Experiment 4: Bioenergy- The Awesome Power of Microbial Metabolism

One of the most important challenges of our time is the development of clean energy technologies to replace fossil fuels. Wind, solar, and geothermal energy along with biofuels all lead the list of promising new technologies for energy production. Microorganisms play a central role in many approaches to biofuels, from using microbes to convert cellulose into ethanol to using microbes to generate electricity in microbial fuel cells. The microbial-based approaches to biofuels all rely on the many diverse metabolic pathways possessed by microbes.

The first known fuel cell producing energy from microbes was built in 1911 by Michael Potter, a botanist studying microbial metabolism. He noticed that microorganisms could produce electricity as they degrade organic compounds. He built a simple microbial fuel cell that produced a small amount of energy. It wasn't until close to seventy years later that scientists revisited the microbial fuel cell as a potentially simple and inexpensive way to generate electricity and interest in microbial fuel cells has built since then. In 2009, "the electric microbe" was named by Time magazines as one of the 50 best inventions of the year. Several researchers around the world are now working to improve microbial fuel cell technology so that it can be brought into practical use. One of the leaders in the field, Bruce Logan, is a professor at Penn State.

The key to microbial electricity production is found in central metabolism. Compared to humans, microbes have a rich and diverse array of energy-generating metabolisms, some of which can be harnessed to produce electricity. Humans are limited to one form of energy metabolism to keep us alive. We eat electron-rich organic compounds that are degraded in a series of reactions and their electrons are transferred to the electron transport chain. The electrons pass through the electron transport chain through a series of oxidation-reduction reactions and ultimately reduce oxygen to water. Energy gained from these oxidation-reduction reactions is used to make ATP, which is a major storage form of energy for the cell. This process is called aerobic respiration and is why we need oxygen to breathe. Without oxygen, electron transport stops and our cells can no longer generate energy. By contrast, many microbes are capable of anaerobic respiration in which they use a variety of terminal electron acceptors other than oxygen. In essence, they "breathe" iron or nitrate or sulfite - whatever the terminal electron acceptor might be. Certain microbes can actually use an electrode as a terminal electron acceptor.

Electricity is generated from a battery by the movement of electrons between the two electrodes, the anode and the cathode. The flow of electrons between the anode and cathode can be used to power your flashlight or iPod or other device. Batteries work through relatively straightforward chemical reactions. Oxidation reactions at the anode produce electrons that are then consumed in reduction reactions at the anode. Microbial fuel cells work on the same principle as a chemical battery. Electrons are generated at the anode by bacteria through anaerobic respiration. The electrons are then used to reduce O_2 to water aerobically at the anode. The anode and cathode are often separated by a membrane that is permeable to ions but not electrons.

In principle, any bacteria that can respire anaerobically could be used as a source of electrons. The main issue preventing the efficient use of many species of bacteria in fuel cells is that the bacteria use terminal electron acceptors for the electron transport chain that are located inside the bacterial cell. The electrons must then be shuttled outside of the bacterium to reach the anode, which is not simple to achieve. However, bacteria can be found that specialize in using an external electron acceptor for anaerobic respiration. These exoelectrogenic bacteria are often found in the soil and can use insoluble compounds in the environment, such as Fe^{3+} particles, as electron acceptors. Species of *Geobacter* actually produce pili that act as bacterial nanowires. Electrons travel from the bacterium, down the pili to the Fe^{3+} particles in the soil, or to an electrode in a fuel cell. *Shewanella* species produce flavins that serve as extracellular electron carriers, carrying electrons from the bacteria to external electron acceptors.

Although microbial fuel cells might sound esoteric and complicated, they can be made quite simply using microbes found in soil. Your average gram of dirt contains nearly a billion microbial cells from thousands of different species. Among these thousands of species are some that are exoelectrogenic. To construct a fuel cell you do not even have to purify the bacteria, a scoop of dirt and the proper placement of electrodes are all that you need.

The power output of a fuel cells depends on a few basic properties. First is the source of soil. Different soil environments favor the growth of different bacteria due to the types of nutrients available, moisture, temperature fluctuations, and oxygen availability. The types and proportion of exoelectrogenic bacteria, therefore, vary from one soil source to another. Another important factor are the types of organic matter available for consumption by the exoelectrogenic bacteria. Some soil-based fuel cells are supplemented by the addition of carbon sources, such as acetate, that are preferred by exoelectrogenic bacteria. Finally the temperature at which the fuel cells are operated affects the power output, since it affects the growth of bacteria that power the fuel cell.

Resources

For information specifically about Microbial Fuel Cells and the MudWatt, read the files posted with this lab manual.
You can find information about bacterial metabolism and respiration in any microbiology textbook.
In the online textbook of bacteriology, look at the chapter on Diversity of Metabolism in Prokaryotes. Focus on the overview of energy-generating metabolism (pg 1) and respiration (pg 4). http://textbookofbacteriology.net/metabolism.html

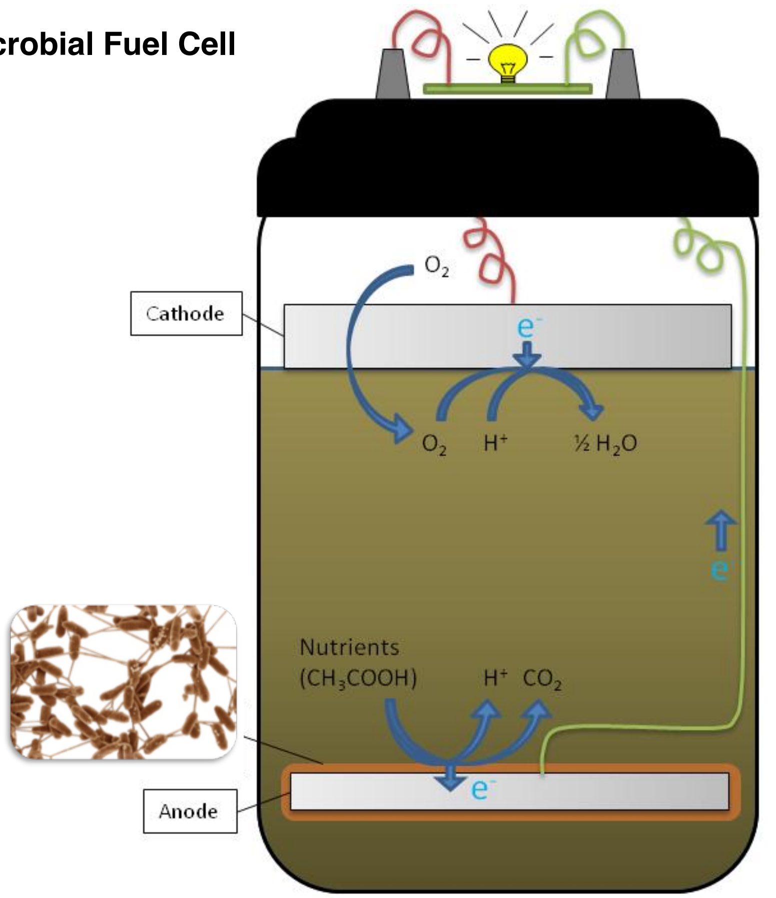
Key Concept

Harnessing microbial metabolism to generate energy.

Challenge

In this lab module, each group will make its own microbial fuel cell using a MudWatt microbial fuel cell kit from Keego Technologies. **As a group decide what soil source and operating conditions you would like to test in your MudWatt.** You also have the option of bringing in additional "food" supplements for your fuel cell - see the document on special ingredients in the MudWatt User Guide. The fuel cells will be used to power a blinking LED light. The frequency of blinking is determined by the power output of the fuel cell. You will measure the power output of your fuel cells throughout the semester to monitor changes in power production. In December we will compile the data as a class and compare the different fuel cells.

The MudWatt Microbial Fuel Cell



References
Lovely, D.R. Bug juice: harvesting electricity with microorganisms (2006) Nat Rev Microbiol 4: 496-508
Logan, B.E. and Rabaey, K. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies (2012) *Science* 337:686-690.
http://www.keegotech.com

Assessment

Throughout the remainder of the semester you should make weekly recordings of the blinking frequency of your MudWatt and the power output. Measure the power output by following the instructions in the protocol section for performing a potentiometry sweep. Record your data weekly in a table like that shown below. At the end of the semester, each group will give an oral presentation on their Microbial Fuel Cell experiment.

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<tr><td colspan="3">Date:</td></tr>
<tr><td colspan="3">Temperature:</td></tr>
<tr><td colspan="3">Blinking frequency:</td></tr>
<tr><th>Resistance</th><th>Voltage</th><th>Power</th></tr>
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