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The microbe electric: conversion of organic matter to electricity

Derek R Lovley

Broad application of microbial fuel cells will require substantial increases in current density. A better understanding of the microbiology of these systems may help. Recent studies have greatly expanded the range of microorganisms known to function either as electrode-reducing microorganisms at the anode or as electrode-oxidizing microorganisms at the cathode. Microorganisms that can completely oxidize organic compounds with an electrode serving as the sole electron acceptor are expected to be the primary contributors to power production. Several mechanisms for electron transfer to anodes have been proposed including: direct electron transfer via outer-surface c-type cytochromes, long-range electron transfer via microbial nanowires, electron flow through a conductive biofilm matrix containing cytochromes, and soluble electron shuttles. Which mechanisms are most important depend on the microorganisms and the thickness of the anode biofilm. Emerging systems biology approaches to the study, design, and evolution of microorganisms interacting with electrodes are expected to contribute to improved microbial fuel cells.

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Introduction

Numerous investigators have suggested that microbial production of electricity may become an important form of bioenergy because microbial fuel cells offer the possibility of extracting current from a wide range of complex organic wastes and renewable biomass. The limitation to wide-spread utilization of microbial fuel cells as an alternative energy source is that, at present, the power densities of microbial fuel cells are too low for most envisioned applications. The only practical applications are sediment microbial fuel cells that extract electrons from organic matter in marine sediments to power electronic monitoring devices [1], and possibly 'sediment

fuel cells in a pot' which can serve as a light source or battery charger in off-grid areas (www.lebone.org). Substantial improvements will be required before other commonly projected uses of microbial fuel cells, such as large-scale conversion of organic wastes and biomass to electricity, or powering vehicles, mobile electronic devices, or households with suitably scaled microbial fuel cells will be possible.

The purpose of this review is to summarize advances in the last two years in the understanding of the microbiology of microbial fuel cells. Significant advancements in increasing the current densities of microbial fuel cells have been made recently by modifying fuel cell architecture and materials while treating the microorganisms as a 'black box' [2]. Additional potential engineering modifications seem promising [3,4,5]. However, new strategies for engineering microbial fuel cells are also almost certain to benefit from a better understanding of the microbiology of microbial fuel cells. Important questions include: 1) what microorganisms are capable of effectively interacting with electrode surfaces; 2) what factors influence the growth and metabolism of these microbes; and 3) what are the mechanisms of electronic communication between microorganisms and electrodes. Answering these questions might also aid in the development of microorganisms with enhanced capacities for power output, which is an under-explored avenue for improvement [4,6,7]. Even with the narrowed focus of this review on microbiology the number of publications in the last two years far exceeds the citation limits of this review format and the author apologizes to the authors that feel their publications were unduly omitted.

Electrode-reducing microorganisms

Microorganisms can interact with both the anode and cathode of microbial fuel cells. Various names for these organisms abound (Box 1), but here they will be referred to simply as electrode-reducing or electrode-oxidizing microorganisms. Most research has been on the electrode-reducing microorganisms that donate electrons to the anode. Evidence that electrons from microbial metabolism can be harvested with electrodes has existed for nearly 100 years, but the 'discovery of bacteria capable of complete oxidation of organic compounds and of efficient electron transfer to electrodes via direct contact was a landmark in the history of this research' [2]. The simple reason for this is that organic compounds must be oxidized to carbon dioxide in order to efficiently extract the available electrons and convert them to current [8]. This is true whether the fuel source consists of complex wastes

Box 1 What's in a name? Standardizing names for microorganisms that interact with electrodes to conform with conventions for discussing other forms of microbial respiration

The nomenclature for microorganisms capable of interacting with electrodes is as yet far from standardized. This is not unexpected in a rapidly developing field, but leads to confusion. Proposed terms for microorganisms that can contribute electrons to fuel cell anodes include: anodophiles [77], exoelectrogens [78], electrogenic microorganisms [2**], anode-respiring bacteria [34], and electrochemically active bacteria [28]. Furthermore, the term electricigens was proposed to specifically refer to microorganisms that completely oxidize organic compounds to carbon dioxide with an electrode serving as the sole electron acceptor [11]. This special distinction is important because electricigens must, by simple stoichiometric considerations, account for most of the current generation from most conceivable organic substrates [8].

For other forms of anaerobic respiration the common convention is to include in the name of the respiratory microorganisms a description of what the microorganisms reduce (i.e. sulfate-reducing microorganisms or Fe(III)-reducing microorganisms, etc. often shortened to sulfate reducers and iron reducers, etc.). Therefore, it is proposed that microorganisms that donate electrons to the anode of microbial fuel cells be referred to as electrode-reducing microorganisms or electrode reducers, for short. Microorganisms accepting electrons from electrodes will be referred to here as electrode-oxidizing microorganisms. Depending on circumstances any given microorganism might function as an electrode reducer or an electrode oxidizer, just as microorganisms can function as either as an iron reducer or an iron oxidizer, depending on environmental conditions.

or biomass mixtures or just simple defined compounds. Thus, the communities contributing to current production may be functionally complex (Figure 1) with even greater phylogenetic diversity, but only the microorganisms capable of complete oxidation of organic compounds can be the most significant direct contributors to power production. The study of this process is in its infancy because the capacity for such microbial metabolism was only recognized within this decade with the finding that microorganisms in the *Geobacteraceae* could carry out this form of respiration [9].

As recently reviewed [3**,10], one strategy to determine which microorganisms contribute to power production from various fuel sources is to identify those microorganisms that selectively colonize anode surfaces. The microorganisms that are most abundant on the anode can depend on the enrichment conditions [3**]. In recent examples, the previously reported selective enrichment of *Geobacteraceae* on the anodes of sediment microbial fuel cells (reviewed in [11]) was observed not only in additional sediment fuel cells [12], but also in fuel cells initiated with a sewage sludge inoculum and fed acetate, glucose, or lactate [10,13]. However, with other inocula, energy sources, and/or fuel cell configurations, other recent studies have found a predominance of *Gammaproteobacteria* [14], *Betaproteobacteria* [15], *Rhizobiales* [16], or *Clostridia* [17] on the anode surface. Under thermophilic

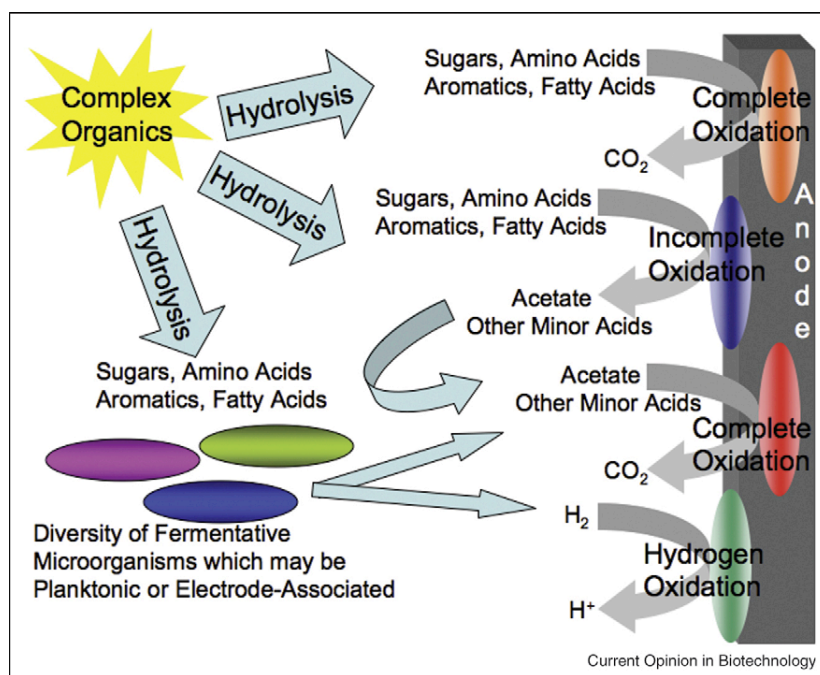
conditions, there was a strong selection for microorganisms closely related to *Thermincola carboxydophila* from a marine inocula [18] and to *Deferribacter* and *Coprothermobacter* species from a wastewater inoculum [19].

Interpretation of the role of the anode-associated microorganisms is sometimes difficult. Surprisingly, many of these more recent studies failed to carry out the necessary control of providing the anode material as a surface for colonization, but not electronically connecting it to the cathode. This control aids in identifying microorganisms that have an affinity for the surface, but do not require it as an electron acceptor. Attachment of microorganisms that are not effective in current production may be especially problematic when the efficiency of conversion of the organic fuel to electricity is low [13]. Under such circumstances many, if not most, of the microorganisms attached to the anode may be using the anode as a convenient surface for attachment, but carrying out forms of metabolism other than electron transfer to the anode, such as methanogenesis or aerobic respiration. Furthermore, when complex organic substrates serve as fuel it is expected that microorganisms that ferment these compounds to simpler substrates that electrode-reducing microorganisms can oxidize will also be components of the anode microbial community [8,10,20]. As recently demonstrated in coculture studies [21**,22] these fermentative microorganisms may have little or no capacity for electron transfer to the anode (Figure 1), but their metabolism is key to powering microbial fuel cells.

The actual electrode-reducing capabilities of particular organisms can best be determined with defined cultures. A common strategy for isolating electrode-reducing microorganisms is to employ Fe(III) as an electron acceptor because many of the most effective current-producing microorganisms are Fe(III) reducers [11,23**,24]. However, not all current-producing microorganisms effectively reduce Fe(III) [8,24,25] and enrichment and isolation with an electrode serving as the electron acceptor may be the most suitable method for recovering such organisms in culture [26*,27,28]. Isolation with more traditional, alternative electron acceptors, such as oxygen for facultative microorganisms [25,29], may be sufficient in some cases. More current-producing microorganisms also continue to be identified by screening microbial cultures already available in culture collections [30,31]. In evaluating the potential for microorganisms to donate electrons to electrodes, it may be important to provide exogenous electron shuttles because some microorganisms may rely on electron shuttles in the environment, or provided by other microorganisms in the community [29], in order to contribute to power production.

Analysis of rates of electron transfer to electrodes at various voltages, anode potentials, external resistances, electron donor concentrations, and cell densities can aid

Figure 1



Simplified model for the conversion of complex organic fuels to electricity. Complex organic matter is hydrolyzed to constituents, which in most cases are primarily fermented, but there are microorganisms that can completely oxidize such compounds with an electrode serving as the sole electron acceptor or incompletely oxidize these substrates with electron transfer to an electrode. Acetate and other more minor fermentation acids can be completely oxidized to carbon dioxide and this will typically be the primary source of electrons for current production. Hydrogen produced from fermentation may also be a source of electrons. Direct electron transfer to the anode is illustrated but indirect electron transfer to the anode via soluble electron shuttles is also possible.

in understanding the power production capabilities not only of pure cultures, but also of mixed communities [32–38,39,40]. Data from such analyses have served as the basis for initial attempts to model current production. This approach may also be helpful in elucidating the biomolecular components involved in electron transfer to anodes, but interpreting the data is complex and should be combined with physiological studies [40].

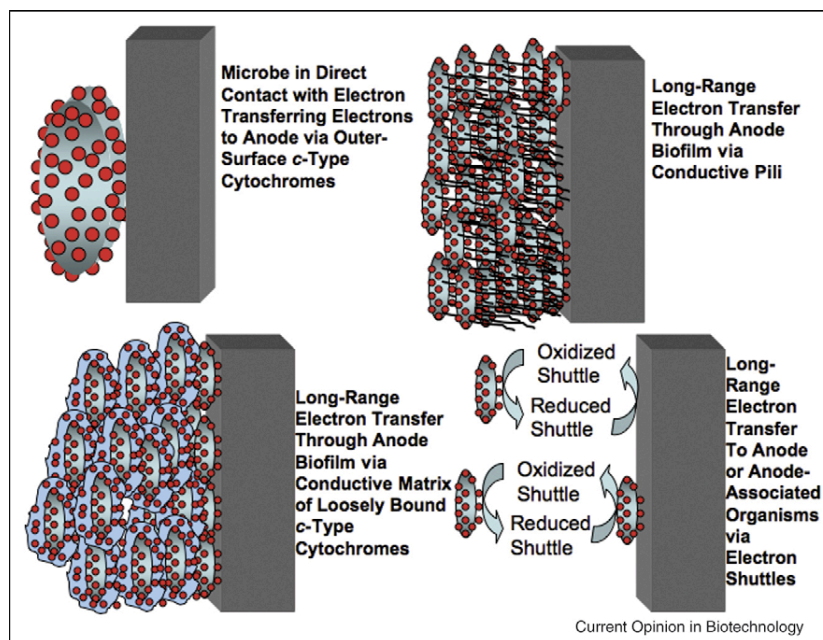
Electrode-oxidizing microorganisms

As recently reviewed [41,42], it has been known for some time that electrodes poised at low potentials can indirectly donate electrons to microorganisms via the production of hydrogen or the reduction of various electron shuttles. Recent examples are the microbially catalyzed reduction of perchlorate [43] or trichloroethane [44] with electrode-reduced anthraquinone-2,6-disulfonate or methyl viologen as electron donors, respectively. However, microorganisms may also directly accept electrons from the cathode surface, functioning as electrode-oxidizing microorganisms. Direct electron transfer from electrodes was first observed with *Geobacter* species utilizing fumarate, nitrate, or U(VI) as an electron acceptor [45–47]. One strain, *G. lovleyi*, can reduce chlorinated compounds with an electrode serving as the sole electron

donor [31] as can several strains of *Anaeromyxobacter dehalogenans* (S Strycharz, unpublished data). Microbially catalyzed reduction of nitrate, U(VI), and chlorinated compounds with electrodes serving as the electron donor offers several potential advantages for bioremediation of these contaminants over more traditional approaches [48]. Nitrate removal from wastewater via direct electron transfer from electrodes to denitrifying microorganisms has also been proposed [49,50], but the microbiology of this process has yet to be investigated in detail.

Other potential electron acceptors at the cathode include oxygen and protons. Microorganisms colonizing cathodes can enhance electron transfer to oxygen [51,52,53], but it has not yet been demonstrated that this is a respiratory mechanism in which electrons derived from the cathode serve as an energy-yielding electron donor for oxygen reduction and there are a variety of other possible mechanisms by which cells might catalyze enhanced oxygen reduction. Reduction of protons to produce hydrogen gas represents a strategy for generating a potentially valuable fuel. A three-step enrichment process resulted in greater hydrogen production from electrodes poised at low potential than nonenriched electrodes, suggesting a selection for proton-reducing microorganisms [54]. The fact that

Figure 2



Proposed mechanisms for electron transfer to the anode of microbial fuel cells. The various methods are not necessarily mutually exclusive. Red dots represent outer surface cytochromes, black lines represent nanowires, and the blue clouds represent the possible extracellular matrix which contains c-type cytochromes conferring conductivity.

carbon monoxide inhibited hydrogen production was consistent with the concept that hydrogenases were involved in the hydrogen production. Microorganisms capable of reducing protons to hydrogen with electrode-reduced methyl viologen appeared to be a component of a mixed culture containing dechlorinating microorganisms [55].

Mechanisms for microbe–electrode interaction

A diversity of mechanisms by which microorganisms may transfer electrons to the anode of microbial fuel cells has been proposed (Figure 2). Initial investigations into the mechanisms of microbe–anode interactions have focused on studies with pure culture models because pure cultures can be genetically modified for functional studies and genome-scale investigations on gene expression and proteomics are more readily interpretable with pure cultures. Pure culture studies are likely to have the most relevance to power production in mixed communities if the pure culture: 1) is representative of those that predominate on anodes; 2) is capable of high current densities; and 3) completely oxidizes environmentally relevant organic electron donors, such as acetate. Two cultures, *Rhodospseudomonas palustris* strain DX-1 [23^{••}] and *Geobacter sulfurreducens* have been reported to be capable of current densities comparable to mixed communities. Of these two, detailed investigations on mechanisms for electron transfer to anodes have only been

reported for *G. sulfurreducens*. Studies on this organism have the additional benefit that it is closely related to organisms that, as noted above, often predominate on anodes and that it is capable of completely oxidizing acetate with an electrode serving as the sole electron acceptor.

On the basis of genome-scale gene expression and genetic studies it has been proposed that cells of *G. sulfurreducens* in direct contact with the anode surface transfer electrons to the anode via c-type cytochromes displayed on the outer cell surface [56^{••},57,58]. This hypothesis is supported by sophisticated spectroelectrochemical studies [59^{••}]. However, *G. sulfurreducens* can form relatively thick (>50 µm) anode biofilms and cells at this substantial distance from the anode contribute to current production [60^{••},61]. Gene expression and genetic studies have suggested that, ‘microbial nanowires’ the electrically conductive pili of *G. sulfurreducens* [62], are important in this long-range electron transfer [60^{••}], but their actual function requires further investigation [63]. A cytochrome that may be easily released into the biofilm matrix may also contribute (KP Nevin, unpublished data). The *G. sulfurreducens* biofilm is conductive, in contrast to previously reported microbial biofilms which act as insulators (N Malvankar, unpublished data). This is consistent with modeling studies which indicate that the current production observed in *G. sulfurreducens* fuel cells would only be feasible with a conductive biofilm [64,65^{••}]. The *G.*

sulfurreducens anode biofilm also has significant capacitance (N Malvankar, unpublished data), which has been attributed to the abundant *c*-type cytochromes which provide substantial electron storage capacity in individual cells [66].

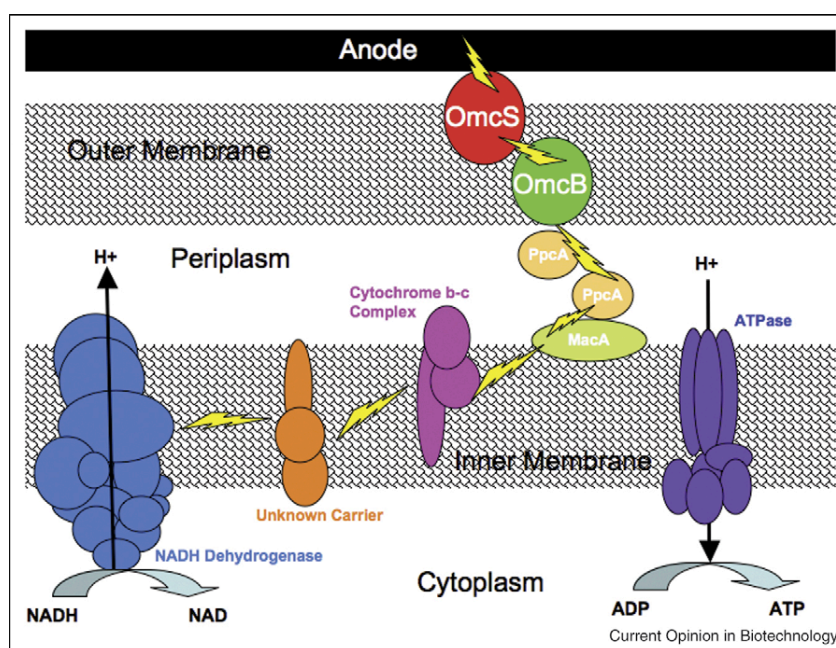
In contrast, studies with *Shewanella oneidensis*, an intensively studied electrode reducer, have suggested that soluble electron shuttles are the mediators for most of the electron transfer to the anode with this organism. This was most clearly apparent from electrochemical analyses that gave a response for *S. oneidensis* that was significantly different from the response resulting from direct electron transfer to the anode by *G. sulfurreducens* [39^{••}]. Riboflavin released from the cells appears to be the source of the shuttle [39^{••},67^{••}]. Therefore, even though *S. oneidensis* produces microbial nanowires [68], direct wiring to the electrode does not appear to be an important conduit for electron transfer to the anode. Differences between *S. oneidensis* and *G. sulfurreducens* in their ability to interact with smooth gold electrodes further suggest different attachment and/or electron transfer mechanisms [69]. Some of the outer surface *c*-type cytochromes, known to be important for extracellular electron transfer in *S. oneidensis* are also important for optimal current production [70^{••}], but this may reflect a requirement for the cytochromes for electron shuttle reduction. Many of the cells contributing to power production in *S. oneidensis* fuel cells

are planktonic and electron transfer over such long distances is only conceivable with electron shuttles [71]. The closely related *Escherichia coli* may also release metabolites that serve as electron shuttles [72] as can a diversity of other organisms [11].

Whether the initial extracellular electron acceptor is an electron shuttle or the electrode itself, an often misunderstood point in the microbial fuel cell literature is that these final electron transfer steps are unlikely to directly yield energy for the microorganism. Energy conservation results from electron transfer and associated proton pumping across the inner membrane, but not from any subsequent electron transfer reactions (Figure 3). Therefore, even though it is often considered that growth yields will be higher with anodes poised at higher potentials, this is unlikely to be the case, just as reduction of Fe(III) forms with different mid-point potentials results in similar growth yields.

Less is known about the mechanisms by which electrode-oxidizing microorganisms accept electrons from cathode surfaces. Electrochemical analysis suggests electron transfer from electrodes to *G. sulfurreducens* proceeds via a different mechanism than electron transfer to electrodes [47]. Microarray analysis of gene expression and gene deletion studies is consistent with this conclusion (Strycharz, unpublished data). Studies with purified

Figure 3



Model for *Geobacter sulfurreducens* electron transfer to the anode of a microbial fuel cell from NADH derived from organic matter oxidation. Energy conservation results from proton pumping associated with inner membrane electron transport. Subsequent electron transfer steps function merely to dispose of electrons. Electron transfer to the anode is depicted via an outer surface cytochrome as has been proposed for cells in direct contact with the anode surface.

proteins that are potential candidates for the electrical contact between the outer cell surface and electrodes [73] are likely to aid in better understanding electron flow in either direction.

Conclusions

Although the microbiology of microbe–electrode interactions is fascinating from a purely biological perspective, most research in this area is ultimately justified by the hope of increasing the power output of microbial fuel cells or developing additional microbe–electrode applications. Just as there is a wide phylogenetic diversity of microorganisms capable of extracellular electron transfer to Fe(III), it is likely that there is an equally diverse range of microorganisms capable of interacting with electrodes [8]. If the appropriate strategies can be devised, it may be possible to recover microorganisms capable of higher rates of electron transfer between microorganisms and electrodes than currently available strains. Genome-scale metabolic modeling [74] coupled with genetic engineering may yield strains that can enhance current production [75]. The capacity to produce current appears to be a fortuitous reaction with no direct natural analog [11] and placing the appropriate pressure to favor the selection of mutations that enhance current production is a promising approach for increasing the power output of microbial fuel cells [8,76]. Furthermore, as the understanding of the range of reactions that microorganisms can carry out with electrodes serving either as the electron donor or the electron acceptor continues to expand the application of microbe–electrode interactions to novel areas such as environmental restoration and production of commodity chemicals may eclipse power production as the most promising uses of this technology.

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