); if the scan is also continued in the reverse direction and comes back to the start potential the method is cyclic voltammetry (CV; Figure 4B). Voltammetry can be used for assessing the electrochemical activity of microbial strains or consortia (*6, 16, 50, 51*), determining the standard redox potentials of redox active components (*7*), and testing the performance of novel cathode materials (*55*). A potentiostat can also be operated in a two-electrode setup to obtain polarization curves or to determine the ohmic resistance using the current interrupt technique (Figure 4A) as described below. In the two-electrode setup, the working electrode connector is connected to the cathode (positive terminal) and both the counter electrode and reference electrode connectors are connected to the anode.

More advanced measurements can be done when the potentiostat is equipped with a frequency response analyzer (FRA), allowing electrochemical impedance spectroscopy measurements (EIS) (*65*). In EIS a sinusoidal signal with small amplitude is superimposed on the applied potential of the working electrode. By varying the frequency of the sinusoidal signal over a wide range (typically 10^{-4} to 10^{6} Hz) and plotting the measured electrode impedance, detailed information can be obtained about the electrochemical system. EIS can be used to measure the ohmic and internal resistance of an MFC (*27, 66*), as well as to provide additional insight into the operation of an MFC. The interpretation of EIS data can be rather complex, however, and therefore EIS techniques will not be discussed further here.

Calculations and Procedures for Reporting Data

Electrode Potential. The potential of an electrode (anode or cathode) can only be determined by measuring the voltage against an electrode with a known potential, i.e., a reference electrode. A reference electrode consists of several phases of constant composition (*65*) and therefore has a constant potential. The standard hydrogen electrode (SHE) or normal hydrogen electrode (NHE), consisting of a platinum electrode in a hydrogen saturated acidic solution (all components at unit activity), has a potential of 0 V. Because the NHE is not a very practical reference electrode to work with in an experimental setup, other reference electrodes are often used. The most popular reference electrode in MFC experiments is the silver−silver chloride (Ag/AgCl) reference electrode, because of its simplicity, stability, and nontoxicity. In a saturated potassium chloride solution at 25 °C the Ag/AgCl reference electrode develops a potential of +0.197 V against the NHE. Also practical, but less common in MFC experiments, is the saturated calomel electrode (SCE, 0.242 V against the NHE). Electrode potentials are often strongly dependent on the pH in the system and it is therefore important to report the solution pH. Preferably, electrode potentials are reported in the literature back-calculated against the NHE (expressed in V or V vs NHE), but are also often reported as a voltage difference against the reference electrode that was used in the study (e.g., V vs Ag/AgCl).

As a consequence of these different methods, the potential of the electrodes appears to vary dependent on the electrode used, the pH, and for the cathode the concentration of the electron acceptor. For example, at pH 7 a typical anode potential is −0.20 to −0.28 V (NHE), equivalent to −0.40 to −0.48 V vs Ag/AgCl. At the same pH a typical cathode potential is 0.30 to 0.10 V (NHE), equivalent to 0.10 to −0.10 V vs Ag/AgCl.

Power. The overall performance of an MFC is evaluated in many ways, but principally through power output and Coulombic efficiency. Power is calculated as

$$P = IE_{cell} \tag{13}$$

Normally the voltage is measured across a fixed external resistor (R_{ext}), while the current is calculated from Ohm's law ($I = E_{cell}/R_{ext}$). Thus, power is usually calculated as

$$P = \frac{E_{cell}^2}{R_{ext}} \tag{14}$$

This is the direct measure of the electric power. The maximum power is calculated from the polarization curve (see below).

Power Density. Power is often normalized to some characteristic of the reactor in order to make it possible to compare power output of different systems. The choice of the parameter that is used for normalization depends on application, as many systems are not optimized for power production. The power output is usually normalized to the projected anode surface area because the anode is where the biological reaction occurs (*6, 31, 34, 67*). The power density (P_{An}, W/m^2) is therefore calculated on the basis of the area of the anode (A_{An}) as

$$P_{An} = \frac{E_{cell}^2}{A_{An}R_{ext}} \tag{15}$$

In many instances, however, the cathode reaction is thought to limit overall power generation (*30, 32*) or the anode consists of a material which can be difficult to express in terms of surface area (i.e., granular material; (*35*)). In such cases the area of the cathode (A_{Cat}) can alternatively be used to obtain a power density (P_{Cat}). The projected surface areas of all components should always be clearly stated, as well as the specific surface area (if known) and the method of its determination.

To perform engineering calculations for size and costing of reactors, and as a useful comparison to chemical fuel cells, the power is normalized to the reactor volume, or

$$P_v = \frac{E_{cell}^2}{vR_{ext}} \tag{16}$$

where P_v is the volumetric power (W/m^3) (*68*), and v is the total reactor volume (i.e., the empty bed volume). The use of the total bed reactor volume is consistent with a tradition in environmental engineering to use the total reactor size as a basis for the calculation. A comparison on the basis of total reactor volume, however, is not always level when comparing two- and single-chambered reactors because there is no "second chamber" for an open air cathode. In such cases it is useful to compare reactors on the basis of the total anode compartment volume. If multiple reactors are operated in concert, for example as a series of stacked reactors, the volume used for the air-space for the cathode (or volume for the catholyte) is then included for the overall reactor volume. Thus, the volume used in the calculation should be clearly stated, and volumes of the individual chambers must always be clearly noted.

Ohmic Resistance Using the Current Interrupt Technique. The ohmic resistance (R_Ω) of an MFC can be determined using the current interrupt technique (*63, 64*) by operating the MFC at a current at which no concentration losses occur. Next the electrical circuit is opened (which results in zero current, i.e., an infinite resistance) and a steep initial potential rise (E_R, Figure 4A voltage differences 2 + 3) is observed, followed by a slower further increase of the potential (E_A, Figure 4A voltage differences 1 + 4) to the OCV. The determination of the steep potential rise after current interrupting requires the fastest possible recording of the potential (up to μs scale) (*64*). Ohmic losses (IR_Ω) are proportional to the produced current and the ohmic resistance. When the current is interrupted the ohmic losses instantaneously disappear. This results in a steep potential rise (E_R) in potential that is proportional to the ohmic resistance (R_Ω) and the current (I) produced before the interruption (Figure 4A; see sections 2 and 3). Using Ohms law, R_Ω is estimated using this approach as $R_\Omega = E_R/I$. The slower further increase of the potential (E_A) to the OCV after the initial steep potential rise gives the electrode overpotentials that occurred during current generation.

Polarization Curves. Polarization curves represent a powerful tool for the analysis and characterization of fuel cells (*63*). A polarization curve represents the voltage as a function of the current (density). Polarization curves can be recorded for the anode, the cathode, or for the whole MFC using a potentiostat. If a potentiostat is not available, a variable resistor box can be used to set variable external loads. Using a periodical decrease (or increase, when starting at short circuit) of the load, the voltage is measured and the current is calculated using Ohms law. To separately study the performance of the system in terms of anode or cathode potentials, a reference electrode is used as described above. When a potentiostat is used to record a polarization curve, an appropriate scan rate should be chosen such as 1 mV s^{-1} (*25*). The polarization curve should be recorded both up and down (i.e., from high to low external resistance) and vice versa. When a variable external resistance is used to obtain a polarization curve, the current and potential values need to be taken only when pseudo-steady-state conditions have been established. The establishment of this pseudo-steady state may take several minutes or more, depending on the system and the external resistance. This condition is only a temporary steady state because over longer times the substrate concentration in the reactor will change due to substrate demand at the anode (unless continuously replenished). This will in turn affect the incidence of substrate/products mass transfer over voltage and current. Care should therefore be taken not to wait too long for the establishment of the pseudo-steady state. Polarization curves can also be obtained over multiple batch cycles, i.e., with one resistor used for the whole cycle, allowing measurement of Coulombic efficiency (see below) for each resistor (see ref *69* for a comparison of these two methods). Long-term recording may risk shifts in the microbial community.

Polarization curves can generally be divided in three zones: (*i*) starting from the OCV at zero current, there is an initial steep decrease of the voltage: in this zone the activation losses are dominant; (*ii*) the voltage then falls more slowly and the voltage drop is fairly linear with current: in this zone the ohmic losses are dominant; (*iii*) there is a rapid fall of the voltage at higher currents: in this zone the concentration losses (mass transport effects) are dominant (solid line, Figure 5A). In MFCs, linear polarization curves are most often encountered (dashed line, Figure 5A). For a linear polarization curve, the value of the internal resistance (R_{int}) of the MFC is easily obtained from the polarization curve as it is equal to the slope (e.g., $R_{int} = -\Delta E/\Delta I = 26\ \Omega$; Figure 5A, dashed line).

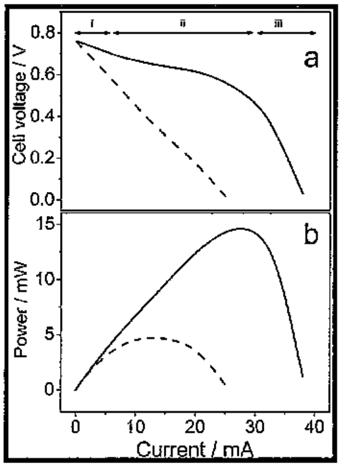

FIGURE 5. Polarization (a) and power (b) curves of a microbial fuel cell operating on starch. The solid curves are the original data (*70*), the dashed curves represent a mathematically manipulated dataset in which the effect of an increase of the ohmic resistance with 20 Ω is illustrated. The increase of the ohmic resistance resulted in a linear polarization curve (dashed line). From the slope of this curve an internal resistance of 30 ohm can be determined.

Power Curves. A power curve that describes the power (or power density) as the function of the current (or current density) is calculated from the polarization curve. Figure 5B (solid line) shows a typical power curve based on a previously reported polarization curve (Figure 5A; *70*). As no current flows for open circuit conditions, no power is produced. From this point onward, the power increases with current to a maximum power point, MPP (14.6 mW, Figure 5B). Beyond this point, the power drops due to the increasing ohmic losses and electrode overpotentials to the point where no more power is produced (short circuit conditions).

In many MFCs the ohmic resistance plays a dominant role in defining the point of the maximum attainable power (MPP), partially due to the low ionic conductivity of the substrate solutions (*71*), but usually to a low degree of optimization in the fuel cell design. The effect of increased ohmic resistance on the shape of a polarization curve is shown in Figure 5A. The solid curve is the original data set, while the dashed curve was calculated by including an additional ohmic resistance of $\Delta R_\Omega = 20\ \Omega$ by subtracting a potential drop of $\Delta E_{IR} = I\Delta R_\Omega$. Increasing the ohmic resistance by this amount produces a polarization curve that is linear (Figure 5A, dashed line), which is typically observed for MFCs. When a polarization curve is linear the slope is equal to the internal resistance (eq 12), which for this example is calculated as $R_{int} = 30\ \Omega$ (dashed line). If the polarization curve is not linear (solid line), a current independent R_{int} cannot be defined and the system is better expressed in term of ohmic resistance R_Ω and the electrode overpotentials $\Sigma\eta_a$ and $|\Sigma\eta_c|$ (eq 11), which can be determined using the current interrupt method or EIS. Increasing the ohmic resistance decreases the MPP from 14.6 to 4.8 mW. A symmetrical semi-cycle power density curve is typical for a high internal resistance MFC limited by ohmic resistance (dashed line, Figure 5B) rather than a fuel cell limited by mass transfer (solid line, Figure 5B). In the case of a symmetrical semi-cycle, the MPP will occur at a point where the $R_{int} = R_{ext}$.

Treatment Efficiency. MFCs have been proposed as a method to treat wastewater, and thus it is important to evaluate the overall performance in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), or total organic carbon (TOC) removal. Other factors may also be important, such as soluble versus particulate removal, and nutrient removal. We focus here on performance in terms of COD removal as it is a common measure for wastewater treatment efficiency, and the COD removal is needed for Coulombic and energy calculations. The COD removal efficiency (ϵ_{COD}) can be calculated as the ratio between the removed and influent COD. This parameter measures how much of the available "fuel" has been converted in the MFC, either into electrical current (via the Coulombic efficiency) or biomass (via the growth yield) or through competitive reactions with alternative electron acceptors (e.g., oxygen, nitrate, and sulfate). As the MFC influent can contain both dissolved and particulate COD, it can be difficult to specify what fraction of the effluent particulate COD was due to biomass produced in the reactor, or untreated COD that was originally in the reactor influent.

Coulombic Efficiency. The Coulombic efficiency, ϵ_C, is defined as the ratio of total Coulombs actually transferred to the anode from the substrate, to maximum possible Coulombs if all substrate removal produced current. The total Coulombs obtained is determined by integrating the current over time, so that the Coulombic efficiency for an MFC run in fed-batch mode, ϵ_{Cb}, evaluated over a period of time t_b, is calculated as (*35*, *52*)

$$\epsilon_{Cb} = \frac{M \int_0^{t_b} I\,dt}{Fbv_{An}\Delta COD} \tag{17}$$

where $M = 32$, the molecular weight of oxygen, F is Faraday's constant, $b = 4$ is the number of electrons exchanged per mole of oxygen, v_{An} is the volume of liquid in the anode compartment, and ΔCOD is the change in COD over time t_b. For continuous flow through the system, we calculate the Coulombic efficiency, ϵ_{Cb}, on the basis of current generated under steady conditions as

$$\epsilon_{Cb} = \frac{MI}{Fbq\Delta COD} \tag{18}$$

where q is the volumetric influent flow rate and ΔCOD is the difference in the influent and effluent COD.

The Coulombic efficiency is diminished by utilization of alternate electron acceptors by the bacteria, either those present in the medium (or wastewater), or those diffusing through the CEM such as oxygen. Other factors that reduce Coulombic efficiency are competitive processes and bacterial growth. Bacteria unable to utilize the electrode as electron acceptor are likely to use substrate for fermentation and/or methanogenesis. It has been observed that fermentative patterns diminish through time during enrichment of the microbial consortium in the MFC (*6*). As long as the anode remains attractive enough for the bacteria due to its potential, alternative electron acceptors will not be used. However, high potential compounds such as nitrate (+0.55 V) will almost certainly be used.

Growth Yield. Cell growth will reduce ϵ_C due to diversion of electrons into biomass. The substrate utilization for growth is measured by the net (or observed) cell yield, Y, calculated as

$$Y = \frac{X}{\Delta COD} \tag{19}$$

where X is the biomass (g COD) produced over time (either t_b or hydraulic retention time). An important advantage of an MFC is the lower cell yield compared to aerobic processes. This is caused by the reduced energy available for biomass growth as a significant part of the substrate energy is converted to electrical power. Reported MFC net yields range from 0.07 and 0.22 g biomass COD (g substrate COD)$^{-1}$, while typical aerobic yields for wastewater treatment are generally around 0.4 g biomass COD (g substrate COD)$^{-1}$ (*26*). The growth rate can be measured directly by determining the biomass (g COD) built up on the electrode surface and discharged in the effluent (for continuous operation). The low biomass production in MFCs is an especially attractive benefit since sludge disposal by combustion (becoming the standard technology in Europe) costs approximately 600 Euros per tonne.

COD Balance. Once the efficiencies for electricity and biomass production are completed, the fraction of COD that was removed by unknown processes, φ, can be calculated as

$$\varphi = 1 - \epsilon_C - Y \tag{20}$$

Loading Rate. When examining the use of MFCs for wastewater treatment, it is useful to examine performance achieved with this new technology in terms of loading rates with those typically obtained in conventional treatment systems. To do this, we calculate the loading based on volumetric loading rates as B_v (kg COD m^{-3} d^{-1}). Typical values for B_v achieved to date are up to 3 kg COD m^{-3} d^{-1} (*18*), compared to values for high-rate anaerobic digestion of 8–20 kg COD m^{-3} d^{-1} or activated sludge processes of 0.5–2 kg COD m^{-3} d^{-1}. These loading rates can be normalized to the total anode volume for comparison with suspended biomass processes (e.g., activated sludge, anaerobic digestion), and to total anode surface area for comparison with biofilm processes. Based on the reported areal short-term peak power productions (*3*, *46*), the anode surface-specific conversion rates for MFCs are up to 25–35 g COD m^{-2} d^{-1}, which is higher than typical loading rates for rotating biological contactors (RBCs; 10–20 g COD m^{-2} d^{-1}; *72*) and comparable to those of high rate aerobic biofilm processes such as the moving bed bio-reactors (MBBRs).

Energy Efficiency. The most important factor for evaluating the performance of an MFC for making electricity, compared to more traditional techniques, is to evaluate the system in terms of the energy recovery. The overall energetic efficiency, ϵ_E, is calculated as the ratio of power produced by the cell over a time interval t to the heat of combustion of the organic substrate added in that time frame, or

$$\epsilon_E = \frac{\int_0^t E_{cell} I dt}{\Delta H m_{added}} \tag{21}$$

where ΔH is the heat of combustion (J mol^{-1}) and m_{added} is the amount (mol) of substrate added. This is usually calculated only for influents with known composition (i.e., for synthetic wastewaters) as ΔH is not known for actual wastewaters. In MFCs, energy efficiencies range from 2% to 50% or more when easily biodegradable substrates are used (*18*, *30*). As a basis for comparison, the electric energy efficiency for thermal conversion of methane does not exceed 40%.

Distinguishing Methods of Electron Transfer

Presence of Mediators. Bacteria can reduce activation losses by increasing their extracellularly oriented mediation capacity. Three pathways are discerned at this point:, direct membrane complex mediated electron transfer (*8*), mobile