

Bioenergy Education Initiative

Levels:

Grades 9-12

Content Areas:

Biology, Chemistry; Physical Science

Lesson Time:

45 -60 minutes

Next Generation Science Standards:

HS-PS3-3

HS-LS2-3

Objectives & Outcomes:

- Students will build a biological battery and generate a measurable voltage.
- Students will be able to calculate electrical power given voltage and resistance.
- Students will understand how bacteria can convert chemical energy into electrical energy.

Contact:

Bioenergy Education Initiative agsci.oregonstate.edu/bioenergy-k-12

Description:

In this experiment students will build a sediment or mud-based battery and learn how bacteria can convert chemical energy, like that in wastewater, into electrical energy. Students will also be introduced to the fundamental principles surrounding energy conversion, microbial metabolism and electricity.

Using This Lesson:

The experiment in this lesson can be done in small groups. The background information has been written so it can be used as reading material for students. Vocabulary words are highlighted in the text.

Questions are provided to promote discussion and critical thinking. Additional, more advanced student questions and activities to expand the lesson are provided. See the Resources page for links to YouTube videos on bioenergy that support this lesson.

Bacteria Power and the Future

Just as the food we eat contains a lot of energy, so does the waste we produce. If we dumped all this waste into the rivers and oceans without removing the energy, we would destroy those important ecosystems. For this reason, all the nutrient rich organics in wastewater must be removed at treatment plants.

The process to remove these organics, or treat the wastewater, requires a lot of energy (3% of all the electricity in the U.S.). Researchers are currently

developing microbial fuel cells as a way to simultaneously treat wastewater and convert the energy into electricity. If this can be done, it may be possible for wastewater treatment to go from a process that uses a lot of energy, to one that could generate energy.



Microbial Fuel Cells and Bacterial Power

Set Up:

Collecting Sediment: To do this lesson you will need to collect sediment from local bay, pond or river. Mud found under stagnant, oxygen depleted water is best. To collect the sediment, lower a bucket into the water and scoop up the black mud. A hydrogen sulfide smell (rotten eggs) is an indicator of anaerobic conditions. This is what you want. The sediment you collect should be saturated, but once it settles the water layer should not be more than ½ of total volume.

Adding Nutrient Solution: Once you have collected your sediment, you will need to add a sugar solution. This solution mimics the nutrient-rich organics (electron donor) found in wastewater which the bacteria in the sediment will convert into electricity. Dissolve five sugar cubes into one cup of water then pour the solution into your sediment.

Directions:

- 1. Build the Electrode: Attach one end of each electrical lead to the opposite ends of the resistor by twisting the resistor wire around the alligator clip. Wrap the resistor wire firmly around the alligator clips to ensure a secure connection. The voltage drop across the resistor allows you to measure the electrical current.
- 2. Attach the Anode: Attach the free end of one of the electrical leads to one of the pieces of carbon cloth. This piece will serve as the anode which will collect electrons released by the bacteria in the mud.
- 3. Placing the Anode: Place the connected piece of carbon cloth (anode) at the bottom of the container. Leave the connected resistor and lead wire hanging over the lip of the container.
- 4. Add the Sediment/ Microbes: Fill the container half way full with anaerobic sediment. The sediment should cover the carbon cloth on the bottom (photo 4). Make sure the carbon cloth is completely buried and remains at the bottom of the container with the lead attached. The resistor wire should hang over the lip of the container. It can be secured with tape. The clip needs to be exposed so you can take measurements. If the sediment is not heavy enough, use a small weight to hold down the anode.
- 5. Attach the Cathode: Attach the other piece of carbon cloth to the free end of the electrical lead. Float the cloth gently on the surface of the water. This piece of carbon cloth needs to be in contact with oxygen. It will serve as the cathode and is the site at which electrons from the anode react with oxygen on the water's surface. It is important to ensure that the cathode and the anode are not touching.

Materials: Per MFC

- 16 oz./475 ml anaerobic soil (stagnant pond mud best and salt water is okay)
- 24 oz./700 ml food storage container
- 2 electrical test leads with alligator clips (6" 10")
- 2 carbon cloth pieces (4" X 4"; can be purchased from Amazon.com)
- 1 resistor (100 kΩ)
- 1 multimeter
- 5 sugar cubes
- 1 cup/ 236.59 ml water

OHM's Law

The relationship between power, resistance (resistance to electrical flow), current and voltage is described by Ohm's Law.



Using the voltage readings from the multimeter and the known resistance of the resistor, students will be able to calculate the electrical current

Additional photos of the steps can be found in the student directions section.

- 6. Let the sediment in the container settle for at least 15 minutes.
- 7. Using the multimeter, measure the voltage across the resistor by touching the clips attached to each end of the resistor. (Note: Students need to select the smallest voltage range on the multimeter 1-3 volts. The voltage may also drift continuously, so use the initial measurements.)
- 8. Record voltage and resistance.
- 9. Using Ohm's law and the equation describing power, calculate the current and power being generated.
- 10. Have students experiment with the MFC and attach them in different ways. Have them record their observations.

Extension 1: Let the solution sit for 24-48 hours and measure the fuel cell voltage again. Voltage should increase over time.

Extension 2: Set up MFC using different types of sediment, or sediment with different levels of sugar added. Compare voltages? Alternatively, compare different types of electron donor additions to the sediment – juice, vinegar, etc.

Extension 3: Connect the MFCs in a series in parallel. This will increase the voltage and students should be able to see the power increase.

Expected Outcomes:

All students should be able to successfully build MFCs. Initial voltage readings should be in the range of 50 – 200 mV depending on the source and anaerobic nature of the sediment/mud. Voltages will tend to decrease at first, then increase slowly over time as **exoelectrogenic biomass** grows. Subsequent feeding should result in higher voltages reaching a maximum of around 0.6 V, and 5 mA.

Make sure the sediment solution gets to sit in the containers for at least 15 minutes. Thirty minutes is even better. Consider taking readings at both 15 and 30 minutes.

The MFC can be successfully run over a long period of time, as long as the anode remains buried in sediment and the appropriate water level maintains separation between the anode and the cathode. Adding things like vinegar should result in high voltage readings because acetic acid is easily used by exoelectrogens. Different types of juice may not result in as high of readings because they must first be fermented by other microorganisms before being used by the exoelectrogens.

The electron donor, or sugar solution, mimics the nutrient rich organics found in wastewater. Let the solution in MFC sit for a couple days and take another reading.

Experiment Questions:

The following are the questions and answers for the student worksheet and some additional advanced level questions. An example of a completed chart using Ohm's law to calculate current and power is below.

Electrical Current Calculation Solution/Example:

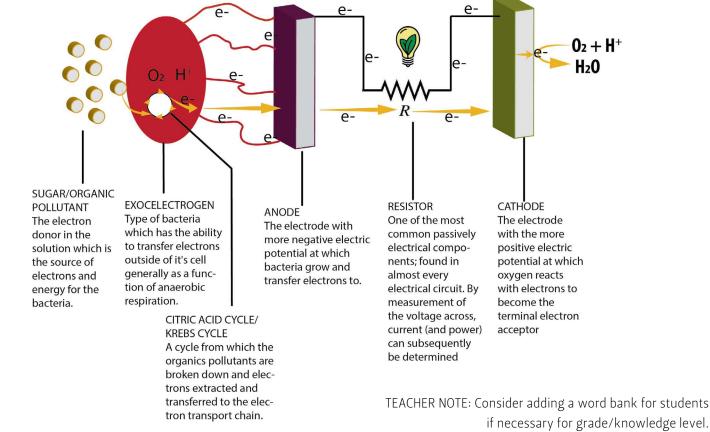
Voltage (mV)	Voltage (V)	Resistance (Ω)	Current (A)	Current (mA)
100*	0.1	10000	0.00001	0.01
*measured				

1 V = 1,000 mV 1 A = 1,000 mA V = IR I = V/R Conversion from mV to V $100 \text{ mV} \times (1 \text{ V} / 1,000 \text{ mV}) = 0.1 \text{ V}$ Calculation of Electrical Current $0.1 \text{ V} / 10,000 \Omega = 0.00001 \text{ A}$ Conversion from A to mA $0.00001 \text{ A} \times (1,000 \text{ mA} / 1 \text{ A}) = 0.01 \text{ mA}$

BASIC LEVEL

- 1. The nutrients and organics in wastewater need to be removed before being put back into water ways or the excess nutrients may cause ______to happen. (Eutrophication)
- 2. Electricity is the flow of electrons. The source of electrons, or electron donor, in this experiment is the _____(Nutrient Solution/Pollutants/Organics).
- 3. The bacteria use the _____cycle to break down the organics. The electrons that are released are then passed through the _____chain and then outside of the cell to the _____. (Krebs, electron transport, anode)
- 4. The anode potential is more _____ than the cathode potential. (Negative)
- 5. At the same resistance an increase in voltage will lead to a ______ in current and power. (Increase)





6. Use the reading material provided to fill in the blanks of the diagram outlining the path of electrons through the MFC.

ADVANCED LEVEL

- 1. What is electricity? How do MFCs and alkaline batteries differ in how they generate electricity?
 - Electricity is the flow of electrons through a circuit. The primary differences between MFCs and alkaline batteries are the source of the electrons and the electron acceptors. In MFCs electrons are released from the organics by bacteria, travel through the circuit and react with oxygen. In alkaline batteries the circuit is completed by connecting the anode and the cathode. In alkaline batteries the negative electrode and source of electrons is typically zinc and the positive electrode and electron acceptor is typically manganese.
- 2. What kind of applications are best suited for using the electricity generated by MFCs? Applications that require small amounts of power over long periods of time.
- 3. What are other means to convert chemical energy in organics into energy? What are advantages and disadvantages in using MFCs to do this?
 - Another means of conversion is through combustion or burning organics. This is a highly effective way to quickly release large amounts of energy compared to an MFC. However, MFCs will not have the same amount of energy lost in terms of heat and will be much more efficient in terms of converting energy into electricity.
- 4. What are the advantages of using an electrode as an electron acceptor versus the naturally present minerals in sediments bacteria generally use?
 - The primary advantage is that the electrode, if connected, provides an inexhaustible electron acceptor as opposed to minerals that will quickly become reduced and unable to be used as an electron acceptor anymore.
- 5. What are some ways current generation could be increased in MFCs?
 - The current can be increased by increasing the surface area for the bacteria and the oxygen reduction reaction. Additionally, if the exoelectrogenic bacteria can be enriched that would further enhance current generation. Providing substrates that are easy for the bacteria to break down quickly would also work.

Microbial Fuel Cells and Bacterial Power

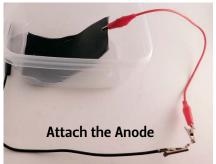
Directions:

- 1. **Build the Electrode:** Attach one end of each electrical lead to the opposite ends of the resistor by twisting the resistor wire around the alligator clip. Wrap the resistor wire firmly around the alligator clips to ensure a secure connection
- **2. Attach the Anode:** Attach the free end of one of the electrical leads to one of the pieces of carbon cloth.
- **3.** Placing the Anode: Place the connected piece of carbon cloth (anode) at the bottom of the container. Leave the connected resistor and lead hanging over the lip of the container.
- 4. Add the Sediment: Fill the container about half way full with sediment. The sediment should cover the carbon cloth on the bottom. Make sure the carbon cloth is completely buried and remains at the bottom of the container with the lead attached. The resistor should hang over the lip of the container. It can be secured with tape. The clip needs to be exposed so you can take measurements. If the sediment is not heavy enough, use a small weight to hold down the anode.

Materials: Per Group

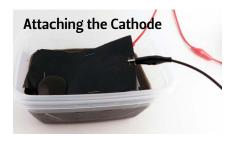
- 16 oz./475 ml anaerobic soil (stagnant pond mud best)
- 24 oz./700 ml food storage container
- 2 electrical test leads with alligator clips (6" - 10")
- 2 carbon cloth pieces (4" X 4")*
- 1 resistor (100 kΩ)
- 1 multimeter







- **5. Attaching the Cathode:** Attach the other piece of carbon cloth to the free end of the electrical lead. Place the cloth gently on the surface of the water. The cathode and anode should not touch.
- 6. Let the sediment in the container settle for at least 15 minutes.
- 7. Using the multimeter, measure the voltage across the resistor by touching the clips attached to each end of the resistor. (Note: Make sure the multimeter is set to its lowest setting. The voltage may drift continuously, so use the initial measurements.)
- **8. Record Voltage and Resistance:** Measure and record voltage and resistance. Using Ohm's law and the equation describing power, calculate current and power. (See worksheet for details)





Measuring Electrical Current in MFCs

Electrons carry a negative electrical charge. The movement of these negatively charged electrons is called **electricity**. In a microbial fuel cell (MFC) electrons are released at the anode so the anode has a more negative charge, also referred to as **electric potential**, compared to the cathode.

The difference between the electric potentials of the two electrodes (**anode** and **cathode**) is referred to as **voltage**, measured in **volts** (V) and represented by **V**. The larger the voltage between the electrodes the more electricity wants to flow. The measurement of electrical flow is referred to as **electrical current** and is measured in **amperes** (A) and represented by **I**. The relationship between voltage and current is described by **Ohm's law** (equation below) and is dependent on electrical resistance, which measured in **ohms** (Ω) and represented by **R**.

Using a water analogy to describe electricity, voltage is the pressure pushing the water through a pipe. A larger voltage means more pressure. Resistance is the size of the pipe; a higher resistance is equal to smaller pipe from which less water can flow through. Current is the measurement of how much water flows.

$V = I \times R$ or I = V/R

To determine electrical current produced by the MFC, measure the voltage on opposite sides of the resistor. Then use the measured voltage and the known resistance of the resistor to calculate current.

Directions:

Use Ohm's law to calculate current and power. Record your answers in the chart below.

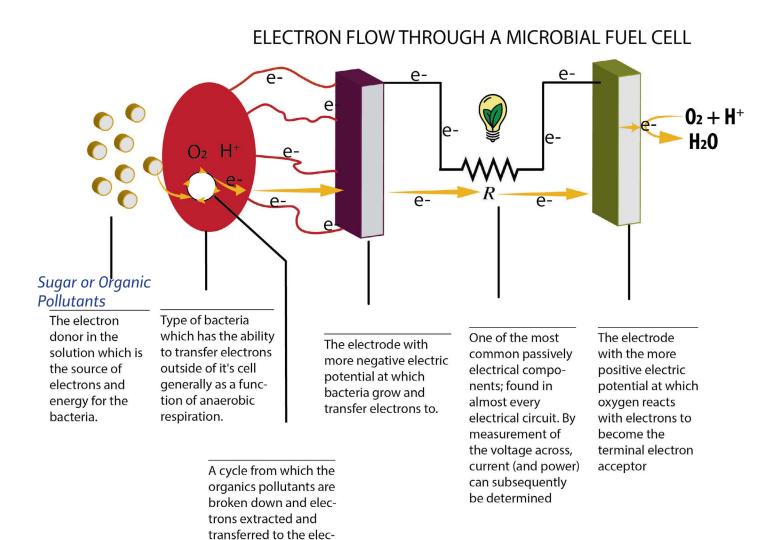
Note: amp (A) = 1,000 milliamp (mA)

Voltage (mV)	Voltage (V)	Resistance (Ω)	Current (A)	Current (mA)

Complete the following statements.

1.	The nutrients and organics in wastewater need to be removed before being put back into water ways or the excess nutrients may cause to happen.
2.	Electricity is the flow of electrons. The source of electrons, or electron donor, in this experiment is the
3.	The bacteria use the cycle to break down the organics. The electrons that are released are then passed through the chain and then outside of the cell to the The anode potential is more than the cathode potential.
4.	At the same resistance an increase in voltage will lead to an in current and power. (Increase)

5. The diagram below outlines the path of electrons through the MFC. Fill in the blanks of the diagram that label each item/and or step.



Advanced Option: Set up MFC again, but this time use a different type of substrate like vinegar or juice. Use Ohm's law to calculate current and power. Record your answers in the chart below. Compare voltages to your initial experiment. Are there any differences? If so, why do you think they have occurred?

tron transport chain.

Voltage (mV)	Voltage (V)	Resistance (Ω)	Current (A)	Current (mA)

The Power in Bacteria

There are many ways to convert different forms of energy into electricity, such as using solar panels (solar energy into electricity), wind turbines (wind energy into electricity), and hydroelectric dams (gravitational potential energy into electricity). Certain bacteria even have the ability to convert the chemical potential energy contained in organic pollutants directly into electricity.

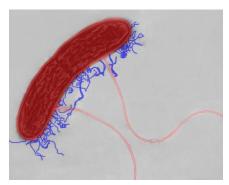
Microbial Fuel Cells (MFC)

While scientists have known that bacteria can produce electricity since the early 1900's, it is only in the last 20 years that researchers have begun to take advantage of the electricity producing bacteria found in most types of sediment using microbial fuel cell (MFC) bioreactors.

Traditionally, biomass like coal or wood has to be burned or combusted to be converted into electrical energy. In a microbial fuel cell many forms of biomass, including food and sewage wastes, are able to be converted directly into electrical energy by bacteria. This direct conversion reduces the amount of energy lost as heat during the conversion processes.

The power MFCs can generate is limited by the rate bacteria can degrade its food sources, but they are very efficient. Almost 90% of the energy in waste streams (sewage, waste water treatment plants) can be converted into

electrical current, significantly higher than combustion processes. The wastewater is also cleaned by microbial reactions in the MFC that prevent the excess nutrients and organics, which cause *eutrophication* and destruction of aquatic habitats, from entering water ways. The electricity produced by the MFC can then be used to power homes, businesses and countless other electrical needs.



Bacterial nanowires: A team of researchers in the U.S. has found clear evidence of a microbe that conducts electricity just like a metal using nanowires. The nanowires are the long strands hanging below the bacteria pictured above.

Wired Bacteria

Why would a bacterium produce electricity? The answer is actually something we can all relate to: the bacteria need to perform respiration, like we do when we breathe.

Once a bacterium absorbs organic pollutants/nutrients (*electron donors*) the organics enter the *Krebs cycle*. During this cycle the electrons in the organics are extracted. The flow of these electrons through the *electron transport chain* (ETC) and ultimately to oxygen turns

a pump in cells that generates the energy the organism needs to live. This process is known as **aerobic respiration**.

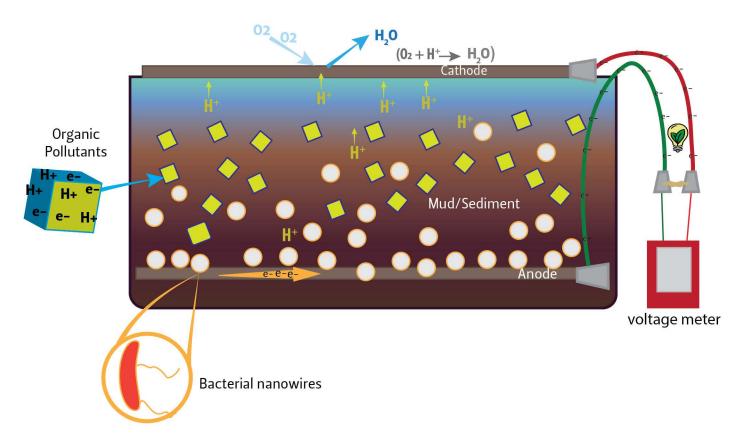
Bacteria that generate electricity live without oxygen in under water environments. They take in food and extract electrons through the citric acid cycle and pass them through the ETC in a similar manner. However, since oxygen is not available, they are forced to use alternate compounds like iron or sulfur as a terminal electron acceptor, instead of oxygen. This process called *anaerobic respiration*.

Sometimes, the only chemicals that are able to accept electrons from these bacteria are solid metals. like iron. Solid metals cannot be transferred into cells like oxygen can, therefore the bacteria are forced to transfer electrons outside of their own cells in order to respire. These bacteria are referred to as exoelectrogens. They do this by growing special microbial **nanowires** that stick out from their cells and act like electrical wires (see image). They enable anaerobic respiration and also allow bacteria to send electrical signals to one another and communicate.

Harnessing Bacteria Electricity

When we feed bacteria they are able to extract the electrons from their food and pass them to a solid metal, like iron.

How Microbial Fuel Cells Work



In microbial fuel cells, an electron transferring surface called an *electrode* is placed at the bottom of the mud where these bacteria live. This electrode is called an *anode*. Another electrode is then connected to the circuit at a more positive electrical potential that attracts the negatively charged electrons.

This electrode is called a *cathode*. At the cathode electrons react with oxygen, which is the *terminal electron acceptor* in the system. This is why the cathode needs to be near the surface of the MFC. The flow of the electrons from the anode to the cathode is electricity and can be used to power electrical devices.

The bacteria then transfer their electrons to the anode electrode instead of the metals in the mud. These electrons have a negative electric charge.

When these electrons are transferred to the anode, a negative electric potential environment is created. In order to generate electricity these negatively charged electrons must flow to a more positive electrical potential environment.

Oxygen in air generates a very positive electrical potential environment. If another electrode (also called a cathode) is placed at the surface of the water where it is exposed to oxygen in the air, and then connected to the anode

by an electrical wire, the electrons will be free to flow from the anode to the cathode. This flow of electrons is called electricity.

While MFCs are expected to see expanded use in the future of wastewater treatment, they are currently employed for powering sensors in ocean environments. NASA has even looked at using MFCs to power small spacecraft due to their ability to generate small amounts of power over a long period of time. Understanding the bacteria that drive MFCs and their interactions with electrodes will lead to many more exciting advancements and applications.

Next Generation Science Standards

DISCIPLINARY CORE IDEAS:

PS3: Energy in chemical processes

LS2: Ecosystems: Interactions, energy and dynamics

PERFORMANCE EXPECTATIONS:

HS-PS3-3:Design, build and refine a device that works within given constraints to convert on form of energy into another form of energy.

HS-LS2-3: Construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.

PRACTICES:

- Planning/carrying out investigations
- Math/computational thinking
- Energy/matter: Flows, cycles, conservation

CROSSCUTTING CONCEPTS

- Systems and system models
- Energy/matter: Flows, cycles, conservation
- Structure and function

Video Resource

Electrifying Wastewater: Using Microbial Fuel Cells to Generate Electricity. BytesizeScience (July 2013). https://youtu.be/ ZotwUJAb8R4

GRANT SUPPORT

This work is part of the Advanced Hardwood Biofuel Northwest project (hardwoodbiofuels.org) and is supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30407 from the USDA National Institute of Food and Agriculture.

Resources:

<u>How to Create a Microbial Fuel Cell - Part 1</u>, You Tube (December 2009). Retrieved from https://www.youtube.com/watch?v=jnJUCORTwt0&list=PL7C91954C5DA5BB35.

<u>Fuel Cell Treats Wastewater and Harvests Energy</u>, Scientific American (July 2012). Retrieved from http://www.scientificamerican.com/article/microbial-fuel-cell-treats-wastewater-harvests-energy.

<u>Ten Bacteria with real-life superpowers</u>, BBC (July 2015). Retrieved from http://www.bbc.com/earth/story/20150730-ten-bacteria-with-superpowers.

<u>Microbial Fuel Cells Q& A</u>, Penn State College of Engineering, Retrieved from http://www.research.psu.edu/capabilities/documents/MFC_QandA.pdf.

<u>Bacterial Nanowires Conduct Like Metals</u>, Physics World (August 2011), Retrieved from http://physicsworld.com/cws/article/news/2011/aug/10/bacterial-nanowires-conduct-like-metals.

<u>Navy Tests an Ocean Sensor that Autonomously Dives and Surfaces Using Microbe Power,</u> Popular Science (July 27, 2011), Retrieved from http://www.popsci.com/technology/article/2011-06/navy-tests-ocean-sensor-powered-microbial-fuel-cell.

Thanks to Dr. Hong Lu at Oregon State University for equipment use.