Dissimilatory Metal Reducers Producing Electricity: Microbial Fuel Cells

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Abstract This chapter provides an introduction to the application of dissimilatory metal reducers in microbial fuel cells. In this type of fuel cells, exoelectrogenic bacteria act as anodic electrode catalysts and enable the direct generation of electricity from e.g., waste waters and other organic carbon sources. The chapter covers the basic thermodynamic principles of electrochemical energy conversion and the interrelations between cell voltage, power density, and efficiencies. Furthermore, important aspects of fuel cell construction are discussed, including reactor design as well as suitable anode materials and catalysts for cathodic oxygen reduction. Special importance is given to fuel cell characterization techniques that allow researchers to evaluate the power output of a microbial fuel cell and distinguish the different loss mechanisms that govern its performance. The chapter closes with a comparison of typical application examples and a perspective on future challenges and trends in the field of microbial fuel cells, also regarding emerging applications beyond electricity generation.

1 Introduction

The observation that microorganisms can produce useful electricity dates back to the beginning of the twentieth century. As early as 1911, M. C. Potter, Professor of Botany at the University of Durham, reported on the "electro motive force" developed by cultures of *Saccharomyces cerevisiae* and *E. coli* growing in vicinity of a platinum electrode (Potter 1911). In 1931, Cohen expanded these observations by

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Fig. 1 Evolution of microbial fuel cell publications since 1983. Data extracted from an ISI Web of Science query with the search term topic = "microbial fuel cell"
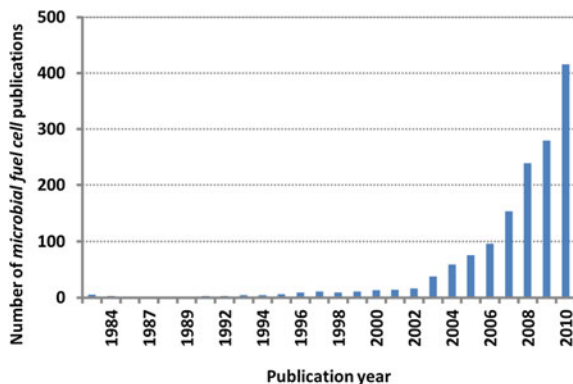

demonstrating that also *Bacillus subtilis* and *Proteus vulgaris* function as an electrochemical half-cell and generate electrical currents (Cohen 1931). However, the concept found no immediate practical application and was thus quickly forgotten. Later, research activities in the field of microbial fuel cells were driven by space and military research during the 1960s, and the use of microbial fuel cells not only as power source but also as toxicity sensors and for the generation of oxygen from CO_2 for blood oxygenation were discussed (Thomson and Brady, 1963). Still, in 1966 Kenneth Lewis concluded at the *Annual Meeting of the American Society for Microbiology* that *the available results indicate that a direct biofuel cell in which the microbial cells are located at the electrode has little value […] the systems are not competitive with conventional power sources* (Lewis 1966).

Nevertheless, with the beginning of the twenty-first century research on microbial fuel cell systems has regained considerable and further growing interest, as illustrated by the number of publications on microbial fuel cells since 1983 shown in Fig. 1. This is not only related to the increasing necessity to develop environmentally friendly and renewable power sources, but also to the discovery of the direct electron transfer capability of *Shewanella putrefaciens* (*oneidensis*) and the consequent demonstration of the first mediator-less microbial fuel cell by Kim et al. in 1999 (Kim et al. 2002; Logan 2008). The following chapter of this book is intended to provide readers from different disciplines with a general overview on the overall concept, technology, design, characterization, and applications of microbial fuel cells, in which exoelectrogenic bacteria act as anodic electrode catalysts. For further reading on microbial fuel cells the detailed and comprehensive textbooks "Bioelectrochemical systems" (Rabaey et al. 2010) and "Microbial Fuel Cells" (Logan 2008) as well as the cited literature accompanying each section of this chapter are recommended.

1.1 Bacteria as Electrode Catalysts—Microbial Fuel Cells

In principle, any chemical reaction involves the transfer of electrons from an oxidizable species ("fuel") to a reducible reaction partner ("oxidant"). This exchange
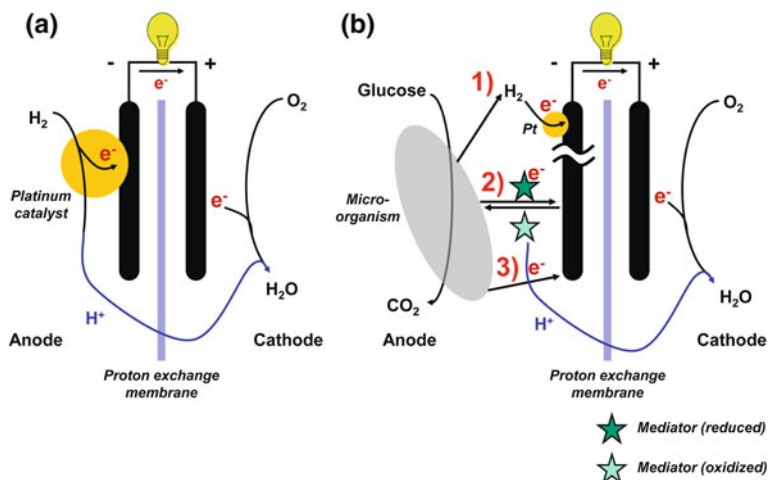
Fig. 2 Fuel cell principles. **a** Conventional H_2–O_2 fuel cell with platinum as anode catalyst. **b** Three different types of microbial fuel cells: (1) indirect, (2) mediated, and (3) mediator-less type. See text for explanations

of electrons can be transformed into a useful electrical current by means of a fuel cell. Here, oxidation and reduction reaction take place at two spatially separated electrodes, connected through an external electrical load circuit, as illustrated in Fig. 2. At the anode, the "fuel" is oxidized, releasing its electrons. These travel through the external load to the cathode, where an "oxidant" (usually oxygen) accepts these electrons and is, thus, reduced. The driving force for the electron flow from anode to cathode is the difference in redox potential between the oxidation and reduction reaction. To enable the electrode reactions of a fuel cell, catalysts are required. As illustrated in Fig. 2, not only noble metals such as platinum, but also the enzymatic systems of living microorganisms can act as electrode catalysts. In practice, three types of microbial fuel cells can be distinguished:

- In *indirect microbial fuel cells* microbes break down a complex fuel into smaller molecules [e.g. glucose into H_2, for example by *clostridium butyricum* (Karube et al. 1977)] which can be easily oxidized on e.g., a platinum electrode. The microbes are not necessarily in direct contact with the electrode and do not directly exchange electrons with it. The disadvantage of these systems is the need for costly noble metal catalysts to enable the oxidation of the microbial fermentation products.
- *Mediated microbial fuel cells* require a mediator or electron shuttle to transfer metabolic electrons from the microorganism to the anode. Examples are the use of *E. coli* or *Saccharomyces cerevisiae* (Bennetto et al. 1983). Typically, mediators are small redox-active molecules [e.g. thionine, viologens, and methylene blue (Chang et al. 2006)] which can diffuse into the microbial cell and can be reduced there. At the anode, they in turn release electrons and are thus oxidized. The main disadvantage of such systems is the cost and loss of mediators in flow-through systems.

- In *mediator-less microbial fuel cells* dissimilatory metal reducing bacteria are used, which possess the ability to transfer electrons using surface exposed proteins. Such microbes can directly live on an electrode and transfer respiratory electrons released upon metabolic oxidation of an organic fuel to the fuel cell's anode. Mediators or electron shuttles are not necessary.
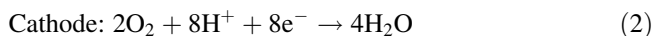
As mentioned before, the first mediator-less microbial fuel cell was demonstrated by Kim et al. in 1999 (Kim et al. 2002) using *Shewanella oneidensis (putrefaciens)*. In the meantime, further exoelectrogenic microorganisms found practical application at the anode of microbial fuel cells. Example organisms are: *Aeromonas hydrophilia*, *Clostridium butyricum*, *Desulfobulbus propionicus*, *Enterococcus gallinarum*, *Geobacter sulfurreducens*, and *Rhodoferax ferrireducens* (Chang et al. 2006). The prime advantage of exoelectrogenic bacteria in the design of microbial fuel cells is the abdication of the otherwise required mediators. Mediators are not only potentially toxic, but can also become a cost factor (Chang et al. 2006). This becomes particularly relevant if large systems or flow-through setups e.g., operated with waste water streams are considered.

In addition to the direct electron transfer via membrane-bound proteins, some exoelectrogenic bacteria are also capable of secreting natural redox mediators (Gralnick and Newman 2007; Marsili et al. 2008), or transferring electrons throughout the biofilm by e.g., bacterial nanowires (Gorby et al. 2006). These capabilities enlarge the number of bacterial cells that are electrically connected to the electrode, and can thus increase the power output of a microbial fuel cell (see also "On the Role of Endogenous Electron Shuttles in Extracellular Electron Transfer" and "Humic Substances and Extracellular Electron Transfer").

1.2 Fuel Cell Voltage, Current, Power, and Efficiency

In general, a fuel cell system is characterized by its voltage, current and power density, as well as efficiency. In the following section, the thermodynamic and electrochemical principles behind these figures are introduced (the textbooks "Fuel Cell Systems Explained" by Larminie and Dicks (2000) and "Microbial Fuel Cells" by Logan (2008) are suggested for further reading).

Using acetate as exemplary *fuel* that is fully oxidized to carbon dioxide and oxygen as *oxidant* (or terminal electron acceptor), the electrode reactions of a microbial fuel cell are

$$\text{Anode: } C_2H_3O_2^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \tag{1}$$

$$\text{Cathode: } 2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O \tag{2}$$

$$\text{Overall: } C_2H_3O_2^- + 2O_2 \rightarrow 2\ HCO_3^- + H^+ \tag{3}$$

Table 1 Values for the standard free energy of formation ΔG_f^0 (Thauer et al. 1977) for different compounds of relevance in the microbial fuel cell context

| Substance	ΔG_f^0 at 25 °C kJ mol^{-1}
O_2	0
H_2O	−237.178
H^+	0
HCO_3^-	−586.85
Acetate$^-$	−369.41
Lactate$^-$	−517.81

Let us first consider the *standard redox potential* (or *standard electromotive force*) ΔE_r^0 of the overall electrochemical fuel cell reaction. According to thermodynamics, this is the maximum voltage a single fuel cell can produce. It can be calculated from the *standard free energy* of the overall reaction ΔG_r^0

$$\Delta E_r^0 = -\frac{\Delta G_r^0}{n\mathrm{F}} \tag{4}$$

wherein $n = 8$ is the number of electrons transferred in the reaction and $\mathrm{F} = 96{,}500 \ \mathrm{C \ mol^{-1}}$ is the Faraday constant (Mortimer 1996). The *standard free energy* of the overall reaction ΔG_r^0 can be calculated from tabulated values (see for instance Table 1) of the *standard free energy of formation* ΔG_f^0 using the relation (Thauer et al. 1977)

$$\Delta G_r^0 = \Sigma \Delta G_f^0 \, (\text{Products}) \, - \Sigma \Delta G_f^0 \, (\text{Educts}) \tag{5}$$

For reaction (3) the *standard free energy* ΔG_r^0 and the standard potential ΔE_r^0 thus calculate to

$$C_2H_3O_2^- + 2O_2 \rightarrow 2\,HCO_3^- + H^+$$
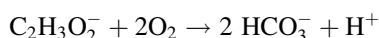
$$\Delta G_r^0 = (2 \cdot (-586.85) + 0) \ \mathrm{kJmol^{-1}} - (-369.41 + 2 \cdot 0) \ \mathrm{kJmol^{-1}}$$
$$= -804.29 \ \mathrm{kJmol^{-1}}$$

$$\Delta E_r^0 = -\frac{\Delta G_r^0}{n\mathrm{F}} = \frac{804.29 \ \mathrm{kJ \ mol^{-1}}}{8 \cdot 96500 \ \mathrm{C \ mol^{-1}}} = 1.042 \ \mathrm{V}$$

In a similar way also the potentials (with reference to the standard hydrogen electrode SHE) of the half-cell reactions, and thus the individual electrodes can be calculated. Consider that according to the IUPAC convention (Logan 2008) the direction of the anode reaction Eq. (1) has to be reversed, so that the products are the reduced species (educts $+ \ e^- \rightarrow$ products). This leads to

$$\text{Anode: } 2\,HCO_3^- + 9H^+ + 8 \ e^- \rightarrow C_2H_3O_2^- + 4\,H_2O$$
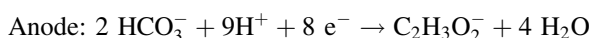
$$\Delta G^0_{\text{anode}} = (-369.41 + 4 \cdot (-237.178)) - (2 \cdot (-586.85) + 9 \cdot 0) \text{ kJ mol}^{-1}$$
$$= -144.422 \text{ kJ mol}^{-1}$$

$$\Delta E^0_{\text{anode}} = -\frac{\Delta G^0_{\text{anode}}}{n\text{F}} = \frac{144.422 \text{ kJ mol}^{-1}}{8 \cdot 96500 \text{ C mol}^{-1}} = 0.187 \text{ V vs. SHE}$$

Cathode: $2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$

$$\Delta G^0_{\text{cathode}} = \left(4 \cdot \left(-237.178 \text{ kJ mol}^{-1}\right)\right) - (2 \cdot 0 + 8 \cdot 0)$$
$$= -948.712 \text{ kJ mol}^{-1}$$

$$\Delta E^0_{\text{cathode}} = -\frac{\Delta G^0_{\text{cathode}}}{n\text{F}} = \frac{948.712 \text{ kJ mol}^{-1}}{8 \cdot 96500 \text{ C mol}^{-1}} = 1.229 \text{V vs. SHE}$$

Since in biological systems the concentrations of the reaction partners are usually different from standard conditions (pH = 0; T = 298 K; concentration of all species at 1 mol L^{-1}) the potential of the overall reaction under non-standard conditions $\Delta E^{0\prime}$ has to be corrected for the actual concentrations of the reaction partners. Assuming a pH of 7, an oxygen partial pressure of 0.2 bar, and the concentrations of acetate and HCO$_3^-$ being at 16.9 and 5 mM, respectively, (Logan 2008) the potential of the overall reaction under non-standard conditions calculates from the concentration of the products and educts to the power of their respective stoichiometric coefficients p and e according to

$$\Delta E^{0\prime}_r = \Delta E^0_r - \frac{RT}{n\text{F}} \cdot \ln \frac{[\text{products}]^p}{[\text{educts}]^e}$$
$$= \Delta E^0_r - \frac{RT}{n\text{F}} \cdot \ln \frac{[H^+]^1 \cdot [HCO_3^-]^2}{[C_2H_3O_2^-]^1 \cdot [O_2]^2} \qquad (6)$$
$$= 1.042 \text{ V} - \frac{8.31 \cdot 298}{8 \cdot 96500} \text{ JC}^{-1} \cdot \ln \frac{[10^{-7}]^1 \cdot [0.005]^2}{[0.0169]^1 \cdot [0.2]^2}$$
$$= 1.104 \text{ V}$$

This correction can also be applied to the individual electrode potentials.

Anode: $C_2H_3O_2^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$
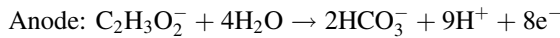
$$\Delta E^{0\prime}_{\text{anode}} = \Delta E^0_{\text{anode}} - \frac{RT}{n\text{F}} \cdot \ln \frac{[C_2H_3O_2^-]^1}{[H^+]^9 \cdot [HCO_3^-]^2}$$
$$= 0.187 \text{V} - \frac{8.31 \cdot 298}{8 \cdot 96500} \text{ J C}^{-1} \cdot \ln \frac{[0.0169]^1}{[10^{-7}]^9 \cdot [0.005]^2}$$
$$= -0.299 \text{V vs. SHE}$$

Cathode : $2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$
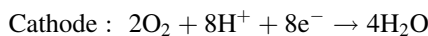
Fig. 3 Schematic
representation of the
polarization behavior of a
complete fuel cell. The
thermodynamic reversible
potential is indicated by a
gray line and differs from the
practical open circuit
potential. With increasing
current (density) losses occur
due to: activation
overpotential (region *A*),
ohmic resistances (region *B*),
mass-transport limitations
(region *C*)
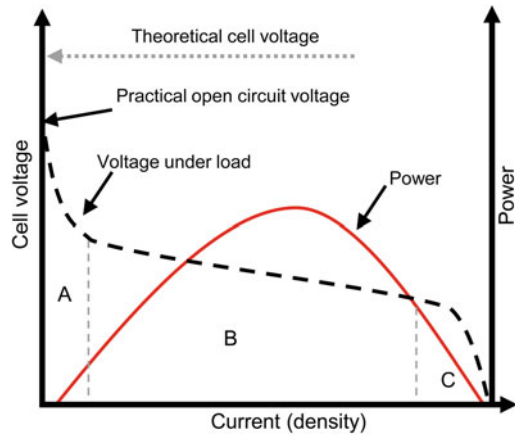
$$\Delta E^{0'}_{cathode} = \Delta E^{0}_{cathode} - \frac{RT}{nF} \cdot \ln \frac{[H_2O]^4}{[H^+]^8 \cdot [O_2]^2}$$

$$= 1.229\,V - \frac{8.31 \cdot 298}{8 \cdot 96500} JC^{-1} \cdot \ln \frac{[1]^4}{[10^{-7}]^8 \cdot [0.2]^2}$$

$$= 0.805\,V\ vs.\ SHE$$

The above-derived theoretical potentials and the overall cell voltage are only an ideal point-of-view. Due to the irreversible losses that occur at the fuel cell electrode the practical cell voltage of the fuel cell is considerably lower than the theoretical thermodynamic voltage. This is illustrated in Fig. 3 and explained as follows.

Already under open circuit conditions (no current flowing) activation losses reduce the practical open circuit voltage of the fuel cell. These stem from the activation energy necessary to drive the electrochemical reactions or in the case of microbial fuel cells the electron transport chain of the bacterial metabolism (Logan and Regan 2006). When current is flowing the practical fuel cell voltage is further decreased by ohmic losses e.g., resulting from the resistivity of the electrolyte and electrodes, and mass-transfer losses e.g., due to the depletion of a reaction partner. The origin of these losses and their influence on fuel cell voltage are discussed in Sect. 3.2 in more detail. In principle, the cell voltage of the fuel cell ΔE_{FC} follows:

$$\Delta E_{FC} = \Delta E_{ocp} - IR_{int} \tag{7}$$

wherein ΔE_{ocp} is the practical open circuit voltage, I is the current, and R_{int} is the sum of the fuel cells internal resistances. It depends nonlinearly on current density, and combines the linear ohmic resistances with the nonlinear polarization and mass-transfer resistances occurring at the electrodes. Furthermore, the electrical power output of the fuel cell at a given current can be calculated. As shown

schematically in Fig. 3, the power output goes through a maximum and follows the equation

$$P = \Delta E_{FC} I \tag{8}$$

1.3 Calculating Power: Normalization Allows for Comparison

From an applicational point-of-view the power output of a microbial fuel cell is the prime figure of interest. It can be easily calculated as the product of fuel cell current and voltage. Since a fuel cell's power output clearly depends on its size some normalization is necessary to be able to compare the performance of several fuel cells that e.g., differ in their size, design, or in the type of electrode materials employed. For fuel cell designs of the essentially 2D nature (e.g. two electrodes laminated to an ion exchange membrane) normalization to the projected electrode area is obviously reasonable. However, in systems where one electrode is considerably more bulky than the other normalization to the volume of one electrode or even the overall fuel cell is a better way of reporting power density.

1.4 Fuel Cell Efficiency

An important characteristic number of a fuel cell is its efficiency, which relates the fuel's energy content to the obtained electrical energy. One way to calculate the efficiency of any energy conversion device is to relate the fuel cell's power output to the energy flow into the fuel according to

$$\begin{aligned} \eta &= \frac{(\text{electrical output power})}{(\text{enthalpy of the fuel}) \cdot (\text{mass flow})} \\ &= \frac{(\Delta E_{FC} \cdot I)}{(\Delta H_{fuel}) \cdot (\text{mass flow})} \end{aligned} \tag{9}$$

While this is a straightforward way of accounting for a fuel cell's efficiency, it requires knowledge about the fuels energy content and in particular about the fuel flow into the fuel cell. A more convenient way to estimate a fuel cell's efficiency is based on the comparison of theoretical to practical voltage. As shown in Eq. (4) only the free energy ΔG_r^o of a reaction is transformed into electricity in a fuel cell. However, the complete energy content of a fuel is described by its enthalpy. If we now assume that all the fuels' energy content (heating value or molar enthalpy) could be transformed into electricity, the corresponding theoretical voltage E_{th} would calculate to

$$\Delta E_{\text{th}} = \frac{-\Delta H_{\text{fuel}}}{n\text{F}} \tag{10}$$

For instance, with the *standard enthalpy* (or heating value, heat of combustion) ΔH_{fuel}^0 (under standard conditions) of complete oxidation of acetic acid to water and CO_2 amounting to -874 kJ mol^{-1} (Lide 2001) this leads to

$$\Delta E_{\text{th}} = -\frac{-874}{8 \cdot 96500} \text{ kJ C}^{-1}$$
$$= 1.132 \text{ V}$$

With the practical fuel cell voltage ΔE_{FC} at its point of operation, a voltage efficiency η_v of the fuel cell system can then calculated according to

$$\eta_V = \frac{\Delta E_{\text{FC}}}{\Delta E_{\text{th}}} \tag{11}$$

In other words, the voltage efficiency η_v describes how much of the theoretical energy content of a fuel is actually converted into electricity, assuming that all of the fuel takes part in the electrochemical reaction. Considering that the operating voltage of an acetate-fed microbial fuel cell is in the range of 400 mV (Nevin et al. 2008), it is obvious that their efficiency cannot be better than 35 %, even when complete fuel utilization is assumed.

The fact that in a fuel cell side reactions occur and not all of the fuel is transformed into electrical current is expressed as coulomb-efficiency or fuel utilization coefficient

$$\eta_C = \frac{\text{coulombs recovered as electricity}}{\text{total coulombs in the substrate}} \tag{12}$$

Knowing these two numbers, the overall efficiency of a fuel cell system (neglecting balance of plant such as fans and pumps) can be calculated according to

$$\eta = \eta_V \cdot \eta_C$$
$$= \frac{\Delta E_{\text{FC}}}{\Delta E_{\text{th}}} \cdot \eta_C = \frac{\Delta E_{\text{OCP}} - IR_{\text{int}}}{\Delta E_{\text{th}}} \cdot \eta_C \tag{13}$$

This simple equation implies an important characteristic of fuel cell systems: with increasing current the efficiency of the fuel cell is decreased. Consequently, high fuel efficiency requires a low current density. This translates into a larger fuel cell to deliver the same power output and thus a high capital cost and space demand of the overall fuel cell system. As a consequence, fuel cell optimization also means reducing the internal resistances of the fuel cell, to enable high current at minimized voltage losses and thus optimize power output and efficiency of the overall system. Important optimization points are the fuel cell reactor design, the choice of electrode materials and electrocatalysts, as described in the following section on fuel cell construction.

2 Fuel Cell Construction

2.1 Reactor Design

The easiest way to construct a microbial fuel cell is to simply place anode (anaerobic) and cathode (aerobic) in separate vessels, connected by an ion bridge. An example of this configuration is shown in Fig. 4a). Here, a proton conducting membrane is situated between the two horizontal tubes interconnecting both vessels, thus preventing crossover of fuel, bacteria, or oxygen between the compartments. While comparably easy to fabricate, the main drawback of this concept is the large distance between the electrodes, which translates into a high internal resistance due to ohmic losses in the electrolyte. Such systems are useful to study e.g., an individual electrode reaction (preferably with the help of a reference electrode, see also Sect. 3.1), but produce only little electrical power.

Often, the anode is comparably thicker in comparison to the cathode, since it must accommodate the bacterial biofilm. This for instance is reflected in the anode concept (Fig. 4b) where graphite fibers in the form of a brush serve as 3D electrode on which the anodic biofilm is formed (Logan et al. 2007). Similarly in the tubular fuel cell concept, a stable cylindrical structure is formed from e.g., an ion exchange membrane (Fig. 4c). The cylinder's interior volume is filled with the anodic matrix (e.g. graphite granules) through which the fuel solution is pumped. The cathode is realized as a relative thin layer e.g., from woven graphite mat laminated to the outside of the cylinder (Rabaey et al. 2005). A limitation of these concepts is the comparably large distance between anode and cathode, leading to large ohmic losses in the electrolyte and consequently reduced power density.

As with conventional hydrogen fuel cells, also with microbial fuel cells the ohmic losses in the electrolyte can be reduced by minimizing the distance between the electrodes, e.g., by placing or laminating the electrodes to the two faces of an ion conducting membrane (Min and Logan 2004). This concept is illustrated in Fig. 4d, and has been adopted by a number of researchers.

A major cost factor in the construction of microbial fuel cells can be the *ion exchange membrane* that serves as a separator between anode and cathode compartment. Typically, it prevents the diffusion of oxygen into the anode compartment, which would decrease the coulombic efficiency of the fuel cell due to unwanted side reactions (see also Sect. 1.4). It thus has to be gas tight, but at the same time allow for the transport of protons from the anode to the cathode compartment. Other functions of the membrane are the prevention of bacteria or fuel leakage (crossover) to the cathode, which may reduce fuel cell performance by unwanted side reactions and fouling. Historically, high-performance materials such as Nafion (a sulfonated Teflon-like polymer), which is used in hydrogen fuel cells due to its excellent proton transport capability, have been applied. However, at the relatively low current densities typical for microbial fuel cells the high proton conductivity of Nafion is not needed. Consequently, researchers have considered cheaper membranes of the cation or anion exchange type (Kim et al.
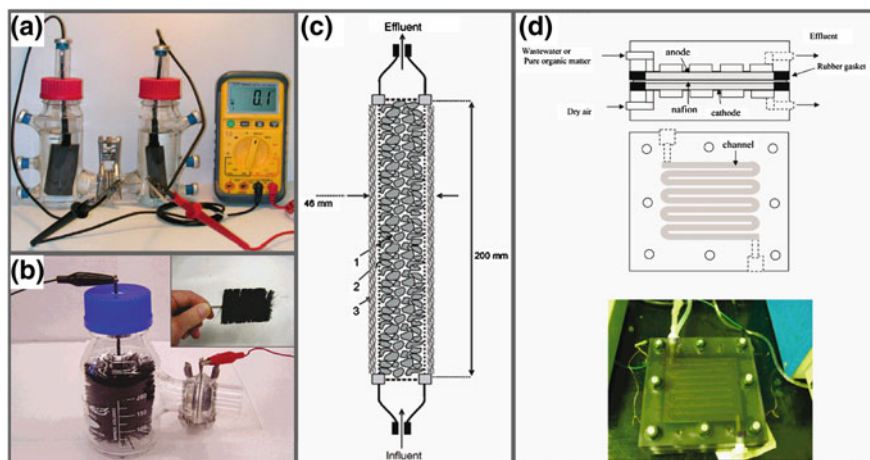
Fig. 4 Comparison of reactors from literature: **a** anode (anaerobic) and cathode (aerobic) in separate vessels each, interconnected by an ion bridge. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Microbiology (Lovley 2006), copyright (2006). **b** Three-dimensional graphite fiber brush anode with large ohmic resistance between anode and cathode. Reprinted with permission from (Logan et al., 2007), copyright (2007) American Chemical Society. **c** Tubular microbial fuel cell with graphite granule anode inside the tube, and a woven graphite mat as exterior cathode. Reprinted with permission from (Rabaey et al. 2005), copyright (2005) American Chemical Society. **d** Flat-plate type microbial fuel cell with minimized ohmic resistance between the electrodes. Reprinted with permission from (Min and Logan 2004), copyright (2004) American Chemical Society

2007), glass fiber mats (Zhang et al. 2011), or even omitted the membrane completely (Li et al. 2011; Liu and Logan 2004). One difficulty with the use of ion exchange membranes in microbial fuel cells is that at neutral pH the proton concentration in the typical electrolyte is much lower than the concentration of cations such as sodium. Consequently, e.g., sodium ions travel preferably over protons through the membrane to achieve charge balance, and the pH in the anodic compartment increases whereas the cathode becomes more alkaline (Rozendal et al. 2006).

For power generation, the use of oxygen from air as the electron acceptor at the *cathode* is almost exclusively considered. In principle, also other chemical compounds such as ferricyanide (Rabaey et al. 2004) can be reduced, which happens at low overpotential and yields comparably high power densities. However, once all the ferricyanide in a fuel cell is reduced it must be regenerated; the concept is thus hardly sustainable. With respect to the design of the oxygen (air) cathode in a microbial fuel cell, the question whether the cathode is operated in submerged or air-breathing configuration is of great importance. In *submerged* operation, the cathode is placed in an aqueous electrolyte containing dissolved oxygen. The advantage of this approach is that this way the electrode surface always is in intimate contact with the electrolyte, which is prerequisite for the electrochemical

reaction to take place. However, the main limitation of this type of electrode is the low (<200 μM) concentration of dissolved oxygen in aerated aqueous solutions. At higher current densities this leads to the increasing dominance of mass-transport limitations on cathode performance, which can also put an upper constraint on the total possible fuel cell current density and power output. Mass transport (or specifically oxygen transport) to the cathode electrode can be greatly augmented by the construction of *air-breathing* or *gas-diffusion cathodes* (Rismani-Yazdi et al. 2008), which is standard in case of conventional fuel cells. Here, the electrochemical reaction takes place at the triple phase boundary which is formed at the interface between electrolyte, electrode, and gas (air) phase.

2.2 Anode Materials

A suitable anode material for microbial fuel cells has to be electrically conductive, biocompatible, chemically inert, and allow for an efficient electron exchange with the microorganism. Furthermore, it has to be porous enough to enable sufficient mass transport to supply reactants and remove microbial reaction products. In the early works with indirect and mediated microbial fuel cells, platinum was the electrode material of choice due to its chemical inertness, electrical conductivity, and electrocatalytic activity toward the oxidation of small organic molecules. However, its drawback is the high cost and consequently alternative materials were sought for practical application of microbial fuel cells as electricity generators.

In practice, carbon-based materials such as graphite felts, fibers, or granules are nowadays dominant (Wei et al. 2011). New and promising materials are carbon fiber electrodes prepared as a 3D network by electrospinning (Chen et al. 2011) or textile fibers modified with carbon nanotubes (Xie et al. 2011). Carbon-based materials can be further improved by the introduction of surface functional groups, resulting in improved anode performance. Examples are the treatment with HNO_3 and ethylenediamine (Zhu et al. 2011) or ammonium gas treatment (Cheng and Logan 2007).

2.3 Electrocatalysts for Oxygen Reduction

Although it is consensus among researchers that the high cost of platinum prohibits its economic use as oxygen reduction catalyst in microbial fuel cells, platinum electrodes often serve as the benchmark in microbial fuel cells, against which other oxygen reduction catalysts can be compared (Birry et al. 2011; Harnisch et al. 2009b; Yu et al. 2009). However, it should be noted that platinum is far from being the optimal catalyst for oxygen reduction under the typical operation conditions of a microbial fuel cell. In particular, the near-neutral pH and interfering substances, such as e.g., chloride (Skou 1973), have a detrimental effect on its catalytic activity. Similarly, also fuel crossover effects, similar to the presence of methanol

at platinum cathodes in direct methanol fuel cells (Convert et al. 2001), can negatively affect the performance of a platinum cathode in a microbial fuel cell. Thus, not only the high cost of platinum, but also its sensitivity to interfering substances sparks new research on alternative catalyst materials (Harnisch and Schroder 2010), as outlined in the following.

Carbon materials are not only suitable as anode material, but also exhibit catalytic activity toward oxygen reduction (Kerzenmacher et al. 2008; Kozawa et al. 1970). In the field of microbial fuel cell cathodes, there are some examples where carbon materials have successfully been employed. For instance, Freguia et al. demonstrated the use of graphite granules as efficient oxygen reduction catalyst in a microbial fuel cell, and put special emphasis on the importance of further research on high surface area materials (Freguia et al. 2007). Zhang et al. demonstrated the use of activated carbon as efficient catalyst for air-breathing cathodes. In phosphate-buffered saline containing acetate as fuel this electrode even exhibited a slightly better performance than a platinum cathode (Zhang et al. 2009).

The catalytic activity of carbon materials can be further improved by chemical treatment, e.g. HNO_3 (Erable et al. 2009). Another possibility to increase the catalytic activity of carbon is the adsorption of *metal macrocycles* such as phthalocyanines (Harnisch et al. 2009a) or porphyrines (Zhao et al. 2005) to carbon materials, sometimes in conjunction with a pyrolysis step. Such catalysts can show performance comparable to platinum (Haoyu et al. 2007); however, their long-term stability demands further investigation. A different class of catalysts is based on *manganese oxide* (Roche and Scott 2009). These materials show only little electrical conductivity, and thus need to be dispersed on support materials such as carbon black. So far, these materials exhibited significantly lower oxygen reduction performance than platinum, but have the advantage of comparably low cost (Roche et al. 2010).

In principle, also a number of *enzymes* would be suitable as catalysts for the cathodic oxygen reduction in microbial fuel cells. Among the reported enzyme catalysts for cathodic oxygen reduction in microbial fuel cells is for instance laccase (Schaetzle et al. 2009). At pH 5, the oxygen-reducing laccase system exhibited a by approx. 50 % higher power density compared to ferricyanide reduction. In the context of conventional hydrogen and methanol fuel cells also the favorable *air-breathing* cathodes using laccase have been reported (Gellett et al. 2010; Shleev et al. 2010). Another option for air-breathing cathodes is *bilirubin oxidase* (Gupta et al. 2011), which compared to laccase has the advantage of higher tolerance toward chloride ions. This aspect is relevant for a number of practical applications where the presence of chloride ions cannot be circumvented, e.g., waste water or sediment fuel cells in the ocean floor. Furthermore, *bilirubin oxidase* has a wide operational pH range (5–8) (Gupta et al. 2011). One of the main limitations of all enzymatic catalysts is their short lifetime in the range of a few weeks, which at present hinders long-term application in fuel cells. Besides immobilization and structural optimization of the enzymes, the continuous production of active enzyme by microorganisms directly at the electrode is a promising approach to extend the lifetime of enzymes in bioelectrochemical systems (Rubenwolf et al. 2011, 2012). Recently, the use of the crude culture supernatant of the fungus *Trametes versicolor*

to supply laccase to a biofuel cell cathode has been demonstrated as a first step toward a self-regenerating enzymatic cathode (Sané et al. 2011).

Besides abiotic catalysts and enzymes, also microorganism can be used to facilitate oxygen reduction at the cathode. Several studies have demonstrated the feasibility of this concept (Clauwaert et al. 2007; Mao et al. 2010; Rabaey et al. 2008; Rismani-Yazdi et al. 2008), but the field is still in its infancy.

3 Fuel Cell Testing

Being a relatively new field of great dynamics, the current practice in microbial fuel cell research still lacks the standardization and established characterization procedures that are now common practice in e.g., the field of conventional fuel cells for automotive and power generation applications [see for instance "The Fuel Cell Testing and Standardisation Network" (Tsotridis et al. 2006)]. The following section aims at providing guidelines to obtain significant, meaningful, and comparable performance data of microbial fuel cells. Furthermore, some dedicated testing device architectures for microbial fuel cells are discussed. Besides the recording of polarization curves, other electrochemical methods such as cyclic voltammetry or impedance spectroscopy can provide more detailed insight into electrode processes and loss mechanisms. However, their description is beyond the scope of this chapter. For a detailed treatise on the application of these techniques, the reader is thus referred to specialized textbooks on electrochemistry (Hamann et al. 2007) and in particular the respective chapters in the book "Bioelecrochemical systems" (Rabaey et al. 2010).

3.1 Electrode Polarization: Insight into Loss Mechanisms

Besides the determination of the fuel cell's maximum power output, the detailed analysis of polarization curves also allows for further insight into the loss mechanisms that affect the power output of the complete fuel cell. Analysis of the polarization behavior of the individual electrodes, furthermore, allows for identification of the limiting electrode reaction, a prerequisite for systematic optimization.

In Fig. 5, the typical polarization behavior of a complete fuel cell and its individual electrodes (potential recorded against a reference electrode) are shown schematically. Starting from their respective open circuit potential (at zero current) the anode is polarized toward more positive potentials, whereas the cathode is polarized toward more negative potentials with increasing current. Consequently, the overall cell voltage (difference between cathode and anode potential) decreases. As shown in Fig. 5, the polarization curve can be divided into three regions, in which different loss mechanisms dominate:
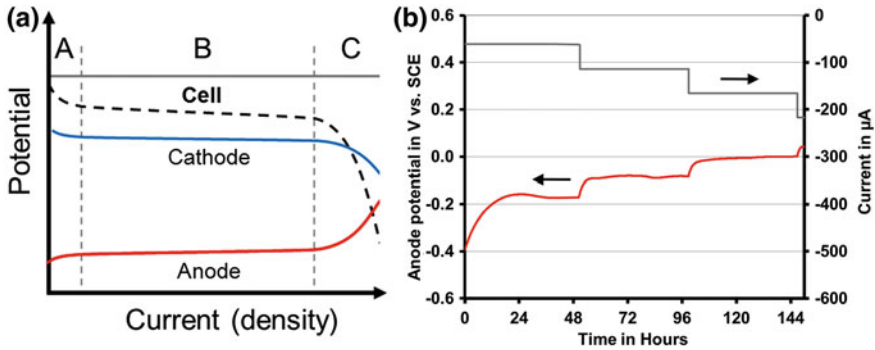
Fig. 5 a Schematic representation of the polarization behavior of a complete fuel cell and its individual electrodes. With increasing current (density) losses occur due to: A activation overpotential, B ohmic resistances, C mass-transport limitations. **b** Evolution of the anode potential (vs. the standard calomel electrode, SCE) during the recording of a polarization curve with stepwise increased load current. Activated carbon cloth anode with 2.25 cm^2 geometric area and *S. oneidensis* under anoxic conditions (Kipf et al. 2011)

- Region A: the pronounced voltage drop at low current densities is caused by the *activation overpotential* necessary to drive the electrochemical reactions at the electrode. These activation losses are related to the catalytic activity of the electrode (materials), e.g., a cathode with a better electrocatalyst will show less activation polarization.
- Region B: with increasing current *ohmic resistances* within the fuel cell dominate the overall polarization and the polarization curve shows a linear dependence between cell voltage and current. These ohmic resistances either result from the ionic resistance of the electrolyte (large spacing between the electrodes) or from electronic resistance within the electrodes or fuel cell connection cables. It can be lowered e.g., by reducing the space between the electrodes or using highly conductive electrode materials.
- Region C: with a further increase in current a sharp drop in cell voltage is observed, which is generally attributed to *mass-transfer* losses. These result from insufficient reactant supply due to limited diffusion or convection, or from the limited turnover number of an enzymatic reaction. The reactant supply at the cathode could be improved for instance by using *air-breathing* instead of *submerged* cathodes (see Sect. 2.1).

An important parameter in the recording of polarization curves is the load change rate. Although the slow load change response of microbial fuel cells is well documented in published experimental results (Fischback et al. 2006; Logan et al. 2006; Rhoads et al. 2005; Walker and Walker 2006), researchers often do not consider that under such conditions a too fast recording of polarization curves leads to an overestimation of performance. This can be prevented when the polarization curve is recorded in a stepwise technique (Kerzenmacher et al. 2009), as shown exemplarily in Fig. 5b for *Shewanella oneidensis* growing on a carbon

anode (Kipf et al. 2011). Due to the slow load change response, it can take up to several hours until the anode potential stabilizes. The stepwise recording of the polarization curve allows identifying the time period after which the fuel cell electrode potentials stabilize under load, and enables the assessment of actual performance values. Furthermore, the performance sustainability can be quantified by calculating the voltage drift (degradation) over a given time period.

3.2 Techniques for the Recording of Polarization Curves

A commonly employed technique to record polarization curves is the use of variable load *resistors*, sometimes combined with the measurement of individual electrode potentials against a reference electrode (Kerzenmacher et al. 2009; Logan et al. 2006). This technique can be easily implemented in any laboratory at low cost, but mandates that always a complete fuel cell with anode and cathode is assembled. This is usually no disadvantage when the characterization of a complete system is intended. However, for the optimization of single fuel cell components, such as the development of an optimized anode structure or the comparison of the current-generating capabilities of different exoelectrogenic organisms this can become time consuming and costly. Furthermore, with a passive resistor load the fuel cell can only be operated until the overall cell potential reaches zero. This implies that great care must be taken to prevent that other components of the fuel cell, e.g., membrane resistance or cathode performance do no limit overall performance before the operational limit of e.g., the anode is reached. This can be circumvented by using *potentiostats*, which enable the forced operation of half-cell electrodes against arbitrary counter electrodes. They allow for the precise control of either cell or electrode potential (potentiostatic mode) or load current (galvanostatic mode) to record polarization curves, both techniques leading to comparable results (Hamann et al. 2007). The galvanostatic technique is particularly useful to maintain a defined reactant consumption rate at the electrodes, and thus to investigate mass-transfer related effects. However, potentiostats provide more functionality than required for the automated recording of polarization curves to perform e.g., cyclic voltammetry or impedance spectroscopy experiments. Consequently, they are only available at comparably high cost, and most research budgets thus do not allow for the procurement of a large number of these devices to establish a parallel characterization environment (Kerzenmacher et al. 2009).

Nevertheless, most experiments with microbial fuel cells are time consuming. For time-efficient characterization and development it is, thus, essential to perform multiple experiments in a highly parallel fashion. One reason is their slow load change response which mandates that polarization curves are recorded at sufficiently slow scan rates to prevent performance overestimation (see Sect. 3.1). In addition, experimental times up to several weeks or months can be required until a stable biofilm or microbial consortium forms at the electrodes. Several research groups thus
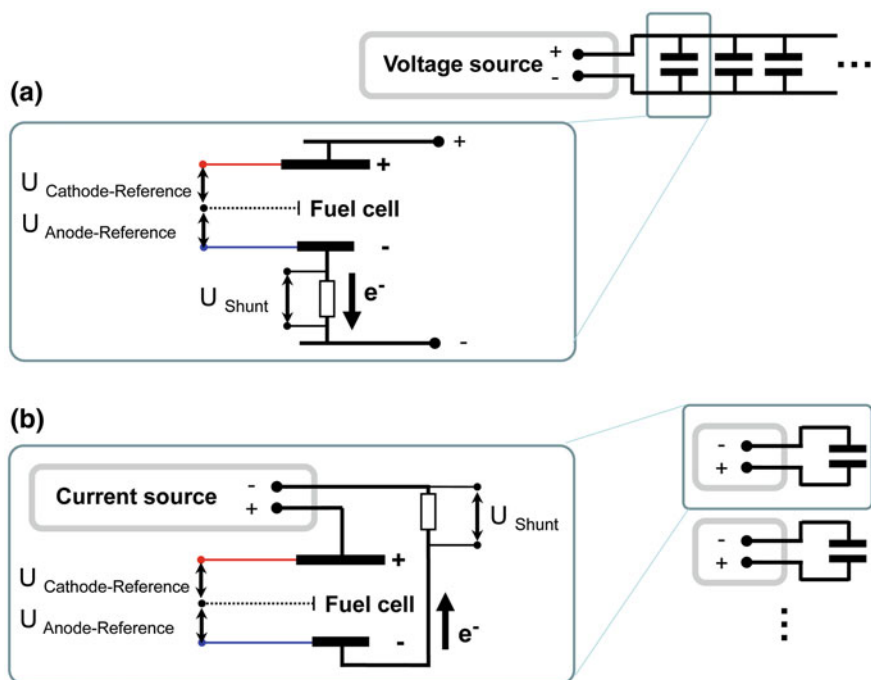
Fig. 6 Two concepts for highly parallel testing devices. **a** Multiple fuel cells are connected in parallel to a single power supply. **b** Each fuel cell is connected to its own current source (galvanostat). In both cases a reference electrode is used to measure the potentials of anode and cathode separately. The voltage drop across a shunt resistor in the circuit is used to measure the fuel cell current. See text for further explanations

developed dedicated testing setups, specifically designed for the intended task of performing many (microbial) fuel cell polarization experiments in parallel.

One of them is the setup for high-throughput material screening developed by Bruce Logan and co-workers (Call and Logan 2011). As shown in Fig. 6 a, it features a standard laboratory power supply as voltage source, to which a number of electrochemical cells (either fuel cells or half-cells) are connected in parallel. The cells themselves consist of small glass vials into which anode, cathode, and optionally a reference electrode are inserted. When a constant voltage is applied a current will flow through each of the cells connected in parallel. Its magnitude depends on the internal resistance of each cell, and is recorded by measuring the voltage drop over a shunt resistor. Polarization curves can be recorded by varying the external applied voltage and measuring the resulting electrode potentials against a reference electrode. The system can work with both, complete fuel cells or mere half-cells where only the electrode of interest is placed into the vial together with an arbitrary counter electrode. Its advantage is its high scalability at low cost: with a single power supply several thousand cells may be operated in

parallel, depending on its current capability (Call and Logan 2011). However, with all the electrochemical cells connected in parallel they are always subjected to the same experimental procedure. Furthermore, the system does not allow for keeping the load current through each cell at a constant value, which can be of relevance when investigating mass-transfer related phenomena.

A more flexible, but also more costly setup is depicted schematically in Fig. 6b (Kerzenmacher et al. 2009). It comprises a number of individually controllable electronic loads, through which a defined load current can be applied to complete fuel cells (or alternatively half-cells with arbitrary counter electrodes). A data acquisition unit is used to individually record the fuel cells' electrode potentials against reference electrodes. The system is fully computerized and features galvanic isolation between the individual channels, which ensures interference-free operation of multiple fuel cells immersed in a common testing solution. This can be of advantage when e.g., a high degree of comparability between the individual experiments is required or when the testing medium itself requires elaborate control mechanisms to keep parameters such as pH and substrate concentration constant.

4 Application Examples

In the following section, some typical application examples for microbial fuel cells are presented. The characteristics and power densities of some microbial fuel cells are compared in Table 2.

4.1 Waste Water Treatment

Waste water treatment combined with electricity generation—this application of microbial fuel cells is probably the most prominent and fascinating for both scientists and non-scientists. In the literature, a number of examples for the treatment of waste water are reported, including landfill leachate (Puig et al. 2011), rice mill waste water (Behera et al. 2010b), municipal sewage (Hays et al. 2011; Lefebvre et al. 2011), and even solid waste (Lee and Nirmalakhandan 2011). Pilot scale plants have already been realized for the application with waste water from a brewery (Logan 2010).

Usually, these systems do not operate with a pure culture or microorganism, but with naturally enriched consortia. Sometimes an inoculum from an already operating microbial fuel cell is used to speed up the formation of a stable biofilm. The anodic community of a microbial fuel cell can differ significantly depending on the type of waste water used (Kiely et al. 2011). In Fig. 7, the bacterial communities of microbial anodes operated with domestic waste and waste water from a winery are compared (Cusick et al. 2010).

Table 2 Some selected characteristics and power densities of microbial fuel cells

| Reference	Electrodes	Substrate	Power density	Remarks
Pure culture systems				
(Watson and Logan 2010)	Anode: carbon fiber brush with S. oneidensis MR-1 Cathode: platinum on carbon	Lactate	332 mW m^{-2} (bottle reactor)	Power density normalized to the projected cathode area, anode much larger than cathode
(Xing et al. 2008)	Anode: carbon fiber brush with Rhodopseudomonas palustris DX-1 Cathode: platinum on carbon	Acetate	2,720 mW m^{-2} (86.6 W m^{-3})	Power density normalized to the projected cathode area, anode much larger than cathode
(Nevin et al. 2008)	Anode: solid graphite with G. sulfurreducens Cathode: platinum on carbon	Acetate	1,900 mW m^{-2} (43 W m^{-3}); Optimized anode: (2.15 kW m^{-3})	Power density normalized to the projected anode area, anode much smaller than cathode
Mixed consortia				
(Watson and Logan 2010)	Anode: carbon fiber brush with waste water inoculum Cathode: platinum on carbon	Lactate	559 mW m^{-2} (bottle reactor) 858 mW m^{-2} (cubic reactor)	Power density normalized to the projected cathode area, anode much larger than cathode
(Cheng et al. 2006)	Anode: carbon cloth with waste water inoculum Cathode: carbon cloth	Glucose	766 mW m^{-2}	Power density normalized to the projected cathode area, equally sized anode
(Shimoyama et al. 2008)	Anode: graphite felt with soil inoculum Cathode: platinum on carbon	Model organic wastewater	899 mW m^{-2} (129 W m^{-3})	Power density normalized to the projected anode area or volume anode, equally sized cathode (flat-plate type)

4.2 Energy-Autonomous Power Supply Systems

A promising application example is the realization of energy-autonomous sensor nodes, powered by benthic microbial fuel cells embedded within the marine sediment (Donovan et al. 2008; Nielsen et al. 2008; Tender et al. 2008). Recently, Tender et al. reported the first demonstration of such a microbial fuel cell in which sufficient electrical power to supply a meteorological buoy was generated (Tender et al. 2008). Their fuel cell was constructed from graphite-plate anodes embedded in the marine sediment and a graphite brush cathode positioned in the overlying water. It delivered 36 mW of continuous electrical energy (16 mW m^{-2} per geometric anode surface) and supplied a set of sensors (temperature, air pressure, and relative humidity) as well as a low-power line-of-sight RF transceiver, which transmitted the data in 5-min intervals. From an economical point-of-view, the concept is attractive, since even in the prototype state the microbial fuel cell's cost is comparable to the cost of changing a conventional battery once a year.

Microbial fuel cells have also been applied to power autonomous robots that feed from the environment. Kelly (2003) first presented their slugbot in 2003 as a robotic predator that autonomically collects snails and carries them to a central fermenter unit. Here, the snails are "digested" in a microbial fuel cell, and the generated electricity is in turn used to recharge the battery packs of the robots. While in this first design the microbial fuel cell had to be stationary due to its size and weight, a later robot called "Eco-BotII" was powered by several onboard microbial fuel cells operating on fuels such as sugar, fruit, and insects (Melhuish et al. 2006). The same research group also suggested the use of microbial fuel cells as power supply for energy-autonomous underwater robots (Ieropoulos et al. 2007).

4.3 Miniature Microbial Fuel Cells, Microbial Sensors and Biobatteries

At present, also a number of miniature microbial fuel cells in mL and μL scale are being developed (Biffinger et al. 2007). Potential applications include e.g., on-chip power supply for lab-on-a-chip systems and microfluidic devices (Wang et al., 2011). Researchers also envision the development of body-implantable microbial fuel cells, situated either in the human body tissue (Wang et al. 2011) or the intestine (Han et al. 2010). However, biocompatibility issues and the associated risk of infection are clearly obstacles for the practical realization of these concepts.

In future, microbial fuel cell technology may be used for the development of biobatteries, intended as power source for mobile devices or distributed sensor networks or in general as an alternative to today's chemical batteries. Biobatteries may also be constructed from fully biodegradable, nontoxic, and low-price materials. Together with advances in biodegradable electronics (Bettinger and Bao
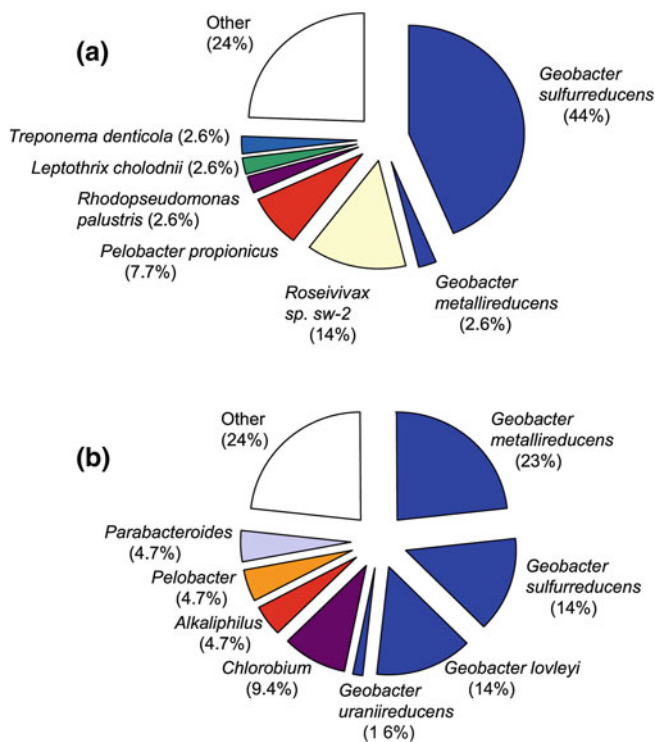
Fig. 7 Anodic bacterial communities of microbial anodes operated with **a** winery waste water and **b** domestic waste water (Cusick et al. 2010). Reprinted from International Journal of Hydrogen Energy 35 (17), Roland D. Cusick, Patrick D. Kiely, Bruce E. Logan, A monetary comparison of energy recovered from microbial fuel cells and microbial electrolysis cells fed winery or domestic wastewaters, 8855–8861, Copyright (2010), with permission from Elsevier

2010) this may pave the way for environmentally friendly disposable distributed sensors ("smart dust") that automatically dissolve after their intended time of operation, and thus do not pollute the environment.

Microbial fuel cells can also serve as sensors (Su et al. 2011) for toxic substances or parameters such as organic carbon or biological oxygen demand (Chang et al. 2004; Di Lorenzo et al. 2009; Kim et al. 2009; Peixoto et al. 2011).

5 Challenges and Future Trends

5.1 Materials, Design, and Testing

Cost is still a major issue when it comes to the practical and commercially successful application of microbial fuel cells. In a recent study, the total acceptable

cost for microbial fuel cell system to become an economically viable option was estimated to be below $\sim 4{,}000$ € per kW. Assuming 2 W m^{-2} as feasible power density of microbial fuel cells in waste water, this corresponds to a maximum cost of ~ 8 € per m^2, including electrodes, membranes, and casings, as well as auxiliaries such as pumps (Sievers et al. 2010). When comparing this figure to the price of a Nafion membrane in the range of several hundred € per m^2, it becomes clear that new and less costly concepts to construct microbial fuel cells with improved performance are needed. This is not only limited to finding new materials for electrodes and membranes, but also casings, cables, and pumps have to be considered.

Furthermore, a microbial fuel cell is worthless without the bacteria actually doing the job. Besides finding new, more powerful organisms and consortia it is necessary to gain a better understanding of the metabolic principles behind microbial electron transfer and electricity generation (Bucking et al. 2010; Nevin et al. 2009; Newton et al. 2009; Schuetz et al. 2009). This may at some point allow researchers to develop synthetic organisms optimized for electricity generation (Nevin et al. 2009; Rosenbaum et al. 2010) from a variety of substrates in a microbial fuel cell. Last but not least, low-cost fuel cell design, operation strategies for improved performance and long-term stability, as well as power conditioning to step up the relatively low fuel cell voltage to grid-compatible levels will be gaining importance on the road toward practical application. Examples are the low-cost microbial fuel cell made from an earthen pot in place of the costly proton exchange membrane (Behera et al. 2010a), or the use of oxygen at the anode to boost current generation by *Shewanella oneidensis* (Rosenbaum et al. 2010).

Probably most important, some standardization is necessary to ensure that meaningful and significant data is obtained that allow for a critical comparison between the results obtained in different laboratories (see Sect. 3). In a nutshell: *use reference electrodes, report polarization data for the individual electrodes, and record polarization curves with a sufficiently low scan rate to prevent overestimation of performance.*

5.2 Beyond Power: Other Applications of Bioelectrochemical Systems

Besides the generation of electricity, microbial fuel cells or more generally bioelectrochemical systems are increasingly considered for other applications (Lovley and Nevin 2011). These include microbial electrolysis cells (Cheng and Logan 2011; Cusick et al. 2011), where an additional voltage is supplied to the fuel cell so that instead of oxygen protons are reduced at the cathode, leading to the production of hydrogen gas. Also desalination cells based on microbial fuel cell concepts have been successfully used to generate fresh water with a lower energy demand than conventional technologies (Kim and Logan 2011). Furthermore, a

bioelectrochemical system can be used to e.g., reduce or immobilize pollutants such as nitrate or uranium in soil or ground water (Gregory et al. 2004; Gregory and Lovley 2005), or to fixate CO_2 and produce valuable organic compounds (Nevin et al. 2011).

In summary, a number of researchers are now of the opinion that successful future application of microbial fuel cells will not only depend on the power output of these systems, but also on additional benefits and added values.

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