# Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency

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The performance of a microbial fuel cell (MFC) depends on a complex system of parameters. Apart from technical variables like the anode or fuel cell design, it is mainly the paths and mechanisms of the bioelectrochemical energy conversion that decisively determine the MFC power and energy output. Here, the electron transfer from the microbial cell to the fuel cell anode, as a process that links microbiology and electrochemistry, represents a key factor that defines the theoretical limits of the energy conversion. The determination of the energy efficiency of the electron transfer reactions, based on the biological standard potentials of the involved redox species in combination with the known paths (and stoichiometry) of the underlying microbial metabolism, is an important instrument for this discussion. Against the sometimes confusing classifications of MFCs in literature it is demonstrated that the anodic electron transfer is always based on one and the same background: the exploitation of the necessity of every living cell to dispose the electrons liberated during oxidative substrate degradation.

## 1. Introduction

Bioelectrochemical fuel cells are electrochemical devices that exploit biological components (often referred to as biocatalysts) to facilitate the generation of electricity. The key advantages of biological fuel cells in comparison to conventional fuel cells are the mild operation conditions (ambient temperature, near to neutral pH) and the virtually unlimited range of potential fuels, for the oxidation of which we lack suitable electrocatalysts. Biofuel cells can be classified into microbial and enzymatic fuel cells that use either whole, living microorganisms or isolated redox enzymes as their respective biocatalysts. The different nature of these biocatalysts leads to essentially different properties and fields of application of enzymatic and microbial fuel cells (MFCs). Thus, the specificity of isolated enzymes allows the abandonment of the conventional separation of the anode and cathode compartment and the miniaturization of enzymatic fuel cells.[1,2] On the other hand, the liability of the isolated enzymes to denaturation and deactivation forbids the use of these fuel cells in harsh environments like sewage. These environments are a major domain of MFCs,[3–5] the emphasis of this publication.

It was 1911 when the occurrence of an electromotoric force between electrodes immersed in bacterial or yeast cultures and in sterile medium in a battery type setup was reported by Michael C. Potter.[6] In this communication Potter came to the conclusion that electric energy can be liberated from the microbial disintegration of organic compounds. Twenty years later Cohen confirmed these results and reported a stacked bacterial fuel cell delivering a voltage of 35 V at a current of 0.2 mA.[7] Although these publications may be considered as the hours of birth of MFCs, it was not until the 1960s that the idea of microbial electricity generation was picked up again, in the framework of the NASA space program, as an opportunity to recycle human waste to electricity during space flights, see *e.g.*, ref. 8. During these research efforts, the complexity of the underlying bioelectrochemical processes became evident, and systematic and long term research programs were demanded.[9,10] Yet, the rapid advances in other energy technologies, as in the field of photovoltaic power generation, again lead to a decreasing interest in MFCs. The latest and most remarkable revival of MFC research began at the end of the 20th century. This ongoing and still increasing upswing is certainly driven by the growing awareness of exhausting resources of fossil fuels, the emerging environmental consequences of their use and of the necessity to develop technologies for a sustainable handling of our environment and our planet's resources.

The course of the development of MFC technology is documented in a series of detailed review papers, *e.g.*, ref. 9–18, that illustrate the search for ways to 'wire' the microbial activity to the fuel cell anode, that is, to transfer electrons from the microorganism to the fuel cell anode. Why is this so difficult? Microorganisms are evolutionarily not designed to dispense energy to power a fuel cell—the majority of relevant redox processes take place buried within the microbial cells, and it is a great challenge and a major research issue to find means to efficiently divert electrons from the metabolism to a fuel cell anode. Various approaches have been proposed. They differ in the nature and the mechanism of the electron transfer from the microorganism to the fuel cell anode. But what is the best, the most promising metabolic path and electron transfer mechanism to produce electricity? Is the total oxidation of a given substrate (the fuel), combined with a quantitative

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electron transfer inevitably a measure for the superiority of a concept,[18,19] regardless of the constraints of the respective microorganisms to low molecular substrates, based on their incapability to utilize complex matter?

So far, no attempts have been made to characterize and compare the electron transfer mechanisms from the viewpoint of their theoretical energy efficiency. Admittedly, this is in fact a difficult endeavor. Many proposed transfer mechanisms are of putative nature and are controversially discussed. Often, the involved redox species are only insufficiently studied and electrochemical data do not exist. Moreover, nowadays the prevailing use of microbial mixed cultures in MFC research (technologically an undoubtedly groundbreaking approach) makes an exact analysis of single electron transfer processes extremely difficult if not impossible.

In this paper, an attempt is made to summarize known electron transfer processes in MFCs and to discuss these processes on a mechanistic and a thermodynamic basis, in order to evaluate their potential to produce electric energy. Here, a comparison of experimental MFC performance data is consciously abstained from, as these data are additionally governed by operational parameters based on the respective fuel cell designs.

This paper is not intended to provide a complete, comprehensive survey. It is rather intended as a critical review, an initiative for a critical discussion and evaluation of the electron transfer processes in MFCs, and as a call for intensified efforts in the fundamental research of these processes.

## 2. Basic considerations

### 2.1 Microbial fuel cells—definitions and general remarks

Generally speaking, a MFC is an electrochemical device in which microbially produced reduction equivalents are utilized to deliver electrons to a fuel cell anode. In the widest sense this definition includes systems in which the microbial reactor is separated from the electrochemical cell, the primary, electrochemically inactive fuel being converted by the microorganisms into a secondary fuel that is separated from the microbial environment and subsequently burned in a conventional fuel cell.[15,20] An example is the fermentative hydrogen generation with the subsequent hydrogen oxidation in a conventional polymer electrolyte membrane (PEM) fuel cell. Such systems are usually referred to as indirect MFCs.

During the last few years, a more restrictive definition has prevailed. It considers only systems, in which the current generating electron transfer process takes place within the microbial environment, as genuine MFCs.[17] As illustrated in Fig. 1, the biocatalyst is located in the anodic fuel cell compartment facilitating the oxidation of the substrate (fuel) as well as the transfer of the liberated electrons to the anode. The anodic performance is inextricably dependent on (i) the nature and the rate of the anaerobic metabolism, and (ii) the nature and the rate of electron transfer from the microbial cell to the anode.

What is the actual task of a microorganism in a MFC? Taking in mind common MFC definitions like: ''Microbial fuel cells (MFCs) are devices that use bacteria as the catalysts to oxidize organic and inorganic matter and generate current''[17] this question may sound trivial. Yet, to understand the mechanisms of current generation, the role of the microorganism has to be more explicitly named: the fundamental task of the microbial cell is to transform an electrochemically inactive substrate (fuel) and its contained chemical energy into a form that is accessible for electrochemical oxidation and thus for conversion into electric energy. For this transformation we make use of the microbial metabolism, or, to be more precise, the microbial catabolism—the energy liberating substrate degeneration.

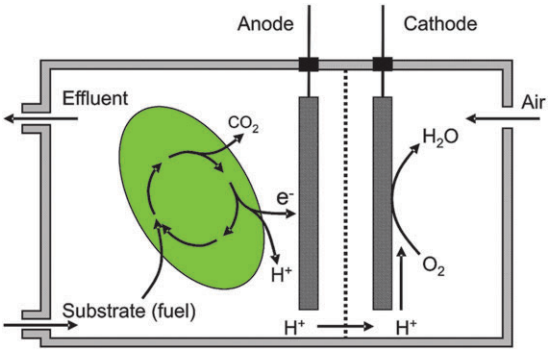



**Fig. 1** Schematic illustration of a microbial fuel cell.

Before discussing the mechanisms *via* which microorganisms and their metabolism can be utilized to produce electricity, the following section will provide a brief, schematic introduction to relevant processes of microbial energy conversion.

### 2.2 Biological energy conversion

Heterotrophic organisms gain the energy for their life from the oxidation of organic compounds or, more precisely, from the free (Gibbs) energy, $\Delta G_{ox}$ of their oxidation. Depending on the involvement of exogenous oxidants (external terminal electron acceptors) two major metabolic pathways can be distinguished: respiration and fermentation. During respiratory substrate oxidation the liberated electrons are transferred *via* a redox cascade, the respiratory chain—their energy gradually decreasing—and are finally transferred to an externally available terminal electron acceptor. The more positive the redox potential of a terminal electron acceptor (with a given substrate—the electron donor), the higher the energy gain for an organism

$$\Delta G^{\ominus\prime} = nF[E^{\ominus\prime}(\text{donor}) - E^{\ominus\prime}(\text{acceptor})] \qquad (1)$$

($E^{\ominus\prime}$ represents the respective biological standard potential; $\Delta G^{\ominus\prime}$ denotes the change of free energy under biological standard conditions.)

Aerobic respiration is the path with the highest energy gain (see, for example, reaction (1a)), but of course it is bound to environments, in which oxygen is available.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 \quad \Delta G^{\ominus\prime} = -2895\ \text{kJ mol}^{-1} \qquad (1a)$$

Under anoxic conditions, specialized facultative or obligate anaerobes utilize other, inorganic or organic, exogenous compounds as terminal acceptors for an anaerobic respiration. Examples for such electron acceptors are nitrate, sulfate,

**Table 1** Biological standard potentials[a] of selected biological electron donors and electron acceptors

| Redox couple | $E^{\ominus\prime a}$/V |
|---|---|
| $CO_2$/Glucose | −0.43[23] |
| $CO_2$/Formate | −0.43[23] |
| $2H^+/H_2$ | −0.42[23] |
| $CO_2$/Acetate | −0.28[23] |
| $CO_2/CH_4$ | −0.24[23] |
| $SO_4^{2-}/HS^-$ | −0.22[23] |
| Pyrovate/lactate | −0.19[23] |
| Fumarate/succinate | +0.33[23] |
| $NO_3^-/NO_2^-$ | +0.43[23] |
| $MnO_2/Mn^{2+}$ | +0.60[24] |
| $Fe^{3+}/Fe^{2+}$ | +0.77[23] |
| $1/2O_2/H_2O$ | +0.82[23] |
| $1/2O_2/H_2O$ | +0.51[25,26b] |

[a] Standard potential, measured at pH 7. [b] Effective (irreversible) potential, determined in MFC experiments (pH 7).

carbon dioxide (methanogenesis), metal ions ($Fe^{3+}$) and fumerate.[21–23] Due to the less positive redox potentials of these oxidants (see Table 1) the energy gain for the organisms are usually considerably lower compared to aerobic respiration.

In the absence of exogenous oxidants, many microorganisms perform fermentation, a biological form of disproportionation, in which parts of the organic substrate serve as electron acceptor and become reduced, whilst other parts are oxidized.[21] As demonstrated in reactions (2) and (3) for the fermentation of glucose to butyrate or acetate,[23] fermentation it is the path with the lowest energy gain for the organisms. In these cases, only 7–8% of the energy content of glucose can be used by the organisms.

$$C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2CO_2 + 2H_2 \qquad \Delta G^{\ominus\prime} = -225\ \text{kJ mol}^{-1} \qquad (2)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \qquad \Delta G^{\ominus\prime} = -206\ \text{kJ mol}^{-1} \qquad (3)$$

This low energy utilization is accompanied by an incomplete substrate oxidation, leaving the major energy content (>90%) of the substrate unused, in the form of energy rich fermentation products.

**2.2.1 Primary and secondary metabolism.** The characterization of the electron transfer mechanisms, discussed later in this paper, requires a differentiation of the species involved in the electron transfer by means of their origin and relevance for the microbial metabolism. Basically, we can distinguish between primary and secondary metabolites, *i.e.*, products of the primary and secondary metabolism, respectively.[21] Primary metabolites are compounds that are essentially connected to the microbial metabolism. They are similar in all groups of living organisms. Relevant for this publication are the major products of the catabolic substrate degradation, such as fermentation products and reduced electron acceptors.

Secondary metabolites on the other hand are usually not directly connected to the main metabolic pathways. Microorganisms as well as all higher organisms produce a great variety of these compounds. They may be specific for a certain organism and may serve very different purposes, such as intercellular communication, antibiotics, *etc.*[27]

### 2.3 Basic requirements for the establishment of an anodic electron transfer—"the link"

What are the basic requirements for the establishment of an electron transfer from a microbial cell to an electrode? Since electrodes are solid entities that cannot penetrate the bacterial cells, a major requirement is that electrons are to be transferred from the inside of the microbial cell membrane to its outside—either *via* the physical transfer of reduced compounds, or *via* electron hopping across the membrane using membrane bound redox enzymes. Regardless of the mechanism, the electron transfer outside of the cell must lead to a redox active species that is capable of electronically linking the bacterial cell to the electrode. This species may, for example, be a soluble redox shuttle, an outer membrane redox protein or a reduced primary metabolite. One may refer to such species as a *mediator*—probably the most adequate expression. Yet, as discussed later in this manuscript, this term is already assigned to electron transfer mechanisms involving diffusional redox shuttles. To avoid confusion, in this publication the respective species will be denoted as *linking species*, or *link*, in order to point out its role in the facilitation of electron transfer.

For an efficient electron transfer the linking species must fulfil the following requirements:

(i) It must be able to physically contact the electrode surface.

(ii) It must be electrochemically active, *i.e.*, it must possess a low oxidation overpotential at given electrode surfaces.

(iii) The standard potential of the linking species, $E^{\circ\prime}_{\text{link}_{ox}/\text{link}_{red}}$, should be as close to the redox potential of the primary substrate, $E^{\circ\prime}_{\text{substr}}$, as possible, or must at least be significantly negative to that of the oxidant (usually oxygen).

The following sections will show that very different mechanisms of electron transfer (involving very different linking species) have so far been identified and exploited. For a basic classification it has to be distinguished whether the linking species—the species that facilitates the electron transfer from the microbial cell to the fuel cell anode—is a soluble compound or is bound to the microbial cell membrane. Similarly to enzymatic fuel cells,[1] it can be distinguished between direct electron transfer (DET), which proceeds *via* membrane bound redox proteins (without the involvement of dissolved species), and mediated electron transfer (MET), which is based on dissolved redox species.

At this point it has to be noted that the term "mediator-less microbial fuel cell", as used in many publications *e.g.*, ref. 28–31 does not necessarily indicate DET as the prevailing electron transfer path. It rather indicates that no artificial redox mediators[15,32–34] are used to facilitate the electron transfer. It does not, however, rule out electron transfer mediation *via* bacterial electron shuttling compounds.

### 2.4 Evaluation of the energy efficiency of anodic electron transfer pathways—basic assumptions

In biotechnological processes (including MFCs) microorganisms are denoted as *biocatalysts*. Referring to MFCs, the use of this term is, strictly speaking, wrong. The definition of a catalyst implies that the catalyst does not appear in the balance of the catalyzed reaction. Yet, in a MFC this is

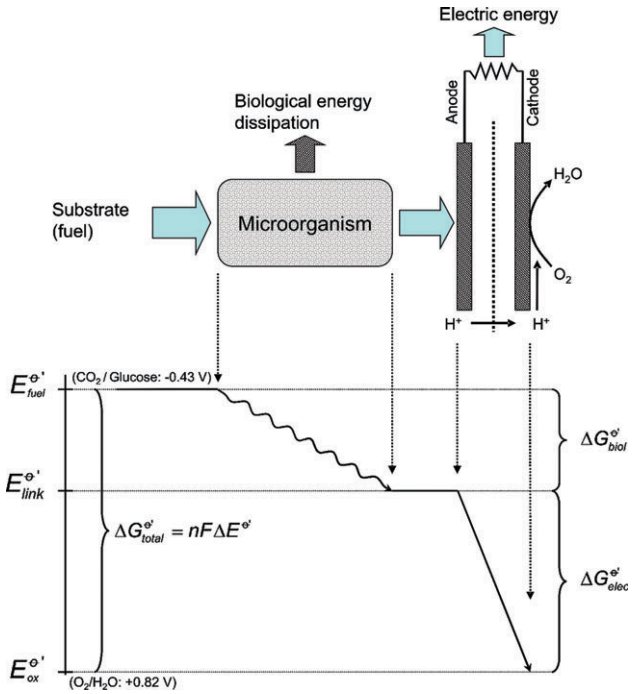



**Fig. 2** Schematic drawing of the energy flux in a microbial fuel cell.

precisely not the case—the microorganism decisively determines the balance of the reaction.

As depicted in Fig. 2, the microorganism may facilitate the conversion of the chemical energy of a substrate into electricity, but it retains a distinct portion of the Gibbs free energy, $\Delta G_{\text{biol}}$, for its own surviving and reproduction (eqn (2)):

$$\Delta G^{\ominus'}_{\text{elec.}} = \Delta G^{\ominus'}_{\text{total}} - \Delta G^{\ominus'}_{\text{biol}} \quad (2)$$

For this reason, the biological energy conversion in a MFC should be referred to as *biotransformation* rather than *biocatalysis*.

The energy gain for the microorganism (hence, the loss of electric energy for a MFC) is by all means wanted and necessary. It allows the maintenance of the bacterial vitality (a prerequisite for the long-term fuel cell operation) and is often the driving force (the wage, so to speak) for the organism to follow certain electron transfer mechanisms. On the other hand, if the biological energy gain becomes too large, the electric energy output may become minute and, instead, stronger cell growth and unwanted biomass formation is likely to occur. As a consequence, suitable metabolic and electron transfer paths have to be found that, at the same time, allow sustainable MFC operation and maximum electric energy output.

In the following sections different known electron transfer mechanisms will be described and discussed with respect to the expected output of electric energy of a respective MFC. This discussion is based on a thermodynamic viewpoint, neglecting fuel cell losses based on ohmic resistances, concentration polarization and kinetic constraints. For the sake of simplification the calculations are based on biological standard or formal potentials of the involved biological and electrochemical redox processes. A more accurate evaluation may require the incorporation of the concentration terms of the Nernst equation, an endeavor that is, at the current stage, difficult to accomplish, since many involved redox processes and species are so far only insufficiently investigated and characterized. The incorporation of the concentration term, however, is not expected to change the overall picture of the anodic electron transfer.

For the characterization of the energy efficiency of the electron transfer mechanisms the full oxidation of glucose with oxygen as the oxidant (reaction (1)) will be used as the reference reaction. The standard free energy of the oxidation of glucose, based on the biochemical standard potentials (pH 7, 25 °C) of −0.43 V for glucose/$CO_2$ and +0.82 V for the oxygen electrode is −2895 kJ per mole glucose. Under practical MFC conditions, the standard potential of the oxygen electrode of 0.82 V cannot be reached. Due to the occurrence of side reactions at the cathode (usually involving impurities in the electrolyte and at the electrode surface) mixed potentials are formed that are generally considerably lower than the expected standard potential.[35] Thus, in MFC studies, effective potentials of the oxygen electrode of usually not higher than 0.510 V (pH = 7, 25 °C, 50 mM phosphate buffer) have been reported.[25,26] Since this potential is more realistic for practical oxygen electrodes, the following discussion will be based on this value as a reference to evaluate given electron transfer mechanisms towards their energy efficiency. Compared to the reversible standard potential, the lower redox potential of experimental oxygen cathodes reduces the free energy of glucose oxidation (eqn (3)) by 25%, to −2176.7 kJ $mol^{-1}$.

$$\Delta G^{\ominus'} = nF[E^{\ominus'}(\text{glucose, } CO_2) - E_{\text{effective}}(O_2, H_2O)] \quad (3)$$

($n$ is the number of electrons (24 for the full oxidation of glucose); $F$ is the Faraday constant)

As depicted in Fig. 2, the amount of electric energy, $\Delta G_{\text{elec}}$, to be drawn from the oxidation of a given substrate, is a function of $\Delta G_{\text{total}}$ diminished by the biological energy dissipation, $\Delta G_{\text{biol}}$ (eqn (2)). $\Delta G_{\text{elec}}$ and $\Delta G_{\text{biol}}$ are connected to each other *via* the redox potential of the *linking species*, $E^{\ominus'}_{\text{link}_{ox}/\text{link}_{red}}$ according to the eqn (4a) and (4b).

$$\Delta G_{\text{biol}} = nF[E^{\ominus'}(\text{glucose, } CO_2) - E^{\ominus'}(\text{link})] \quad (4a)$$

$$\Delta G_{\text{elec}} = nF[E^{\ominus'}(\text{link}) - E_{\text{effective}}(O_2, H_2O)] \quad (4b)$$

In eqn (4a) and (4b) the variable $n$ denotes the total number of electrons transferred *via* a given electron transfer path.

For glucose, the maximum number of electrons transferable is 24, corresponding to a coulombic efficiency of one. Based on the nature of the microbial metabolism, often only a limited number of electrons are transferable *via* a certain mechanism. Here, $n$, and thus the expected coulombic efficiency, is considerably lower. In the following sections, this circumstance will be taken into consideration by assuming theoretical coulombic efficiencies. For an evaluation of the electron transfer independently of the total energy yield (independent on $n$) the value $\Delta G_{\text{elec, } n=1}$ will be used to denote the free energy for the transfer of one electron (eqn (5)).

$$\Delta G_{\text{elec, } n=1} = F[E^{\ominus'}(\text{link}) - E_{\text{effective}}(O_2, H_2O)] \quad (5a)$$

All redox potentials in the following sections refer to biological conditions (pH 7). Thus, biochemical standard potentials ($E^{\ominus\prime}$) or formal potentials ($E'$) of the respective redox species are used, which represent the standard potentials at pH 7.

## 3. Electron transfer in microbial fuel cells—discussion

### 3.1 Direct electron transfer (DET)

The direct electron transfer takes place *via* a physical contact of the bacterial cell membrane or a membrane organelle with the fuel cell anode, with no diffusional redox species being involved in the electron transfer from the cell to the electrode. Since living cells are generally assumed to be electronically non-conducting, such a transfer mechanism has long been considered impossible. The direct electron transfer requires that the microorganisms possess membrane bound electron transport protein relays that transfer electrons from the inside of the bacterial cell to its outside, terminating in an outer-membrane (OM) redox protein that allows the electron transfer to an external, solid electron acceptor (a metal oxide or an MFC anode). In the focus of the discussion are c-type cytochromes, multi-heme proteins especially evolved with sediment inhabiting metal reducing microorganisms such as, *e.g.*, *Geobacter*,[19,36] *Rhodoferax*[37] and *Shewanella*[38,39] that, in their natural environment, often have to rely on solid terminal electron acceptors like iron(III) oxides. In the case of these organisms the MFC anode can conveniently resume the role of the solid electron acceptor (Fig. 3A).

As mentioned, the DET *via* outer membrane cytochromes requires the physical contact (adherence) of the bacterial cell—and of the cytochrome—to the fuel cell anode, with the consequence that only bacteria in the first monolayer at the anode surface are electrochemically active.[19] The MFC performance is thus limited by the maximum cell density in this bacterial monolayer. For example, maximum current densities as low as 0.6 μA $cm^{-2}$, 3 μA $cm^{-2}$ and 6.5 μA $cm^{-2}$ have been achieved for MFCs based on *Shewanella putrefaciens*,[40] *Rhodoferax ferrireducens*[37] and *Geobacter sulfurreducens*,[41] respectively.

Recently it has been demonstrated that, *e.g.*, some *Geobacter* and the *Shewanella* strains can evolve electronically conducting molecular pili (nanowires) that allow the microorganism to reach and utilize more distant solid electron acceptors,[42,43] These pili also allow the organisms to use an electrode that is not in direct cell contact as its sole electron acceptor (Fig. 3b). The pili are connected to the membrane-bound cytochromes, *via* which the electron transfer to the outside of the cell is accomplished. The formation of such nanowires may allow the development of thicker electroactive biofilms and thus higher anode performances. Thus, Reguera and co-workers reported a ten-fold increase of fuel cell performance upon nano-wire formation of *Geobacter sulfurreducens*.[44]

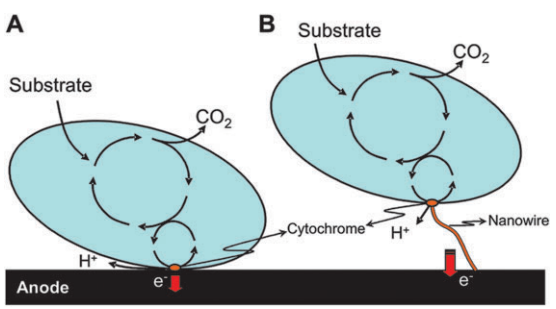



**Fig. 3** Illustration of the DET *via* (A) membrane bound cytochromes, (B) electronically conducting nanowires.

The evaluation of the energy efficiency of the DET is difficult since unambiguous information is still very scarce. Unfortunately, many papers in which the DET from living bacteria to an electrode has been clearly identified, no exact evaluation of the redox potential of the involved species has been undertaken. Thus, Bond and Lovley[41] used the open circuit potential of a *Geobacter sulfurreducens* colonized electrode, growing on acetate, to determine the redox potential of the DET as −0.17 V. At open circuit, however, the redox potential of a metabolizing anaerobic bacterial culture will shift considerably towards negative potentials (up to several hundred mV), due to the strong shift of the concentration term of the Nernst equation towards the reduced species. Thus, the reported open circuit potential may not be equal to the formal potential of the cytochrome based electron transfer.

The potential values provided in Table 2 have been reported by the group of Kim, who studied the electrochemical activity of various bacterial strains by means of cyclic voltammetry. The redox potential of the different bacterial species is virtually identical, with a mean value of 0 V (*vs.* NHE), and the shape of the cyclic voltammograms is very similar for all species (not shown here). The authors ascribe the redox activity to the electron mediation *via* outer membrane cytochromes,[45] which are believed to be responsible for the DET. The assumption is supported by the fact that the reported data are in part based on the measurement of washed and freshly re-suspended bacterial cells, which should exclude the presence of bacterial mediators. The high degree of similarity of the electrochemical data of the Kim group of the different microbial strains[40,45,46] may lead to the assumption that the electron transferring outer membrane cytochromes are very similar, and are not specific to a special bacterium.

Since the DET *via* bacterial nanowires (Fig. 3b) is reported to proceed *via* the membrane bound cytochromes[18] it will, for the following considerations, be assumed that the same standard redox potentials apply for the cytochrome and nanowire based electron transfer. As depicted in Table 3, the formal potential of the cytochrome was used to determine the free energy of the electrochemical oxidation.

**Table 2** Formal potentials measured for DET of different bacterial species

| Bacterial strain | $E'$/V[a] |
|---|---|
| *Shewanella putrefaciens* IR-1 | 0.01 [40] |
| *Shewanella putrefaciens* MR-1 | −0.02 [40] |
| *Shewanella putrefaciens* SR-1 | −0.01 [40] |
| *Aeromonas hydrophila* PA 3 | 0 [45] |
| *Clostridium sp.* EG 3 | 0 [46] |

[a] Determined as mid-peak potential in cyclic voltammetry; pH 7.

**Table 3** Theoretical energy efficiency of DET

| Linking species | $E'$/V | $\Delta G'_{elec,\ n=1}$/kJ mol$^{-1}$ | $\Delta G'_{elec,\ n=1}/\Delta G'_{total,\ n=1}$ (%) | $n$ | $\Delta G'_{elec}$/kJ mol$^{-1}$ | $\Delta G'_{elec}/\Delta G'_{total}$ (%) |
|---|---|---|---|---|---|---|
| Outer membrane cytochrome | 0 | −49.2 | 54.2 | 24[a] | −1181.0[a] | 54.2 |

[a] Theoretical (maximum) number of electrons derivable from a full oxidation of glucose.

For one electron, $\Delta G'_{elec}$ is −49.2 kJ mol$^{-1}$, which corresponds to 54% of the respective $\Delta G'_{total,\ n=1}$ value for the oxidation of glucose. This is a comparably low value indicating a high degree of biological energy dissipation.

In a number of reports it is assumed that the coulombic efficiency of the DET *via* cytochromes (and supposedly nanowires) may reach 100%, *i.e.*, that all electrons, liberated from the oxidation of a given substrate, can be harvested at the anode.[18,19] For this case it follows that the total yield of electric energy to be gained from the complete oxidation of glucose is equal to the value for one electron, 54%.

In reality, only very few of the organisms capable of DET are even able to feed on complex substrates as glucose. To my knowledge, only *Rhodoferax ferrireducens*[37] has so far been reported to utilize glucose, other microorganisms, especially *Geobacter* and *Shewanella* strains, cannot use complex substrates and have to rely on low-molecular organic acids and alcohols—provided by fermenting bacteria.[18] This, of course, can be expected to substantially lower the overall energy conversion efficiency.

### 3.2 Mediated electron transfer (MET)

Some scientists consider DET to be the first (and only) choice for an efficient current generation in MFCs.[18] Yet, so far the performance (in terms of current and power densities) of pure DET systems has often been even orders of magnitude below that of systems involving/or based on mediated electron transfer.

As illustrated in the following sections, MET mechanisms may represent an effective means to wire the microbial metabolism to a fuel cell anode. Very different approaches have been proposed, and they can be classified by the nature of the mediating (or linking) redox species.

**3.2.1 MET *via* exogenous (artificial) redox mediators.** In the following paragraph an approach is described that, due to a number of severe disadvantages, has—with the exception of some fundamental research—been generally abandoned. The approach will be briefly outlined, without going into the discussion of the energetic aspects.

In 1930 Cohen stated that although bacterial cultures, when grown anaerobically, may exhibit a strongly negative potential, the produced current is generally minute.[7] He ascribed this low current generation capacity to a lack of "electromotively active oxidation–reduction products". As a solution to this problem he proposed the introduction of inorganic or organic substances of the type potassium ferricyanide or benzoquinone to facilitate the electron transfer from cultures to immersed electrodes. The approach was reanimated in the 1980s especially by Bennetto and coworkers and found many supporters. A large number of compounds, the majority based on phenazines, phenothiazines, phenoxazines and quinones were investigated for their suitability and behavior as MFC mediators[32–34,47–50] (see Table 4).

An interesting spin-off of these studies was the research into so-called gastrobots (or ecobots), *i.e.*, MFC powered robotics systems.[51,52]

The greatest disadvantage of the use of exogenous redox mediators is, beside the usually low current densities (10–100 μA cm$^{-2}$), the necessity of a regular addition of the exogenous compound, which is technologically unfeasible and environmentally questionable and leads to the general abandonment of the approach.

In the following paragraphs it will be demonstrated that MET does not require the use of artificial redox compounds. As in the case of DET, a number of pathways can be exploited that microorganisms have evolved for the disposal of electrons originating from substrate oxidation.

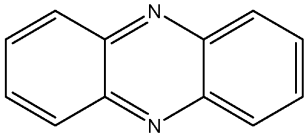
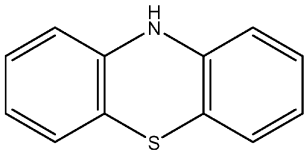
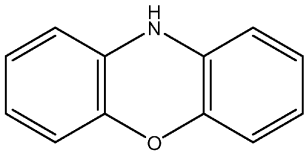
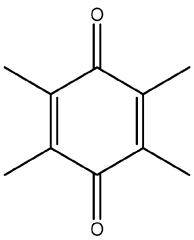
**3.2.2 MET *via* secondary metabolites.** Often microorganisms grow under conditions in which neither soluble electron acceptors are available nor solid electron acceptors are in direct reach (for DET). An example is the conditions that rule within thick biofilms, where, *e.g.*, oxygen diffusion into the depth of the film is limited and the cell is not in direct contact with a solid electron acceptor. Here, the microorganism may either use externally available (exogenous) electron shuttling compounds like humic acids or metal chelates, or can itself even produce low-molecular, electron shuttling compounds *via* secondary metabolic pathways.[53–55] Examples for such secondary metabolites, which have been shown to be involved in extracellular electron transfer processes, are bacterial phenazines like pyocyanine and 2-amino-3-carboxy-1,4-naphthoquinone, ACNQ (see Table 5 and 6).[53]

Very different microbial strategies for such mediated transfer exist, and the reader may be referred to an excellent review paper by Hernandez and Newman[53] in which these strategies are discussed.

For MFC applications, the secondary metabolites (endogenous redox mediators) are especially of great interest, as their synthesis makes the electron transfer independent of the presence of exogenous redox shuttles. The mediator serves as a reversible terminal electron acceptor, transferring electrons from the bacterial cell either to a solid oxidant (the MFC anode!) or into aerobic layers of the biofilm, where it becomes re-oxidized and is again available for subsequent redox processes. One molecule can thus serve for thousands of redox cycles (Fig. 4). Consequently, the production of small amounts of these compounds (directly in the anodic biofilm) enables the organism to dispose of electrons at sufficiently high rates. Especially in batch cultures, these redox mediators effectively facilitate the electron transfer and increase the efficiency of current generation.[58,59]

**Table 4** Selection of exogenous redox mediators used for microbial fuel cells[a]

| Substance class | Redox mediator | Redox potential $E^{\ominus\prime}$/V |
|---|---|---|
|  **Phenazines** | Neutral Red | −0.32 |
| | Safranine | −0.29 |
| | Phenazine ethosulfate | 0.06 |
|  **Phenothiazines** | New Methylene Blue | −0.02 |
| | Toluidine Blue O | 0.03 |
| | Thionine | 0.06 |
| | Phenothiazinone | 0.13 |
|  **Phenoxazines** | Resorufin | −0.05 |
| | Gallocyanine | 0.02 |
|  **Quinones** | 2-Hydroxy-1,4-naphthoquinone | −0.14 |
| | Anthraquinone-2,6-disulfonate | −0.18 |

[a] More detailed information can be found in the following review papers: ref. 14,15,20.

The identification of the extracellular electron shuttling compounds appears to be highly challenging, and so far, only the involvement of pyocyanine and phenazine-1-carboxamide, produced by *Pseudomonas aeruginosa* in the electron transfer to an MFC anode has been proved.[59] Further, it has been discussed that quinone-type redox shuttles support the long distance electron transfer of *Shewanella* species like *Shewanella oneidensis*[55] to electrodes or to solid electron acceptors like iron(III) oxide. The latter organism, however, has also been discussed to use overlapping transfer mechanisms[60] involving DET *via* c-type cytochromes[39] and electronically conducting nanowires[42] (see section 3.1).

Table 6 provides an overview about the energy efficiency of the bacterial MET. So far, only the phenazine paths have been proven for MFCs,[59] but the involvement of other redox shuttling compounds like ACNQ seems likely. Due to a redox potential that is more negative than that of the OM cytochromes (Tables 2 and 3) the amount of extractable electric energy is higher than that of the DET. It has been argued,[18,19] that this high efficiency may be confined to batch systems, whereas in open (flow) systems a steady loss of mediators may occur, leading to a decreasing value of $n$ and thus to a decreasing coulombic and energetic efficiency. Recent studies however suggest that such losses may be low due to a confinement of the redox shuttles within the biofilm *via* electrostatic forces.[56] The production of these electron shuttling compounds is, however, probably energetically expensive, leading to additional biological losses.

**3.2.3 MET *via* primary metabolites.** In contrast to the secondary metabolites the production of reduced primary metabolites is closely associated with the oxidative substrate degradation. Naturally, the total amount of reduction equivalents produced matches the amount of oxidized metabolites. To be utilizable as a reductant for anodic oxidation the metabolite has to fulfil certain requirements. Its redox potential should be as negative as possible (but within the limit imposed by the oxidation potential of the substrate) and it must be accessible for electrochemical oxidation under MFC conditions.

In principle, two major anaerobic metabolic pathways can lead to the formation of reduced metabolites suitable for MFC utilization: anaerobic respiration and fermentation.

**Table 5** Selection of extracellular bacterial (endogenous) redox mediators

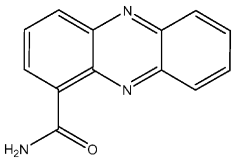
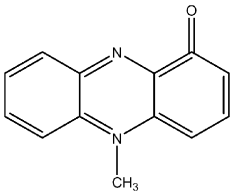
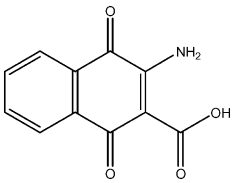
| Name | Structure | Redox potential, $E^{\ominus\prime}$/V |
|---|---|---|
| Phenazine-1-carboxamide |  | −0.115 [56] |
| Pyocyanine (phenazine) |  | −0.03 [57] |
| 2-Amino-3-carboxy-1,4-naphthoquinone (ACNQ ) |  | −0.071 [53] |

**Table 6** Theoretical energy efficiency of MET

| Linking species | $E'$/V | $\Delta G'_{\text{elec, } n=1}$/kJ mol$^{-1}$ | $\Delta G'_{\text{elec, } n=1}/\Delta G'_{\text{total, } n=1}$ (%) | $n$ | $\Delta G'_{\text{elec}}$/kJ mol$^{-1}$ | $\Delta G'_{\text{elec}}/\Delta G'_{\text{total}}$ (%) |
|---|---|---|---|---|---|---|
| Phenazine-1-carboxamide | −0.115 | −60.3 | 66.5 | 24[a] | −1447 | 66.5 |
| Pyocyanine | −0.03 | −52.1 | 57.4 | 24[a] | −1250 | 57.4 |
| ACNQ | −0.07 | −55.9 | 61.7 | 24[a] | −1343 | 61.7 |

[a] Theoretical (maximum) number of electrons derivable from a full oxidation of glucose.

### Anaerobic respiration

So far, only a few examples of the purposeful utilization of anaerobic respiration for MFC operation (Fig. 5A) have been reported. In principle, any terminal electron acceptor that has a redox potential sufficiently negative to that of the oxygen electrode, that is reversibly oxidizable, and that is soluble in water in its reduced and oxidized form, can be utilized to establish the anodic electron transfer in a MFC. As illustrated in Table 1, the standard potential of $Fe^{3+}/Fe^{2+}$ is too positive for an anodic redox mediator. However, the redox potential of the $Fe^{3+}/Fe^{2+}$ couple can be significantly shifted towards negative values *via* the preferable complexation of $Fe^{3+}$ with respect to $Fe^{2+}$ (*e.g.*, with humic acids), which may allow the use of the Fe(II)/(III) system for MFC electron mediation.

With a biological standard potential of −0.22 V, the sulfate/sulfide redox couple is thermodynamically the most suitable system (reaction (4)).

$$SO_4^{2-} + 8H^+ + 8e^- \underset{\text{Anode}}{\overset{\text{Bacteria}}{\rightleftharpoons}} S^{2-} + 4H_2O \qquad (4)$$

Sulfate reduction is a common respiratory path amongst anaerobic bacteria,[21] and especially in waste water based MFCs[61] and benthic fuel cells[62] sulfide oxidation represents an important electron transfer mechanism. An example for an isolated sulfate reducing bacterium used as a biocatalyst for MFC operation is *Desulfovibrio desulfuricans*.[63]

From the biological standard potential of −220 mV (see Table 1) the free energy, $\Delta G'_{\text{elec},n=1}$, of −70.4 kJ can be

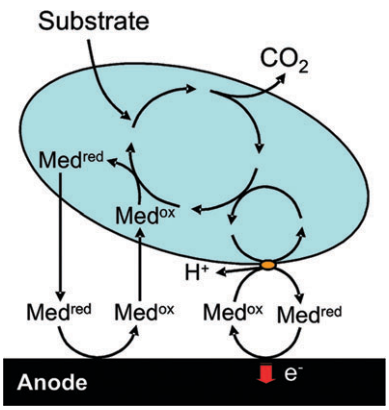



**Fig. 4** Simplified, schematic illustration of MET *via* microbial secondary metabolites. Two possible redox mechanisms have been proposed: shuttling *via* outer cell membrane cytochromes and *via* periplasmatic or cytoplasmatic redox couples.

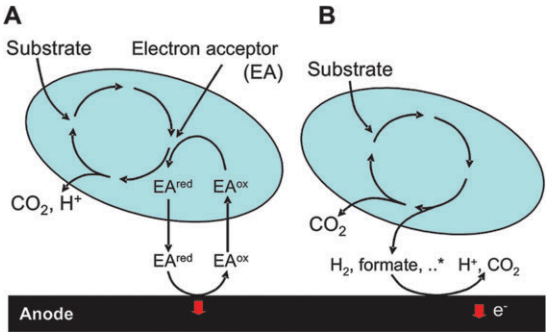



**Fig. 5** Simplified, schematic illustration of MET *via* microbial primary metabolites (A) *via* reduced terminal electron acceptors (use of anaerobic respiration), (B) *via* oxidation of reduced fermentation products.

derived. This is a high value that may be promising for a MFC application. For a theoretical, 24 electron transfer (full oxidation of glucose) this would yield 1690 kJ (energy efficiency of 77.6%). Yet, sulfate reducing bacteria are unable to metabolize carbohydrates. They depend on a co-colonization with fermenting bacteria that provide low-molecular organic acids and alcohols. Further, many sulfate reducers cannot completely degrade the substrate,[21] which further lowers the energetic yield. Additionally, the electrochemical re-oxidation of sulfide to sulfate is difficult, since metallic electrodes are easily poisoned by sulfide due to its strong and often irreversible adsorption. Also, the electrochemical oxidation is usually hampered by the formation of solid sulfur, inhibiting further oxidation. To solve this problem, Habermann and Pommer used porous electrodes impregnated with cobalt hydroxide in order to bind and thus store sulfide ions as cobalt sulfide and thus enrich sulfide within the electrode.[63] The resulting cobalt oxide/cobalt sulfide impregnated electrode possessed a high catalytic activity towards the oxidation of sulfide to sulfate.

### Fermentation

More intensively studied than anaerobic respiration is the use of fermentation for MFC operation. Thus, a large variety of fermentative and photo-heterotrophic processes result in the production of energy-rich reduced metabolites such as hydrogen (see, *e.g.*, reactions (2) and (3)), ethanol or formate. These compounds can be oxidized directly in the microbial medium, provided electrocatalytic anodes are used to facilitate the oxidation (Fig. 5B) and measures are taken to prevent a scavenging of the metabolites by other, *e.g.*, biological, processes.

First reports on the use of fermentation products for direct electricity generation were given by Karube and coworkers who utilized immobilized, hydrogen producing cultures as biocatalysts and platinum as an electrocatalyst for hydrogen oxidation.[64,65] Due to the susceptibility of the platinum electrodes to poisoning and deactivation the reported power densities were rather low. Considerable progress was achieved by the development of sandwich electrodes consisting of platinum, protected from poisoning reactions by an overlay of conductive polymers like polyaniline or its fluorinated forms. With current densities of up to 1.5 mA $cm^{-2}$ these electrodes considerably improved the performance of MFCs.[66,67] They offered access to exploiting a great number of heterotrophic, photoheterotrophic and even purely photosynthetic microorganisms and the access to complex carbohydrates like starch and cellulose for current generation in MFCs.[68–71] In a further significant advancement, the expensive noble metal electrocatalyst was replaced by tungsten carbide, an inexpensive yet effective and robust electrocatalyst. This development went along with a further increase in the anodic performance to 3 mA $cm^{-2}$ maximum current density, and a maximum power density of 586 μW $cm^{-2}$.[72]

With a biological standard potential of −420 mV hydrogen represents the most suitable electron carrier. For one electron, the free energy of oxidation ($\Delta G'_{elec,n=1}$) is −89.7 kJ $mol^{-1}$ (Table 1), which is close to that for the oxidation of glucose. Yet, the greatest disadvantage is the limited hydrogen yield of dark fermentation. As shown in reaction (3) the maximum hydrogen yield—achieved *via* acetate fermentation of glucose—is four moles per mole of glucose. Based on this value, the maximum energy yield, $\Delta G'_{elec}$, is 717.9 kJ per mole glucose, corresponding to an efficiency of 33%. Different strategies have been proposed to overcome this limitation and to increase the coulombic and energy efficiency. An electrochemical approach is the electrocatalytic oxidation of additional, energy-rich reduced organic fermentation side products.[73] First promising results have been achieved using tungsten carbide electrodes for the oxidation of fermentation products like formate ($E^{\ominus\prime}$ = −0.43 V), and lactate ($E^{\ominus\prime}$ = −0.19 V).[72,74]

A second, biological approach focuses on a combination of dark fermentation with, *e.g.*, photofermentation in order to increase the hydrogen yield and thus the coulombic and energy efficiency.[71] As an example, photoheterotropic non-sulfur purple bacteria like *Rhodobacter sphaeroides* have been used to increase the substrate conversion efficiency by exploiting the remaining organic acids of the dark fermentation as a resource for photobiological hydrogen production.[71] The application of photobiological pathways, however, may be difficult to establish, since it requires special photo-reactors and sufficient amounts of light. A further, potential approach may represent the combination of fermentation based MFCs with fuel cells based on, *e.g.*, DET. The latter often require the pre-digestion of carbohydrates—a thus feasible combination.

## 4. Conclusions

What is the best anodic electron transfer mechanism for MFCs? At the current stage of knowledge, this question can not be satisfactorily answered. Many issues are still to be addressed, and the evaluation is highly complex. The anodic electron transfer always has to be discussed taking into account the nature and the rates of the metabolic processes of the used microbial species and their capability to utilize certain substrates. Thus, every electron transfer path has its advantages and disadvantages. As an example, DET usually allows very high coulombic efficiencies but combined with a comparably low free energy of the electron transfer reaction. Further, purely DET based systems have so far delivered only very low current and power densities, which would therefore

require extremely large electrode surface areas for sufficiently high power outputs. Also, the limitation of underlying species like *Geobacter* to low-molecular substrates like acetate and butyrate has to be taken into account. Primary metabolite mediated MFC systems on the other hand allow high current and power densities and, based on the diversity of exploitable microorganisms, a great variety of utilizable substrates. Yet, these systems are usually affected by comparably low coulombic efficiencies, based on the formation of electrochemically inactive side products.

In the case of MFCs based on electrochemically enriched bacterial mixed cultures the combination and interaction of different electron transfer mechanisms and redox species can lead to a rather complex electrochemical behavior (see, *e.g.*, cyclic voltammograms in ref. 75). Often, relatively negative redox potentials are observed[75] that cannot currently be ascribed to a particular mechanism or redox species. Other studies even indicate the capability of anodic biofilm cultures to adapt their metabolism and the mechanisms of electron transfer to changes in the applied anode potential in order to maximize the biological energy gain.[76]

These different aspects clearly show the need for considerable research efforts to better understand the processes of the bioelectrochemical energy conversion, an understanding that is of crucial importance for the further development of this exciting technology. They emphasize the complexity of an evaluating discussion of the electron transfer and biotransformation processes in MFCs and show that statements, in which the supremacy of, *e.g.*, DET *via* "electricigens" is claimed simply on the basis of coulombic efficiencies are of only limited value.

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