



How Do Bacteria Produce Power in a Microbial Fuel Cell?

https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p032/microbiology/bacteria-microbial-fuel-cell

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Experimental Procedure

Microbial Fuel Cells in Science Fairs

Working with microbial fuel cells involves growing soil bacteria. Because of this, many science fairs, including those associated with the International Science and Engineering Fair (ISEF) have requirements which need to be met *before* you start your project. We recommend you:

- Check with your teacher or science fair coordinator about any requirements
- Read the Science Buddies [Microorganisms Safety Guide](http://www.sciencebuddies.org/science-fair-projects/references/microorganisms-safety) (<http://www.sciencebuddies.org/science-fair-projects/references/microorganisms-safety>) to learn how to safely handle bacteria

Setting up the Microbial Fuel Cells and Bacteria Count

You will assemble two identical microbial fuel cells that contain the same soil material. Later in the procedure you will provide one of them with a sugar food source and compare its power output and bacterial quantities to the control microbial fuel cell with no treatment.

1. First, watch the video or follow the step-by-step instructions to see how to assemble the microbial fuel cells.

<https://www.youtube.com/watch?v=5iMw7-GIJFE> (<https://www.youtube.com/watch?v=5iMw7-GIJFE>)

2. Prepare your soil mud.
 - a. Place a plastic strainer or colander over a large mixing bowl.
 - b. Measure a total of about 2 cups (about 500 milliliters [mL]) of your soil for each of the microbial fuel cells into the strainer (4 cups in total). Gently shake the strainer over the bowl so that the soil is strained and any small, hard particles (such as rocks, pebbles, twigs, etcetera) are removed from the soil. You will likely need to be patient; it may take several minutes to strain the soil. When you are done, you should have about 400 g of fine, sifted soil in the bowl.
 - i. It is important to remove these particles from the soil because they can *aerate* the soil and inhibit the desired bacteria from growing (the bacteria do not want to be exposed to oxygen).
 - c. Add distilled water and mix it in until your topsoil mud feels like cookie dough. Add more water if the mud is too crumbly, or add more topsoil if the mixture feels too wet. For 400 g of soil you will likely need about 1 cup (250 mL) of water.
 - d. When you have prepared your topsoil mud, set it aside and wash your hands.
 - e. Take a sample of your prepared soil for measurement of the initial bacteria quantity.
 - i. Sterilize a metal spoon by dipping it in rubbing alcohol, shaking off the excess alcohol, and holding it into a flame (from a candle or gas stove). Wait until the flame on the spoon burns out and let the spoon cool down.
 - ii. Label a mini cup with the date and time, and use your sterilized spoon to scoop about 2–3 spoons full of your prepared soil (you will need 0.5 g) into the cup. Close the lid and set it aside for now.
3. Carefully take the MFC pieces out of the box and lay them out. Identify the different components and use masking tape and a permanent marker to label one of the devices with "control" and the other one with "sugar treatment."
4. Put on the gloves that came with the MFCs and start assembling the first microbial fuel cell.
5. Take out the green and orange wires that came with the MFC electrodes. Bend each wire where the plastic part ends so that each wire is now at a 90° angle (shaped like a capital "L"), as shown in the video above.
6. Remove the MFC anode from its bag. (The anode is the thinner, black, felt-like circle.)
 - a. **Safety Note:** The MFC's cathode and anode (its *electrodes*) are made of a conductive material called *graphite fiber*. Do not put the cathode or anode near electronics or power plugs, and do not disperse the fibers in the air, as the fibers will cause electrical shortages when in contact with electronics.
7. Straighten the metal part of the green wire and carefully insert it into the anode, as shown in Figure 3. Make sure the wire goes in straight and does not poke out on the top or bottom sides of the anode.

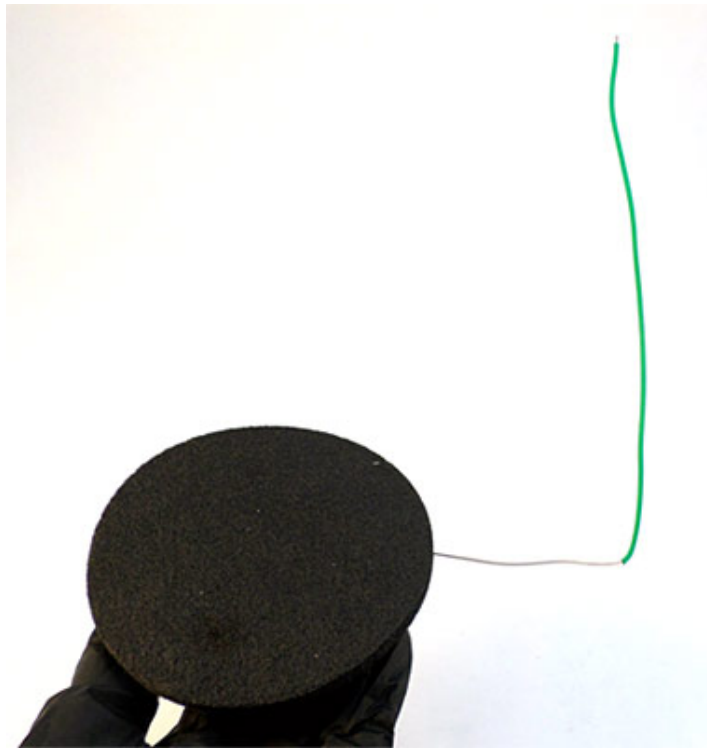


Figure 3. Insert the metal part of the green wire into the anode circle.

8. Repeat steps 6 and 7 using the cathode (the thicker, black, felt-like circle) and the orange wire (which is shorter than the green wire).
9. Take the soil mud that you prepared in step 2 and use it to fill the first MFC vessel (that came with your kit) up to the line next to the "1" on the plastic vessel (marking 1 centimeter [cm]). Once filled, pat the mud so that its surface is smooth.
 - a. *Tip:* You may want to cover the surface you are working on with old newspapers to prevent mud from getting on it.
 - b. When you are finished, rinse the mud off your gloves and dry them (but do not take them off yet).
10. Put the anode on top of the mud in the vessel, as shown in Figure 4.
 - a. The green wire from the anode should be sticking up. The green wire should *not* be stuck down in the mud.
 - b. Gently press the anode flat against the mud so that no air bubbles are under the anode. *Note:* Removing the air bubbles is important as the trapped oxygen can prevent the formation of an anaerobic bacterial biofilm and reduce the power output of your microbial fuel cell.



Figure 4. Place the anode on top of the 1 cm of mud.

11. Use more soil mud to fill the vessel up to the line next to the "5" mark (marking 5 cm). Once filled, again pat the mud so that its surface is smooth.
 - a. Run the green wire along the side of the vessel.
 - b. Rinse the mud off your gloves and dry them.
12. Gently place the cathode on top of the mud and press it as flat as you can, as shown in Figure 5.
 - a. The orange wire from the cathode should be sticking out of the top side.
 - b. Do not let any mud or liquid cover the top of the cathode.
 - c. It is best to arrange the cathode so that its orange wire is about 1–2 cm to the left of the green wire.
 - d. Let the mud rest in the vessel for a few minutes. Then carefully pour off any excess liquid.



Figure 5. Add the cathode on top of the 5 cm of mud.

13. Use a clean paper towel or rag to wipe any mud off the vessel's rim. Then take off your gloves.
14. Take the white plastic lid and pass the wires through the small holes in the lid. Arrange the wires so that the orange wire is on the left and the green wire is on the right when the semicircular indentation on the lid is facing the front. Then carefully snap the lid onto the plastic vessel.
15. Take out the *hacker board* (the small green circuit). Attach it into the lid's rectangular indentation.
16. Locate the "+" and "-" ports (i.e., holes) on the hacker board. Plug the cathode's wire (orange) into the "+" port and the anode's wire (green) into the "-" port.
17. Locate ports 1 and 2 on the hacker board. Plug the black/blue *capacitor* (the small, cylindrical item with two longer metal prongs) into these ports. The black/blue capacitor's longer prong should go into port 1 and the shorter prong into port 2. *Note:* The orientation of the capacitor is important. If you reverse the short and long prongs, it will not work and might even damage the capacitor. You may need to bend the capacitor's longer end slightly so that the capacitor's prongs fit into the ports well.
18. Plug the red LED below the capacitor into ports 5 and 6. (Ports 3 and 4 will remain empty.) The LED's longer prong should go into port 5 and the shorter prong into port 6. **Important:** The LED will only work if inserted in this orientation. If you accidentally reverse the prongs, the LED will not light up. You may need to bend the LED's longer end slightly so that the LED's prongs fit into the ports well.
19. Make sure that the wires, capacitor, and LED are all securely in place. The assembled hacker board on the top of the MFC should now look like Figure 6.

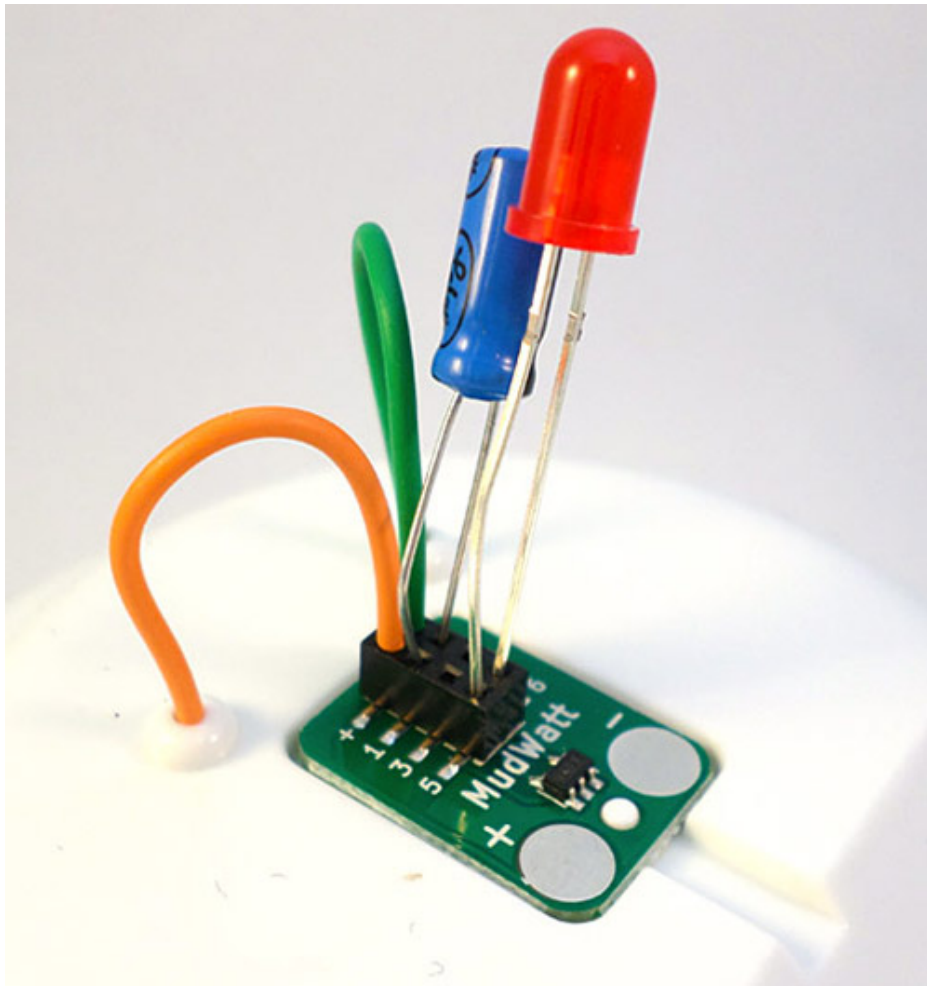


Figure 6. When you have finished assembling your MFC and its hacker board, the top should look like the one in this image.

20. After assembling the first MFC, set up the second microbial fuel cell using the rest of the prepared soil from step 2.
21. Once both microbial fuel cells are assembled, set the MFCs indoors, at normal room temperature (about 19–25° Celsius [C], or 66–77° Fahrenheit [F]), in a place where they will not be disturbed. The MFCs should remain in the same location the entire time after you set them up because if they are moved, this could disrupt the growth of the bacteria. It should take about 3–7 days before the red LEDs on the hacker board start blinking, but you will start taking measurements before that, as described in the next section. *Note:* Temperature variations can cause changes in the power output of the microbial fuel cells due to different bacteria activities.
22. Now you can start doing your initial bacteria count using the plate count method. To do that, put your gloves on, take the cup with the set aside topsoil that you prepared in step 2.e. and continue as follows.
 - a. Prepare three mini cups for the serial dilution and label them 10^{-2} , 10^{-4} , and 10^{-6} . *Note:* 1 g of soil contains millions or even trillions of individual bacteria. Because these are impossible to count, you will do a dilution series of your soil in order to get a small number of colonies on each plate.
 - i. Disinfect the area that you are working on with rubbing alcohol.
 - ii. Sterilize a metal spoon by dipping it in rubbing alcohol, shaking off the excess alcohol, and holding it into a flame (from a candle or gas stove). Wait until the flame on the spoon burns out and let the spoon cool down. Meanwhile, sterilize the medicine droppers by immersing the outsides in rubbing alcohol and then sucking up and expelling fresh rubbing alcohol several times. Wait for them to dry completely before using.
 - iii. Fill each cup with 49.5 mL of distilled water. You can use the digital scale to fill the cups with water until they weigh 49.5 grams.
 - iv. Weigh 0.5 g of your prepared soil and add it to the cup that says 10^{-2} . Use a sterilized spoon to stir the mixture well.
 - v. Suck up half a medicine dropper of the soil water mixture and add it to the cup that says 10^{-4} . Mix with a sterilized spoon.
 - vi. Using the same dropper, take a half of the dropper full of the new mixture from the 10^{-4} cup and add it to the 10^{-6} cup. Stir the solution with a sterilized spoon.
 - b. Take two of the nutrient agar plates and label the bottoms of the plates with the date, the sample volume, and the dilution factor. You will plate half a medicine dropper of the 10^{-4} dilution and 0.5 mL of the 10^{-6} dilution.
 - i. Pipette 0.5 mL of the 10^{-4} dilution onto the surface of the nutrient agar plate. Spread the liquid over the surface of the agar with a sterile cotton swab until it has soaked into the agar and the surface is mostly dry. Then close the

- lid of the plate.
- ii. Repeat the inoculation using the second sterile medicine dropper and the second plate with 0.5 mL of the 10^{-6} dilution.
 - iii. Keep all your dilution cups, as well as the rest of the soil, in a cooler or refrigerator until you do the plate count. Make sure all your cups are clearly labeled and kept away from food.
 - c. Incubate the plates, inverted, for 24–48 hours in a warm place. Make sure to keep the same incubation time for all your plates throughout the experiment.
 - d. After incubation, count the number of colonies on both plates and record the numbers in your lab notebook. The number of bacteria you will find on your plates will depend on the soil you chose. An example is shown in Figure 7. *Note:* Only count plates that have between 25 and 250 colonies on them. If there are less than 25 or more than 250 colonies on both of the plates, you need to re-plate from a different dilution (depending on your results, this could be 10^{-3} , 10^{-5} , or 10^{-7}). You can use the leftover soil or prepared dilutions (10^{-2} , 10^{-4} , and 10^{-6}) that you kept in the refrigerator/cooler to make other dilutions.

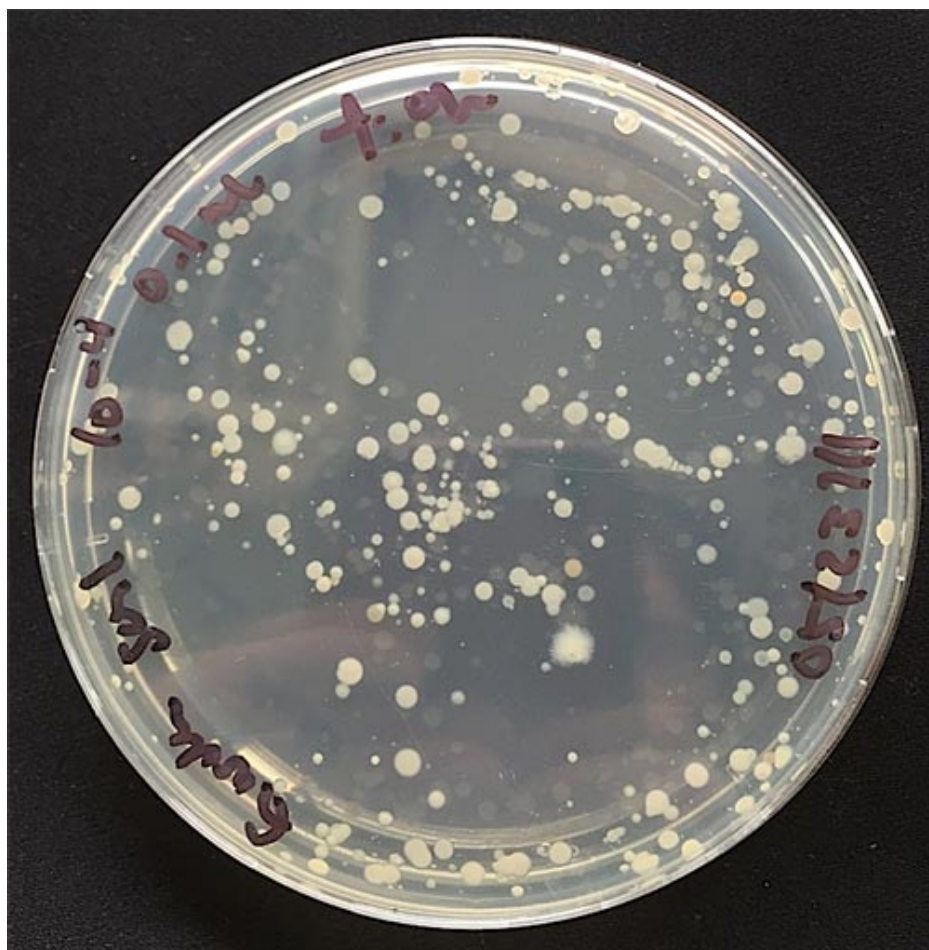


Figure 7. Count the number of bacteria colonies on each of your nutrient agar plates.

Measuring Power Output and Adding Sugar

You will measure the power output for both of your microbial fuel cells every day. Once the power output seems to have stabilized, you will add a small amount of sugar to one of the microbial fuel cells and use the other one as a control. The sugar should change the power output of the MFC. How do you think the MFC's power output will be affected?

1. One day after setting up your microbial fuel cells, check to see if the LEDs are blinking. Most likely, they will not be, but check to make sure. Watch the LEDs for 2 minutes to see if they are blinking.
 - a. If one or both of the LEDs are blinking, time how many seconds apart the blinks for each one are.
 - i. To do this, start a stopwatch as soon as you see the LED blink and stop the stopwatch when the LED blinks again.
 - ii. If the LED is blinking faster than once every 5 seconds, do not time the seconds between blinks, but instead time the blinks per second. Time a 10-second interval and count how many times the LED blinks in this period and then divide this by 10 to get blinks per second.
 - iii. Repeat step 1.a.i. or 1.a.ii. two more times so you have three total counts.
 - iv. Record your results for each MFC in your lab notebook in a data table like Table 1. (If you counted blinks per second, as in step 1.a.ii., change the heading from "Seconds Between Blinks" to "Blinks per Second.") Calculate

the average for your three counts and record that, too.

Seconds Between Blinks				
Day	Count #1	Count #2	Count #3	Average
1				
2				
3				

Table 1. Each day, check both MFCs to see if the LEDs are blinking. If they are, record how many seconds elapse between the blinks (or how many blinks there are per second) for each, making three separate counts. Record the results for each microbial fuel cell in a data table, like this one, in your lab notebook.

2. Next, measure the power output for both of the MFCs using the multimeter that comes with the Microbial Fuel Cell kit. You can either watch the video below or follow the step-by-step instructions. If you need help using a multimeter, consult the Science Buddies reference [How to Use a Multimeter](http://www.sciencebuddies.org/science-fair-projects/references/how-to-use-a-multimeter) (<http://www.sciencebuddies.org/science-fair-projects/references/how-to-use-a-multimeter>), as well as the instructions that came with your multimeter.

<https://www.youtube.com/watch?v=p2q6BGJuxRk> (<https://www.youtube.com/watch?v=p2q6BGJuxRk>)

- a. To measure the MFC's power output, remove the capacitor and LED from the hacker board. Then remove the orange wire from the "+" port and plug it into port 3. This means that the orange wire should be in port 3, the green wire should still be in the "-" port, and all other ports should be empty.
- b. Place a **resistor** between ports 5 and 6, as shown in the video above. For the resistors, the orientation does not matter.
 - i. Several resistors come in the MFC kit. Start with the largest-capacity resistor, which will be a 4.7 k Ω resistor. (Ω , the capital Greek letter Omega, is the symbol for **ohms**, the unit used to measure resistance. 1 kilo-ohm, or 1 k Ω , is 1000 ohms.)
 - ii. Resistors' values are labeled using color-coded bands. Use Figure 8 to determine the resistance for each resistor.
 1. *Tip:* If you want, you can confirm the resistance of any resistor using your multimeter by setting it to measure resistance (usually a " Ω " symbol for ohms) and connecting the multimeter's leads on the wire ends of the resistor.

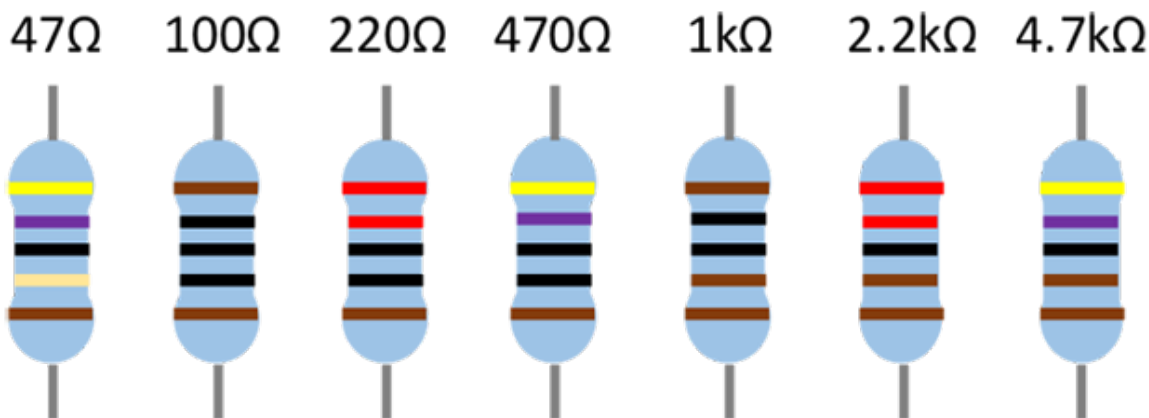


Figure 8. Use this resistor color chart to determine the resistance for each resistor.

- iii. Leave the resistor plugged in for 5 minutes.
- c. After the resistor has been plugged in for 5 minutes, use your multimeter to measure the **voltage** across the resistor.
 - i. Make sure the multimeter's black wire is plugged into the "COM" port and its red wire is plugged into the "V Ω MA" port on the multimeter.
 - ii. Set the multimeter to measure DC voltage. This is marked as "V" with a straight line next to it. Specifically turn the

- dial to "2000 m."
- iii. Clip the multimeter's red lead to the resistor's metal wire that is plugged into port 5. Then clip the multimeter's black lead to the resistor's metal wire that is plugged into port 6, as shown in the video. Read the multimeter's screen to see what the voltage is (in millivolts [mV]).
 - iv. If the voltage seems to be changing a little—such as decreasing slightly over the period of a few seconds—watch the readings on the multimeter for a few seconds more until they stabilize (and stay the same for a few seconds). Use the stabilized value.
 1. If the readings are still changing after several seconds, or if your readings are 0 mV, make sure all of the wires are correctly and securely plugged into the circuit (both the cathode and anode wires, and the resistor's wires), disconnect the multimeter's leads from the resistor, and come back in another 5 minutes. Then repeat step 2.c.
 - v. Record your results for both of the microbial fuel cells in your lab notebook in a data table like Table 2.

Day and Time:		
Resistance (ohms)	Voltage (mV)	Power (μ W)
4700		
2200		
1000		
470		
220		
100		
47		

Table 2. In your lab notebook each day, create a data table like this one for each microbial fuel cell to record your voltage measurements. Do not forget to write down the date and the time you started taking measurements on the top line.

- d. Disconnect the multimeter's clips from the resistor. Remove the resistor.
- e. Repeat steps 2.b.–2.d. until you have tested the MFC with all of the resistors in the kit. Start with the resistor with the largest resistance value and end with the resistor with the smallest resistance value.
3. Once you have finished taking your voltage measurements for the first MFC, plug the wires, the capacitor, and LED back into the hacker board, as described in [steps 16–18](#) ([#step16](#)) in the [Setting Up the Microbial Fuel Cells and Bacteria Count](#) ([#mfc-setting-up](#)) section and repeat the voltage sweep (step 2 and 3) for the second microbial fuel cell.
4. Calculate the power output (in microwatts, or μ W) for each resistor and both microbial fuel cells. You can calculate this by using a derivation of Ohm's law, as described in the [Introduction](#) ([#background](#)), Equation 1.
 - a. **Note:** It is important to convert the voltage measurements into power output measurements. The power output depends on the resistors you use, so you cannot determine how well the MFC is performing by just looking at the voltage measurements alone; they need to be converted into power for them to be meaningful.
 - b. To use Equation 1, you will need to convert your voltage readings from millivolts (mV) to volts (V). To do this, divide the millivolt values by 1000 to give you volts. For example, if you had a voltage reading of 45 mV, this would equal 0.045 V.
 - c. Using Equation 1, your answer will be in **watts** (W). Convert watts to microwatts by multiplying your answer by 1,000,000.
 - d. Once you have calculated it, record the power for each resistor in the data table (such as Table 2) in your lab notebook.
5. Determine what the **peak power** of your MFCs is.
 - a. In your data tables for both microbial fuel cells, look at the power produced using each resistor. The peak power is the highest power produced by any of the resistors.
 - b. If you want to visualize this, you can plot your data for the day on a graph, putting the resistance of the resistors on the x-axis (horizontal axis) and the power on the y-axis (vertical axis) for both MFCs. A sample graph is shown in Figure 9.
 - i. You should see a curve, with the peak power at the top of the curve, as shown in the sample graph.
 - c. Make a note in your lab notebook of what the peak power for both MFCs is each day, by circling or highlighting this value in your data table.
 - d. Although it will not be explored in this science project, you might like to know that the peak power tells you what the

internal resistance of your MFC is. The resistor that gives the peak power is closest to the internal resistance of the MFC. This may change a little over time.

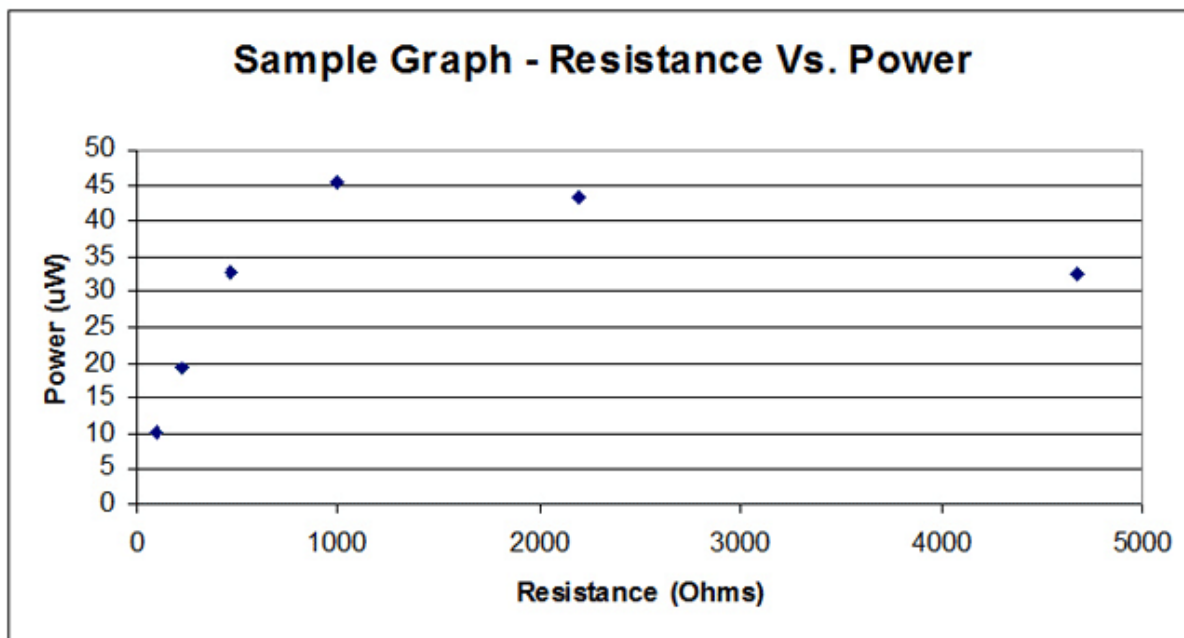


Figure 9. This sample graph shows possible power output data using the resistors with the MFC. In this sample, the peak power was found using a 1 kΩ resistor, and the peak power is about 45.5 µW.

6. Repeat steps 1–5 each day until it looks like the power output (the peak power) for both of your microbial fuel cells is stabilizing.
 - a. Take these measurements around the same time every day. This will limit variables affecting your results (such as changes in temperature).
 - b. For step 1, it should take 3–7 days for the LEDs to start blinking. However, even if the LEDs never blink, you may still be able to do this science project; be sure to continue to take the power output measurements every day.
 - i. *Tip:* See the [Frequently Asked Questions](#) (#help) section for what to do if the LEDs do not blink, or if they were blinking and unexpectedly stopped blinking.
 - c. For step 2, you should see the power output slowly increase.
 - i. For each day, make a data table like Table 2 for each MFC in your lab notebook in which to record your results, and use them to determine the peak power.
 - d. After about 7–14 days, the power output should stabilize.
 - i. It may stabilize anywhere between 10 µW and 200 µW or more. A lot depends on the topsoil you are using, and other factors. Wherever it stabilizes, it should make enough power to blink the LED at least once every 30 seconds.
 1. *Tip:* If the power output seems low, see the [Frequently Asked Questions](#) (#help) section for suggestions on what to check and try.
 - ii. When it stabilizes, the peak power should not change by more than about 10% for at least three days in a row.
 1. Do not worry if your peak power changes by a little more than this. If it has been at least 14 days and when you graph the peak power (as described in the step 6.d.iii) it looks like it is stabilizing (meaning, it is not steadily increasing or steadily decreasing from day to day), then it has probably stabilized enough.
 2. Keeping this in mind; if it still does not look like your peak power is stabilizing, see the [Frequently Asked Questions](#) (#help) section for suggestions on what to check and try.
 - iii. *Tip:* Making graphs of your data as you collect it for both MFCs may help you see if the power output is stabilizing. If you do this, put the date on the x-axis and the power output (peak power) for each day on the y-axis. Does the peak power appear to be stabilizing?
 - iv. The time between LED blinks should also stabilize.
7. Once it appears that the power output has stabilized in both MFCs, carefully open up both microbial fuel cells and add sugar solution to the one you labeled "sugar treatment," and distilled water to the one you labeled "control."
 - a. Take your measurements for the day, as usual, before starting the treatments.
 - b. On the digital scale, weigh 0.5 g of sucrose (table sugar) into a mini cup and add 10 mL of distilled water. Mix with a sterilized spoon to dissolve the sugar completely.
 - c. Prepare another mini cup and only add 10 mL of distilled water.
 - d. Unplug the anode and cathode from the hacker board and carefully remove the lid of the first microbial fuel cell, which you labeled "sugar treatment."
 - e. Put your gloves on and gently lift up the cathode, being careful not to get any mud on top of the cathode.
 - i. **Safety Note:** Remember, the MFC's electrodes are made of a conductive material called graphite fiber. Do not put the cathode near electronics or power plugs, and do not disperse the fibers in the air, as the fibers will cause electrical shortages when they come in contact with electronics.

- f. Take a sterilized spoon and use it to dig into the top 1–2 cm of the soil. Be careful not to dig all the way down to the anode, as this disturbs the anode biofilm. Scoop about 1 teaspoon of soil into a fresh mini cup, labeled with time, date, and microbial fuel cell type (sugar treatment or control) for the bacteria plate count.
- g. After you have taken the soil sample, use a freshly sterilized medicine dropper to suck up some sugar solution from the cup and spread it on top of the mud, mix it in, and then dig around inside of the mud, adding all of the sugar solution and mixing it in as you go. You want to mix the sugar as evenly into the mud as possible, but *do not disturb the anode*—leave it in place (as well as its wire and the mud below the anode).
 - i. *Note:* Disrupting the anode could damage the developing biofilm and interfere with your results.
- h. Wait 5 minutes to let the sugar soak into the mud a little. (If you immediately put the cathode back on the mud, the cathode will likely soak up much of the sugar solution). Flatten the mud surface with a spoon again before you re-assemble the MFC.
- i. Then assemble the MFC exactly as you put it together before, following the instructions from the "Setting Up the Microbial Fuel Cells and Bacteria Count" section to make sure that the wires are twisted together properly and everything is reconnected to the hacker board correctly.
 - i. Specifically, this will be following [steps 16–18](#) ([#step16](#)) from the previous section.
 - ii. Do not get any mud on the top of the cathode. If you do, carefully wipe it off, being careful not to grind it into the cathode.
- j. Repeat step 7 with the "control" microbial fuel cell, but this time add 10 mL of just distilled water without the sugar.
8. Use the bacteria plate count method again to measure the numbers of bacteria in both microbial fuel cell soil samples, following [step 22](#) ([#step22](#)) of the [Setting Up the Microbial Fuel Cells and Bacteria Count](#) ([#mfc-setting-up](#)) section above.
 - a. *Note:* Remember that depending on your previous bacteria counts, you will have to adjust the dilutions that you plate on the nutrient agar plates. In this step you probably do not have to plate the 10^{-4} dilution but rather 0.5 mL of the 10^{-6} and 10^{-7} (or even up to 10^{-10}) dilution.
 - b. Again, keep the soil as well as the dilutions in the fridge until you have counted the bacteria in case you get too few or too many colonies and need to re-plate.
9. Starting the day after you add the sugar, continue monitoring the power output of both microbial fuel cells by repeating steps 1–5 each day until it looks like the power output (the peak power) of both microbial fuel cells is stable again (as described in step 6). Shortly after it stabilizes, the power output may then clearly change again.
 - a. Take these measurements at the same time every day.
 - b. For each day, for each MFC, make a data table like Table 2 in your lab notebook in which to record your results, and use them to determine the peak power. *Note:* It is possible that the resistor you use to determine the peak power will change slightly. Make a note of this in your lab notebook if it happens.
 - c. Depending on the exact conditions of your experiment—which can be affected by the soil you use—the power output could take anywhere from several days to over a week to stabilize after adding the sucrose.
 - i. When the power output is stabilized, the peak power may not change by more than about 10% for at least three days in a row. How much the power varies when it is "stabilized" can depend on the amount of power being produced by the MFC.
 - ii. Overall, if it has been about two weeks after adding the sugar and the peak power has not been steadily decreasing or increasing each day for at least the last week, then it has probably stabilized enough.
 - iii. After it has stabilized, the power output may then clearly change again (steadily decreasing or increasing each day).
 - iv. *Tip:* Making a graph of your data as you collect it for each MFC may help you see if the power output is stabilizing. If you do this, put the days on the x-axis and the power output (peak power) on the y-axis. Does the peak power appear to be stabilizing?
 - v. The time between LED blinks should also stabilize.
10. Once the power output stabilized for both of the microbial fuel cells, take a final sample for the bacteria plate count following [step 22](#) ([#step22](#)) of the ["Setting Up the Microbial Fuel Cells and Bacteria Count"](#) ([#mfc-setting-up](#)) section above.
 - a. *Note:* Remember that depending on your previous bacteria counts, you will have to adjust the dilutions that you plate on the nutrient agar plates. In this step, you probably do not have to plate the 10^{-4} dilution, but rather 0.5 mL of the 10^{-6} and 10^{-7} (or even up to 10^{-10}) dilution.
 - b. Again, keep the soil, as well as the dilutions, in the refrigerator/cooler until you have counted the bacteria in case you get too few or too many colonies and need to re-plate.

Analyzing Your Results and Continuing Explorations

1. Make two graphs of your data for each microbial fuel cell, one showing how the power output changed over time and one showing how the frequency of LED blinks changed over time.
 - a. For the graph showing power output over time, put the number of days after setting up the MFC on the x-axis and the peak power output (in μW) on the y-axis.
 - b. For the graph showing the frequency of LED blinks over time, put the number of days after setting up the MFC on the x-axis and the blinks per second on the y-axis.
 - i. If you recorded the time between blinks in your data table, convert this to blinks per second by taking the data for the average seconds between blinks that you collected each day and calculate what 1 divided by this number is. For example, if your LED blinked an average of once every 15 seconds, 1 divided by 15 is 0.067, which is the number of blinks per second it made.
2. Analyze your graphs.

- a. What happened to the power output and frequency of the LED blinks after adding the sugar? Was there an increase or decrease in power output? How large was the change? How did the power output compare to the control MFC?
 - b. How quickly did the measurements stabilize after adding the sucrose? When they stabilized, were they higher or lower than they were originally, before adding the sugar? Were they higher or lower than the power output of the control MFC?
 - c. When was the power output and blinking frequency the highest for each of the MFCs? What was their peak power at this time?
 - d. Based on your results, do you think it would be feasible to use sugar as substrate to help generate power? Why or why not?
3. Look at the numbers of bacterial colonies that you counted for each time point in your treated microbial fuel cell and control. Calculate the number of bacteria in 1 g of that soil using Equation 2.

Equation 2:

$$\text{Number of bacteria in 1g of soil} = \frac{\text{Number of colonies}}{(\text{Volume plated [mL]} \times \text{Dilution})}$$

You can graph the number of bacteria in a bar graph, showing the time (initial, before and after treatment) on the x-axis, and the number of bacteria on the y-axis for both MFCs. Did the number of bacteria increase, stay stable, or decrease over time? Did the addition of sugar change the number of bacteria compared to the control?

4. At this point in your experiment, there are several ways to continue your explorations. Check out the [Variations](#) (#makeityourown) section for some ideas.

Frequently Asked Questions (FAQ)

FAQ for this Project Idea available online at

https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p032/microbiology/bacteria-microbial-fuel-cell#help.