A Clean Energy Teaching Tool: Microbial Fuel Cells!

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Microbial Fuel Cells (MFCs) have garnered recent interest due to their potential to remediate industrial and municipal wastewater while generating sustainable electricity. MFCs use anaerobic microbes to degrade organic materials and transport the resulting electrons outside their cells to an external electron acceptor, the anode of the fuel cell. These cells can produce measurable electric currents and power a small device. That microbes can produce electricity surprises students and indicates how the sciences are compartmentalized in students' minds. This exercise emphasizes the connections between microbiology, chemistry and ecology and enhances students' understanding of microbial ecology and microbial electrochemistry.

Keywords: microbial fuel cells, clean electricity, microbial ecology, wastewater treatment, experimental design, inquiry-based learning

Introduction

Microbial fuel cells (MFCs) allow us to explore the intersection of microbiology, ecology and electrochemistry in a real world environmental application of microbiology to wastewater remediation and generation of sustainable, renewable electricity. This laboratory exercise is open-ended and allows students to explore the factors that limit or enhance production of electricity by bacteria in an experiment that they design and conduct.

Although so far, we have used this exercise in introductory microbiology, it could also be used in an advanced ecology or biotech lab as long as the microbial aspects were sufficiently explained. The MFCs are set up early in the semester and the students record voltage and current weekly. After some weeks the students present their data to the class in a brief 5 minute presentation.

Microbial Fuel Cells

A fuel cell is similar to a battery in that it converts chemical energy into electricity. A battery's useful life is limited by the amount of chemicals or reactants that it contains, and once they are consumed, the battery is dead. However, in a fuel cell, the reactants are continuously replenished, which allows the fuel cell to operate indefinitely. There are several different types of fuel cells, but in general terms, a fuel cell consists of two compartments that are separated by a membrane or salt bridge. Each compartment contains an electrode, which is where the electrochemical reactions take place. The anaerobic compartment contains the anode, and this is where the fuel, e.g., hydrogen, in a typical fuel cell, loses electrons (oxidation). The aerobic compartment contains the cathode, where oxygen gains electrons (reduction). The electrons pass from the anode to the cathode through a wire and are used to power a load such as a motor. The electrolyte allows for ions to pass between the anode and the cathode, and there is usually a polymeric "membrane" that does not allow either the fuel or oxygen to pass between the compartments containing the anode and cathode. If this were to happen, the efficiency of the fuel cell would be greatly reduced. In order for the electrochemical reactions to occur at a useful rate, the electrodes used in a fuel cell need to contain catalytic materials, such as platinum.

Microbial fuel cells operate via the same general principles, but use microbes to catalyse the oxidation of organic matter rather than a metal/inorganic catalyst. In contrast to normal respiratory processes in most microbes where the transfer of electrons occurs within the microbial cell, in the anaerobic environment at the MFC anode, exoelectrogenic bacteria are able to transfer electrons outside of their cells (Melnuk et al. 2011). Rather than directly binding to the oxygen molecules in aerobic respiration, the electrons are brought outside of the microbial cells to the anode of the fuel cell. Electrons are then transferred via an external wire to the cathode where oxygen is reduced, as in a conventional fuel cell.

There are several different ways to construct a MFC, but we will focus on a single-chambered design. In

a single-chambered cell, a membrane is not necessary as sediments/soil provide the barrier between the anode and the cathode, and slow the diffusion of oxygen, creating an anaerobic environment at the anode. The soil also allows for the movement of protons and other ions.

Sediments or soil in MFCs also provide the exoelectrogenic bacteria, which are the source of the electrons, but also the organic matter that they oxidize for electricity. Some of the microbes already present in the sediments used will be able to use the organic matter in the sediments under anaerobic conditions and will, over time, grow to sizable populations and begin to transfer electrons to the cathode.

Microbial fuel cells are increasingly being viewed as a possible efficient and inexpensive way to generate sustainable and renewable electricity (Logan et al. 2006). MFCs have proven to be good remediators and are effective in cleaning and decontaminating polluted soils and water (Logan et al. 2006). Some wastewater treatment plants in the US treat 33 billion gallons of wastewater which costs \$25 billion annually (Wang et al. 2008). Creating a MFC large enough to run as an industrial wastewater plant would significantly reduce the costs of this cleaning system and generate enough electricity in the process to potentially power 500 homes (Wang et al. 2008). These large scale plants are still relatively new ideas and have yet to be tested, since factors such as size, the amount of microbes in contact with the electrodes, and the microbial species will all affect cell performance (Wang et al. 2008).

Student Outline

Objectives

Explain how microbial fuel cells work Construct a microbial fuel cell Develop an hypothesis about a factor affecting electricity production by a MFC Design an experiment to test the impact of this factor on electricity production Document the results of your experiment

Introduction

Microbial fuel cells (MFC) are, similar to batteries, but use microbes naturally existing in natural sediments (often from the bottom of lakes) to produce a current. As well as generating electricity, MFCs can be used as bio-remediators by digesting organic matter in polluted and contaminated waters (Logan, et al. 2006). There is hope that scaled-up MFCs could be an efficient and cost-effective way to treat wastewater as well as produce electricity (Wang et al. 2008).

Natural sediments, like soil, contain millions of bacteria and other microbes per gram. The microbes of particular interest here are known as exo-electogenic microbes. If an appropriate surface is provided under anaerobic conditions as the final electron acceptor, electrons produced from the oxidation of organic materials will flow to the surface and a current can be generated if there is an electrical connection to another surface in an aerobic environment. These two surfaces are the electrodes of the fuel cell. The anaerobic surface that receives electrons from the microbes is the anode, while the aerobic surface is the cathode. At the cathode, oxygen is reduced to form water. The microbially mediated oxidation of organic matter is the electron source and thus the electrical circuit in the fuel cell results from the electrons that are transferred from microbes to the anode, and then to the cathode via a titanium wire. There are several mechanisms bacteria use to transfer the electrons to the anode, and it may involve direct contact between the microbes and the anode or indirect contact.

Voltage (V) is the difference in electrical potential between 2 points. 1 V = 1 J/C, i.e., the energy per unit charge, which explains why voltage is better, a higher voltage means the cell can do more work. Similarly, current is effectively an electron count, since 1 A = 1 C/s. Current, measured in amperes is the flow of electric charge, in this case, carried by electrons. Preliminary testing of these MFCs showed that initially the voltage and current are very low, since it takes time for the microbial populations to recover following the disturbance due to construction of the MFC and it also takes time to re-establish an anaerobic environment. After approximately 1 week, typical voltages range between 0.40 and 0.80 V and typical currents range between 0.30 and 1.5 milliamps. As a class we hope to observe differences between the MFCs depending on the sediment source, environmental conditions and fuel cell configuration.

Although we are hoping to observe a voltage produced by microbes, a voltage can also be produced in the absence of microbes due to differences in oxygen concentration between the anode and the cathode. Another possibility is that the measured potential difference is due to a corrosion cell if the wire used to construct the cell is corroding at the anode. To be sure we are observing voltage due to microbes, it would be necessary to compare 2 identical MFCs, one of which had been autoclaved. Only if there was a voltage in the non-autoclaved cell and no voltage in the autoclaved cell could we be sure our voltage was solely due to the presence of microbes.

Methods

Procedure

Before assembling the fuel cells, each pair of students will need to choose what variable they would like to investigate, e.g., temperature, sediment source, or the amount of light the cell is exposed to, etc (see below for more details). Each pair of students will make two MFCs, using 2 pop bottles and the materials listed below, and each MFC will receive a different treatment. For example, if you decided to test the effect of temperature, you could keep one cell at room temperature, and the other in the fridge. These fuel cells will then be left to run for a number of weeks in order to record readings of voltage and current once every week.

Setting up a Microbial Fuel Cell

- 1. Cut the top of the pop bottles off
- 2. Label the bottles with your names, sediment source, water source & treatment
- 3. Cut 2 2.5 cm slits on opposite sides of the top edge of each of the bottles & level the sediment
- 4. Add 3-5 cm depth of the sediment to the bottom of the bottles & flatten it the sedmiment
- 5. Cut 4 6 x 6 cm pieces of carbon felt pad using scissors; these are the electrodes
- 6. Cut a 20 cm piece of Ti wire for each anode & a 15 cm piece of Ti wire for each cathode
- 7. Loop one end of a wire through each electrode

- 8. Make sure you have as much contact between the wire & the electrode as is possible while still leaving enough wire free for connecting to the multimeter
- 9. Place the anode on top of the sediment
- 10. Run the free end of the wire up the side of the bottle & through one of the slits
- 11. Fill the bottles to within about 5 cm of the slits with sediment, making sure the anode is completely covered
- 12. Add water to cover the top of the sediment about 2-3 cm deep
- 13. Place the cathode on top of the sediment so half of it is in the water & the other half is exposed to air
- 14. Run the free end of the cathode wire through the other slit
- 15. Cover the top of each bottle with a piece of wax paper/plastic film to reduce evaporation
- 16. Tape the wires to the sides of the bottles & label the anodes & the cathodes
- 17. Check that the anode wires are not touching the cathode pads or wires; if they are, it will cause a short & result in no voltage
- 18. See Figure 1 for a completed microbial fuel cell
- 19. Next lab, & for several weeks you will assess the voltage & current produced by your MFCs & record the measurements.
- 20. To take measurements, attach the red multimeter wire to the cathode & the black voltmeter wire to the anode
- 21. Turn the dial on the multimeter to 20 V on the voltage side and record the voltage
- 22. Turn the dial on the multimeter to 20 milliamp on the current side and record the current

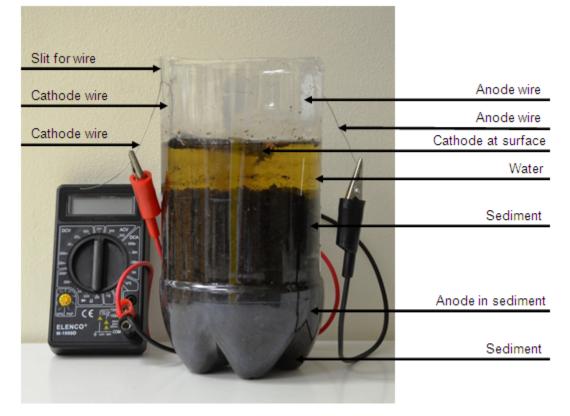


Figure 1. Microbial fuel cell with multimeter.

Preparing Common C Substrate Solutions

- 1. To prepare a 10 mM solution of ethanol, use a micropipette to add 583 μ L of absolute ethanol to a 1 L volumetric flask and dilute to volume with distilled water.
- 2. A 500 g/L glucose solution is prepared by dissolving 500 g of glucose in 1 L of distilled water.
- 3. A 10 ppm glucose solution of glucose is prepared by dissolving 10 mg in 1L of distilled water.
- 4. A 10 ppm glutamate solution can be prepared by dissolving 11.5 mg of monosodium glutamate in 1 L of distilled water.

- 5. A 2.5 g/L sodium acetate solution is prepared by dissolving 2.5 g of anhydrous sodium acetate in 1 L of distilled water, or by dissolving 4.15 g of sodium acetate trihydrate in 1 L of distilled water.
- 6. A 500 g/L glucose solution is prepared by dissolving 500 g of glucose in 1 L of distilled water.

Isolating Bacterial Strains or Communities

Standard isolation methods for anaerobes can be used to isolate exo-electrogenic bacteria from sediments or soils. Serum bottles with rubber septa containing anaerobic media are typically used. Media are purged with nitrogen gas or other oxygen-free gas. The anaerobic medium is inoculated with soil slurry by needle through the septum and followed by gentle orbital shaking for several weeks. If desired, the anodes can be inoculated with these microbes by submerging them in the soil slurry until a biofilm has developed.

Troubleshooting Circuits

The fuel cells are expected to have a voltage between 0.5 & 0.8V. If the cells show a voltage of zero, or near zero, check the following:

- 1. Was "live" sediment/soil and water used to make the MFC? If sterilized potting soil or heavily chlorinated water was used, microbial populations will be very low or absent and electricity will not be produced.
- 2. Has the cell been allowed to sit for at least a week? It takes time for the bacterial colonies to develop, and also for the anode to become anaerobic as the microbes in the soil consume the oxygen introduced during the assembly of the cell.
- 3. Is the wire for the anode touching either the cathode wire, or the cathode itself? If so, this will create a short circuit because electrons can travel directly from the anode to the cathode without having to go through the multimeter.
- 4. Has the anode come out of the sediment? If the anode has been dislodged, it is no longer in an anaerobic environment, and the cell will not function.
- 5. Is the cell still showing no voltage? It's possible that there is a poor connection between the wire and the electrode. It's easiest to check the cathode first, but if the anode connection is the problem, then the fuel cell will need to be disassembled and reassembled.

Potential Research Areas

Microbial fuel cells are at the intersection of microbiology, ecology and electrochemistry and offer a wide range of potential research questions. Of course, the main point of MFCs is to obtain the highest and/or most sustainable electricity production. Increased electricity production could be a result of increases in microbial growth and activity through modifications to either abiotic or biotic parameters affecting microbial growth or through the addition of C substrates. Modifications that optimize the electrochemical properties of the fuel cell could also increase electricity production. Students will work in pairs to design an experiment to test the impact of a particular parameter on electricity production.

Abiotic Factors Affecting Microbial Growth

pH: Although the literature suggests that many exo-electrogenic bacteria function optimally at lower pHs, in practice it is often difficult to manipulate soil pH. Soils & sediments are heavily buffered against pH changes by their mineral content. It is easy to add an acid or base but the effects tend to decrease over time. Once the cell is assembled, it is impossible to adjust the pH without dissembling the cell in order to mix the soil with the acid/base being added, and so this experimental variable is best avoided.

Temperature: Since growth and activity of all organisms varies with temperature, it is logical to expect that varying the temperature of the MFC would change microbial activity and thus electricity production. Based on the origin of the sediments, and what you know about relationship between the optimum and maximum temperatures for most organisms, what temperature might be optimal or suboptimal for the functioning of these MFCs?

Water: Water is essential for all microbes as well as to maintain the electrolyte in the MFC. Does the depth of water over the sediment/soil surface affect MFC electricity production? What happens to voltage and current when the MFC dries out? How wet does the sediment need to be for the MFC to function?

Oxygen: Because oxygen is reduced at the cathode, the performance of the MFC is dependent on the oxygen concentration in the solution adjacent to the cathode surface. What is the effect on voltage and current when a tube from an aquarium pump is placed near the cathode?

Light: Photosynthetic microorganisms, such as cyanobacteria, as well as producing electricity for themselves, also leak excess carbohydrates into their environment. These carbohydrates can be a source of organic substrates for exo-electrogenic bacteria. Also, photosynthesis produces oxygen which is essential at the cathode. How might the addition of

photosynthetic microbes to the cathode compartment affect electricity production? Does electricity production increase when these photosynthesizers are exposed to more light, higher intensity light or different wavelengths of light?

Nutrients: The availability of nutrients such as nitrogen, phosphorus or potassium is critical for all organisms. Microbial growth is often limited by the amount of these nutrients available. Even if C is abundant, the lack of N or P might mean that the organism couldn't use the available C. Does the addition of a nutrient increase microbial growth and activity and hence, electricity production?

Organic matter (OM) Content of Sediments: The organic materials in sediments or soils provide the substrate to fuel the growth and activities of microbes, including exo-electrogenic bacteria. Low amounts of organic compounds in sediments or soils may restrict the growth and activity of microbes. Does the use of sediments/soils from marshes or bogs, which have higher OM than those from agricultural fields produce more electricity?

Sediment/Soil Source: Other properties of sediments and soils beyond the organic matter content can also affect microbial activity. For example, salty, acidic, or alkaline sediments/soils may provide non-optimal conditions for microbial activity. As an extension of the questions on the importance of organic matter content, is there a difference in electricity production due to difference sources of high or low OM sediments/soils? For example, between forest soils & marsh soils or salty and agricultural soils.

Anaerobic vs Aerobic sSediments: Since the exo-electrogenic bacteria are anaerobic, the use of anaerobic sediments and soils (eg from beneath ponds or lakes) could contain more exo-electrogenic bacteria than aerobic sediments and soils and increase microbial activity. Do sediments/soils from anaerobic environments produce more electricity than those from aerobic environments?

Biotic Factors Affecting Microbial Growth

Enrichment of Sediments or Soils with Exo-electrogenic Bacteria: Different species of bacteria use different substrates, have different growth rates and have varying abilities to transfer electrons to the MFC anode. Therefore, isolation of strains or communities of microbes may enhance or reduce the electricity production of the cell, as indicated by the voltage and current measurements. Does enrichment of a MFC with a single strain improve electricity production compared to enrichment with a diverse community of microbes?

Inoculation of Anode with Exo-electrogenic Bacteria: To be effective, exo-electrogenic bacteria must grow on the anode, forming a biofilm and covering as much surface area as possible. Does prior inoculation of the anode increase the amount of electricity produced? Does inoculation of the anode before the MFC is assembled cause the cell to produce electricity sooner?

Available C Sources

Addition of C Compounds: Like other microbes, sediment and soil exo-electrogenic bacteria have been shown to use a wide variety of C substrates. Among these are easily digested monosaccharides such as glucose, moderately digested compounds such as acetate, cysteine, ethanol and starch and more resistant compounds such as cellulose, lignin and chitin. Would an easily digested substrate stimulate microbial growth and activity to produce more electricity or produce it more rapidly or for longer than a resistant substrate? How would different substrate concentrations affect microbial activity and electricity production? What effect would particle size of resistant substrates have on the timing and sustainability of microbial electricity production?

Addition of Carbohydrates from Plants: Plants, like other photosynthesizing organisms, leak excess carbohydrates from their roots into the soil which enhances microbial growth and activity in the root zone. How does the root depth of plants affect the degree of stimulation of microbial activity and hence electricity production? Do roots growing near the cathode affect electricity production differently than roots growing near the anode?

Addition of Industrial or Municipal Wastes: One significant potential use of MFCs is to clean up wastewaters since the microbes involved use organic substrates. Wastewaters could include municipal sewage (don't try this at home!), food processing wastes, brewery wastewaters, starch processing waste waters, potato pulp waste (Gezginci & Uysal 2016; Tian et al 2017). How does electricity production vary when the microbes are supplied with a source of diverse C compounds, such as waste water, compared with a single C source such as glucose?

Use of Microbes from Wastewaters: Wastewaters such as municipal sewage contain not only an abundance of carbon sources, but also an abundance of the microbes which can degrade them. Does the addition of wastewaters alter MFC electricity production through the addition of C sources or through the addition of microbes or both? Any wastewater that is readily available in your area (e.g. whey, any food processing wastewaters, pulp mill effluent, brewery waste, etc) would be interesting to test, although the normal cautions when using any wastewaters involving human or animal sewage would apply.

Optimizing the Fuel Cell

Electrode Size: The current generated by a fuel cell is dependent on the surface area of the electrodes. A larger anode provides more locations where bacteria can transfer electrons, while a larger cathode provides more sites where oxygen can be reduced. Electrode size can be changed by increasing the size of each electrode or increasing the number of electrodes of the same size. What effect does an increase in total size of the anode or cathode have on electricity productid**Distance between the Electrodes:** The resistance of the MFC is reduced when the distance between the electrodes is minimized (Abbas et al 2017). However, if the anode is too far up in the sediment column, it will be in a less anaerobic environment and the function of the fuel cell will be impacted. What is the optimum distance between the anode and cathode in your MFC?

Type of Electrodes & Wire: Carbon felt works very well as an electrode material because of its high surface area for microbial growth, and low electrical resistance. Plastic foam that has been impregnated with carbon, e.g., aquarium filter pads, is unsuitable because the connectivity of the carbon particles is very poor leading to a high electrical resistance. Ti wire is required because it does not corrode in the MFC. Steel wire will readily corrode and create a corrosion cell. A potential difference will be measured, but it will not be due to microbial activity. Do other types of electrodes and wires affect electricity production?

Location of Cathode: The performance of the fuel cell is dependent on the ability of oxygen to reach the cathode. Does the amount of electricity generated change if the cathode is fully submerged or partially exposed to the air?

Oxygenating the Cathode: A limiting step in the generation of electricity by MFCs is the rate of oxygen reduction to water at the cathode (Saba et al 2017). How does the addition of algae or cyanobacteria or other photosynthesizing microbes near the cathode affect electricity production?

Materials

Each lab section should be supplied with markers, scissors, and foil/plastic film/wax paper to cover the MFCs and reduce evaporation. To measure voltage and current, multimeters will be necessary, and to help the students visualize the electricity production, capacitors and LEDs are useful (www.magicalmicrobes.com/collections/spares-accessories/products/mudwatt-spare-hacker-pack).

Each pair of students will supply 2 2L pop bottles, about 2L of soil, sediment or muck (NOT potting soil), and about 2L of non-chlorinated water from a natural source, e.g. rain barrel, pond, creek, lake, etc.

Each pair of students will require 4 6 cm x 6 cm carbon felt electrodes (<u>www.fuelcellearth.com</u>). Both the $\frac{1}{4}$ " and $\frac{1}{8}$ " thick carbon felt will work, but the thicker variety is preferred. Each pair will also need 120 cm of 24 gauge titanium wire.

Notes for the Instructor

Overview

This exercise draws on students' microbiological, chemical and ecological knowledge and is an interesting way for students to explore the connections between these disciplines.

This is an open-ended exercise which allows students to develop an hypothesis and design an experiment to investigate the impact of a wide variety of different parameters on the generation of electricity by microbes. All the normal abiotic and biotic factors that affect microbial growth and therefore their exoelectrogenic activity are relevant here. Also the conformation of the MFC itself can affect electricity production.

Logistics

This exercise is set up at the beginning of the semester to allow time for the microbial communities to develop and start to produce electricity. Set up is relatively rapid, taking less than 30 minutes and can be done at the end of the regular lab. However, students will need time to discuss their hypothesis and experimental design with each other and the lab instructor, either in lab or outside of lab. Also, more time will be required by those students who decide to isolate specific microbes or inoculate the anode before setting up the MFCs.

If the experiment lasts several weeks or all semester, students will have to check their MFCs regularly to make sure they are consistently wet to maintain function. This is particularly important if students are testing the effects of water depth. A non-chlorinated source of water should be provided, e.g. distilled or RO water or water from rain barrels, ponds etc. Assessment of this exercise could include a lab report or exam question. Since one of the purposes of the exercise is for the students to make connections between different disciplines, short (5 minute) presentations by each group sharing what they tested and discovered would also be appropriate.

Our experience suggests that students can be less than diligent in checking their MFCs. Encouraging them to use online signup sheets can help keep them on track.

Experimental Design

We have observed that readings from ostensibly "identical" MFCs containing the same sediment or soil can be pretty variable. So, although we have suggested that students work in pairs and together produce 2 MFCs, it might be more advantageous to work in larger groups (4 or more) and have more replicates of the control and experimental MFCs. Available storage space for the MFCs will affect group size and number of replicates.

Troubleshooting

There are very few problems with the set up for a MFC, but those that occur are almost always related to circuits.

Circuits

The fuel cells are expected to have a voltage between 0.5 & 0.8V. If the cells show a voltage of zero, or near zero, check the following:

- 1. Was "live" sediment/soil and water used to make the MFC? If sterilized potting soil or heavily chlorinated water was used, microbial populations will be very low or absent and electricity will not be produced.
- 2. Has the cell been allowed to sit for at least a week? It takes time for the bacterial colonies to develop, and also for the anode to become anaerobic as the microbes in the soil consume the oxygen introduced during the assembly of the cell.
- 3. Is the wire for the anode touching either the cathode wire, or the cathode itself? If so, this will create a short circuit because electrons can travel directly from the anode to the cathode without having to go through the multimeter.
- 4. Has the anode come out of the sediment? If the anode has been dislodged, it is no longer in an anaerobic environment, and the cell will not function.
- 5. Is the cell still showing no voltage? It's possible that there is a poor connection between the wire and the electrode. It's easiest to check the cathode first, but if the anode connection is the problem, then the fuel cell will need to be disassembled and reassembled.

Assessment

We suggest brief presentations of results in lab to allow students to gain a broader understanding of a variety of factors that affect MFC performance. Other assessment possibilities include exam questions, or complete lab reports.

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