Microbial Fuel Cells: Plug-in and Power-on Microbiology

These devices already prove valuable for characterizing physiology, modeling electron flow, and framing and testing hypotheses

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If scientists have their way, “green” beer won’t be limited to St. Patrick’s Day celebrations anymore. Breweries are taking their wastewater, which is rich in organic material, and turning it into electricity with bacteria in microbial fuel cells (MFCs). MFCs can generate valuable commodities from a variety of organic wastes that are abundant and essentially free—bacteria have generated electricity from industrial wastewaters, sewage, and even sediment. In MFCs, bacteria act as living catalysts to convert organic substrates into electricity. While this technology may sound like an answer to our energy crisis, MFCs are not yet viable for most applications. Ongoing research is dedicated to optimizing their performance, with only recent attention being given to the microbial details of waste-to-wattage conversion.

MFCs may well have a place in the future energy paradigm, as well as in bioremediation and industrial-chemical and hydrogen production. For MFCs to be considered more than a lab novelty, standardization of data expression is necessary to allow reliable and accurate comparison of results. Advances in the hardware, operation, and microbial components are also needed. However, MFCs are valuable research tools for characterizing the physiology and ecology of extracellular electron transfer, modeling electron flow in complex microbial ecosystems, and framing and testing ecology theory.

Denizens of Power: the Role of Bacteria in MFCs

Generating power in MFCs depends on oxidation-reduction (redox) chemistry. MFCs contain anodic and cathodic compartments, each of which holds an electrode separated by a cation-permeable membrane (Fig. 1). In the anode chamber, microbial substrates such as acetate (an electron donor) are oxidized in the absence of oxygen by respiratory bacteria, producing protons and electrons. The electrons are passed through an electron transport chain (ETC) and protons are translocated across the cell membrane to generate adenosine triphosphate (ATP).

Summary

- Microorganisms may be harnessed through fuel cells to convert organic materials into electricity, hydrogen, or industrially useful chemicals, or to remediate polluted environmental sites.
- In a typical microbial fuel cell (MFC), microbes transfer electrons through a traditional electron transport chain onto an electrode surface generating electricity while producing a proton motive force for ATP generation.
- MFC-based research continues to expand knowledge about the diversity of extracellular electron transfer processes, mechanisms used in such processes, and biofilm ecology.
- Although gram-positive bacteria can generate electrical energy in MFCs, how they transfer electrons without outer membranes is a mystery.
- Bioelectrical reactors do not produce electricity but furnish electrons to reduce remediation targets such as uranium, perchlorate, chlorinated solvents, and nitrate.

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Electrons and protons exiting the ETC typically pass onto a terminal electron acceptor such as oxygen, nitrate, or Fe(III). However, in the absence of such acceptors in an MFC, some microorganisms pass the electrons onto the anode surface. Difference in redox potentials (i.e., the ability of a compound to donate or accept electrons, denoted $E_0$ and measured in volts) between the electron donor and the electron acceptor is the determinant of the potential energy available to the microorganism for anabolic processes. In an MFC the electrochemical redox potential difference of the anode and cathode determines how much energy is available.

Electrons produced in an MFC flow from the anode through an external electrical circuit to the cathode to generate electrical current. While electrons move externally, protons diffuse from the anode to the cathode via the cation membrane to complete the internal circuit (Fig. 1). At the cathode, the electrons and protons combine to reduce the terminal electron acceptor, which in many applications is oxygen. Therefore bacteria in the anode are physically separated from their terminal electron acceptor in the cathode compartment.

The electrical power (measured in watts) produced by an MFC is based on the rate of electrons moving through the circuit (current, measured in amps) and electrochemical potential difference (volts) across the electrodes. Many factors affect current production, including substrate concentration, bacterial substrate oxidation rate, presence of alternative electron acceptors, and microbial growth. Electrochemical potential, on the other hand, depends on the redox couple between the bacterial respiratory enzyme or electron carrier and the potential at the anode, which is determined by the terminal electron acceptor in the cathode and any system losses. (Fig. 1).

For bacteria to produce electricity in MFCs, the cells need to transfer electrons generated along their membranes to their surfaces. Yet, very little is known about bacterial interactions with electrodes. While anodes and cathodes can function in bacterial respiration, research has been fo-
cused on understanding microbial anodic electron transfer. Anode-respiring bacteria catalyze electron transfer in organic substrates onto the anode as a surrogate for natural extracellular electron acceptors (e.g., ferric oxides or humic substances) by a variety of mechanisms (Fig. 2).

Bacteria transfer electrons to anodes either directly or via mediated mechanisms. In direct electron transfer, bacteria require physical contact with the electrode for current production. The contact point between the bacteria and the anode surface requires outer membrane-bound cytochromes or putatively conductive pili called nanowires. Although direct contact of an outer-membrane cytochrome to an anodic surface would require microorganisms to be situated upon the electrode itself, direct electron transfer mechanisms are not limited to short-range interactions, as nanowires produced by Geobacter sulfurreducens have been implicated in electron conduction through anode biofilms more than 50 μm thick. In mediated electron transfer mechanisms, bacteria either produce or take advantage of indigenous soluble redox compounds such as quinones and flavins to shuttle electrons between the terminal respiratory enzyme and the anode surface.

Power Tools of Microbiology: MFC Technology Advances Microbial Research

Besides generating energy, MFCs are powerful research tools. With electrical current a proxy for bacterial activity, MFCs are controlled systems for addressing a range of questions about extracellular electron transfers. MFC-based research continues to expand knowledge about the diversity of extracellular electron transfers, mechanisms used in such processes, and biofilm ecology.

Microbial research from MFCs has revealed an expansive diversity of bacteria that transfer electrons onto external electron acceptors. Until recently, knowledge of electricity-generating bacteria was limited to bacteria that transfer electrons to solid metals, thus phylogenetically confining most MFC studies to members of the Proteobacteria. However, culture-independent studies of MFC anode biofilms indicate that the diversity of such microbial communities far exceeds that of the available electricity-producing isolates, suggesting that many organisms with this capability are yet to be discovered. This knowledge has spurred interest in using a variety of alternative inoculum sources, operating conditions, and isolation methods to increase the known diversity of electrode-reducing organisms.

For example, in our lab we are using MFCs operated at 55°C, at which temperature the anode-reducing species Geobacter and Shewanella spp. cannot survive. In this way we find anode communities dominated by gram-positive species and have isolated novel organisms from three of the five most dominant populations identified by 16S rRNA gene clone libraries. Characterization revealed that the isolates use species-specific mechanisms for electricity production, emphasizing not only the phylogenetic diversity that exists in active MFCs but also the phenotypic diversity within a single community to perform the same function, i.e., transfer electrons onto an electrode surface.

One isolate, Thermincola strain JR, a member of the Firmicutes, produces current comparable to that of the original MFC community and greater than either Geobacter or Shewanella species in similarly designed MFCs. Furthermore, this is the first example of electrical power production by a gram-positive bacterium without exogenous mediators. A second isolate, Geobacillus strain S2E, also a member of the Firmicutes, is the first member of this genus reported to respire using solid-phase iron oxides.

Our results indicate that microbial analyses from MFCs can result in the discovery of bacteria that are proficient at energy generation and have unique metabolic functions. The significant enrichment of gram-positive bacteria in our systems may signify a new ecological role for these organisms in respiration of insoluble electron acceptors. How these gram-positive bacteria, which lack outer membranes, transfer electrons extracellularly is a mystery.

To optimize MFC performance, we need to learn more about these electrode-reducing microbial communities. Molecular approaches characterizing the microbiology communities in these systems are needed to reveal the phylogenetic diversity as well as the activity of electrode-respiring communities. Little is known about population-level interactions within the anode community, but it is foreseeable that these types of interactions can be reinforced or controlled to
increase substrate utilization or electron transfer efficiency. Such research could also prove useful for determining the fate, transport, and bioremediation of metals in the environment. Future studies could use MFCs set at different reduction-oxidation potentials to better understand the effects of redox potential and electron donors on microbial community structure and activity.

Elucidating Extracellular Electron Transfer Mechanisms

MFCs as a research tool have expanded our knowledge of bacterial electron transfer mechanisms. Unlike natural external electron acceptors such as Fe(III) or Mn(IV), anodes in MFCs do not participate in mineral dissolution reactions, and electron transfer rates can be quantified. Anodes also provide a stable source of electron acceptor and do not generate reduced products that can interfere with downstream genomic or proteomic applications. Additionally, colonized anodes can be adapted to detect the presence, redox potential, and reversibility of electroactive components in biofilms.

The power of MFCs to elucidate mechanisms of solid-phase electron transfer was convincingly demonstrated in 2008 by researchers at the University of Minnesota. Applying cyclic voltammetry techniques to anode biofilms, they showed that *Shewanella* spp. excrete flavins which function in anode electron transfer and metal chelation and may aid in adhesion to anode surfaces. Since *Shewanella* use outer membrane cytochromes and putatively transfer electrons through conductive nanowires, this work shows that extracellular electron transfer mechanisms are not mutually exclusive within a single species. This may account for observed discrepancies in research findings by different laboratories. Understanding how bacteria attach to anodes could allow the design of more efficient electron transfer systems. Genetic and metabolic engineering of electrode active bacteria, including the overexpression of essential cytochromes or shuttling compounds, could increase current production.

Modeling and Framing Biofilm Ecology

Consistent with their behavior in nature, bacteria in MFCs form biofilms on the anode surface. Because MFCs measure real-time bacterial activity and detect redox-active components, they provide a platform for addressing questions about biofilm ecology. For instance, on the basis...
of mathematical models to describe anode biofilms, Kato Marcus and collaborators at Arizona State University in Tempe determined that the entire anode biofilm is electrically conductive and that biofilm density and detachment are important factors in electrochemical performance.

With these modeling results as a starting point, recent efforts are aimed at increasing the active biomass capable of electron transfer to the anode surface without altering mass transfer events or the physical environment within the anode biofilm. Researchers at the University of Massachusetts found that biofilms formed under increased shear contain a higher density of active bacteria, increasing MFC performance threefold. Future modeling studies could highlight discrepancies between predicted and observed power production in MFCs, suggesting abiotic and biotic areas of improvement.

Beyond modeling, we can explore the environmental and biological cues involved in biofilm formation and dissolution. The role and temporal dynamics of quorum-sensing compounds on anode colonization and current production have not yet been evaluated in MFCs, which is unfortunate given that many signaling molecules may also function as electron-shuttling components in mediated electron transfer. MFCs provide an ideal platform to gain a better understanding of the attachment, succession, dissolution, and interspecies interactions that occur within biofilms.

A Current Affair: Ongoing Research and Challenges

MFC research endeavors are increasing each year. Much attention is dedicated to optimizing power generation. Hardware and operational constraints, rather than microbial activity, primarily contribute to limitations in MFC power densities. Improvements in MFC design and materials have significantly improved reactor performance by 10,000-fold since 1999. Despite this advance, a further increase of 10- to 100-fold is required for MFCs to be considered for practical applications.

A major focus is on reducing internal reactor resistance and increasing cathodic reaction efficiency to maximize power in lab-scale systems. However, in such systems the cost of component materials and operation far exceeds the value of energy generated. Economic feasibility studies of large-scale implementation of MFC technology require pilot-scale applications to evaluate the design, construction, operation, and microbial restrictions. We need to know which aspects of MFCs will scale linearly and which will not, and cheaper and more durable electrodes and cation-permeable membranes are required.

Pilot-scale MFCs are being put to the test at Anheuser-Busch Inc. and Foster’s breweries, of the United States and Australia, respectively. Researchers at the two brewing companies separately are evaluating whether MFC technology can be used to treat organic-rich soluble wastewater while producing electricity. Thus, in September 2007 researchers from the University of Queensland and from Foster’s began a 1,000-L MFC experiment to address some of the questions surrounding scale-up (Fig. 3a).

Full of Potential: Future Applications of MFC Technology

Microbes participate in a vast array of biochemical reactions to satisfy their energy and resource demands. The microbial metabolism in MFCs can be harnessed for bioremediative, industrial, and hydrogen production applications to produce valuable end products in an environmentally responsible way.

The conversion of biomass, especially organic waste, to energy is considered an essential part of a sustainable global energy portfolio. A variety of potentially valuable underutilized energy sources exist in the United States. For example, assuming that its organic material is completely oxidized to carbon dioxide, human waste could produce 34 billion kWh of energy annually. This represents an enormous untapped energy source. Moreover, MFCs can generate electricity from cellulose. Thus it is feasible for MFCs to treat wastewater from biofuel processing, removing waste material to recycle water while generating electricity. Coupling these technologies to minimize production costs and increase energy recovery could help make “green energy” profitable and sustainable.

While the use of MFCs for wastewater treatment is in its infancy, MFCs as batteries for environmental sensors is nearing practical use. In contrast to traditional batteries, MFCs powered by organic matter in sediments offer advantages as power sources because they can gener-
ate energy without a need for recharging. These types of MFCs, called benthic unattended generators (BUGs), have been used in inaccessible areas such as river and ocean sediments. Operation is technically simple; a graphite plate is deployed into the anoxic sediment (anode) that is electrically connected to another graphite plate in the overlaying aerobic water (cathode) (Fig. 3b and 3c). Although power density is minimal, incorporating a capacitor in the electrical circuit stores the produced BUG energy for use in short bursts. Using this approach, a BUG deployed in creek sediment powered an environmental sensor and a wireless data transmitter to monitor air and water temperature and transmit this data to a shore-based receiver.

MFCs have applications beyond electricity production. MFCs are used to power cathodic reduction reactions for bioremediation or industrial processes. Since electricity is not being harvested—the biologically generated current is used to stimulate microbial metabolism on a cathode—these systems are not considered fuel cells, but are called bioelectrical reactors (BERs). An external power source usually provides the reducing equivalents in these systems, but a biological anode may be used. Cathodes have served as electron donors for bacterial reduction of bioremediation targets such as uranium, perchlorate, chlorinated solvents, and nitrate. This technology could be applied to remediate other contaminants including toxic metals, dyes, pesticides, and herbicides.

BERs in which reducing equivalents are produced at the anode may also yield industrially important chemicals such as hydrogen peroxide, sulfur, and butanol. Using BERs to produce fuels such as propanol and butanol from organic waste is very appealing. In this process, organic waste with a sugar content too low to allow ethanol production would be microbially fermented in the absence of an electron acceptor into volatile fatty acids (VFA). These VFA can be fed to the cathode compartment, where bacteria would use the electrons supplied from the cathode to reduce VFA into propanol and butanol. This process, using hydrogen rather than MFC cathodes as a source of reducing equivalents, is feasible, according to Kirsten Steinbusch of the Wageningen Institute for Environment and Climate Research in the Netherlands. Specific research hurdles include evaluating the use of current rather than hydrogen for reducing equivalents, fine-tuning concentrations of VFA and electrons for favorable thermodynamic conditions, and developing methods for separating the desired end-products from the reactor liquor.

In addition to powering BERs, MFCs can also be modified to produce hydrogen gas.
With transportation fuels accounting for up to 25% of global fossil fuel consumption, alternative, sustainable fuel sources are needed. Microbial electrolysis cell (MECs), which like MFCs are based on bacterial oxidation of organic substrates occurring at the anode and electrons flowing to the cathode, can generate renewable hydrogen from waste materials. In MECs an electrochemical potential achieved in the anode is supplemented with an additional ~250 mV from an exogenous source so that electrolysis of water occurs at the cathode, producing hydrogen. Over the past two years research in this area has advanced significantly, with the amount of hydrogen generated per mol of oxidized glucose nearing the U.S. Department of Energy’s target for technology viability. Hydrogen production in reactors using existing technology is too low to make large-scale MEC’s likely in the immediate future, but a combination of improved reactor design and treatment of organic-rich wastewaters makes this an attractive proposition for the future.

SUGGESTED READING


