A CURRICULUM for HIGH SCHOOL SCIENCE EDUCATION:
MICROBIAL FUEL CELLS:
A Living Battery

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This work was made possible through a NSF CAREER grant to Lars Angenent (#0939882).
MICROBIAL FUEL CELLS: A Living Battery

MOTIVATION

Microbial electrochemistry provides a unique method for promoting an interdisciplinary approach to teaching science. Most science curricula today, adapting to the growing demand to align course content to state standards, veer away from the integration of biology, chemistry, and physics. Many times, this does a great disservice to our students. The life processes studied in these courses defy our attempts to simplify and categorize them—they are infinitely complex, and often these complex problems require the integration of biology, chemistry and physics to fully explain them. In a typical high school science course, the student acquires fragmented ideas (factoids) about the subject of study, and will vary in the amount of information they have acquired. Frequently, the average student has great difficulty in conceptualizing the patterns and relationships between the various science disciplines. For example, students can master the basics of oxidation-reduction from their introductory chemistry class, yet when called upon to be familiar with the metabolic pathways of cellular respiration in a biochemistry unit, fail to recognize how redox chemistry governs the biological processes.

Our goal is first to demonstrate how an integrated complex system (e.g., a microbial fuel cell) can be used to successfully teach science (biology/chemistry/physics), and then progress to how science can be used to solve complex problems (e.g., energy-neutral wastewater treatment). Essentially, we hope to provide opportunities for students to transform factual knowledge into usable information for problem solving. Often, this type of investigation not only stimulates the curiosity of students in biotechnology and its relation to the basic sciences, but also encourages a more general flexibility of scientific thinking in their interpretations.

Demonstrations of microbial electricity devices often capture the attention of high school audiences, and the intention of this curriculum is to provide the tools for instruction and some relatively simple experiments that can be performed in a high school laboratory. Some over-simplification of the basic biochemical and microbiological processes is perhaps inevitable, and where necessary references to standard texts should be tailored to the needs of your students. Where applicable, attention will be drawn to the rationale for each activity by describing how the students can link their understanding of each process to the understanding of a functional MFC.
INTRODUCTION

As a result of global population growth and cumulative industrialization processes, two tremendous challenges in our world today are the growing demand for energy, and ecologically and environmentally sound methods for waste disposal. While, arguably, we possess adequate methods for waste treatment and disposal, they are quite energy-intensive processes. Some current research, especially into microbial fuel cells, asks the question if wastewater treatment can be transformed into energy producing processes. As fossil fuels are exhausted, could smaller, tailored solutions designed for individual energy demands come to replace the huge, centralized power plant? In our evolving energy conscious environment, could wastes ever come to play a distinguished role as a resource for the provision of electrical power? These are questions researchers in the field of microbial fuel cells hope to answer. Wastes, contrary to conventional wisdom, are not useless – as the name may imply – rather, they are valuable energy sources. Municipal wastewater possesses an organic load of ~200 mg/L COD, and while this is not enough raw energy for wastewater to ever be a major producing source, there are some that argue the treatment could become an energy-neutral process rather than energy consuming. Special industrial wastewaters, such as from the food industry (dairy, brewery, sugar industry), being much richer in organic content, may potentially hold an even more promising energy production future.

A novel approach to facilitate direct energy production from wastewater is the microbial fuel cell. The first studies into the application of direct transformation of chemical into electrical energy by the exploitation of microbial processes arose during the 1970s. Mainly because of the abundance of fossil oil and gas, it received only temporary attention. It was not until the 1990s, when interest in sustainable and renewable energy sources expanded, that the idea was revived and the research intensified. Although much work remains to be done at the research level to unwrap the biochemistry of the electrical activity of microorganisms, recent studies of microbial fuel cells have greatly advanced our understanding of microbial electricity generation. Additionally, studying these microbial fuel cells can lead to a deeper understanding of electron transfer processes in general and the application of this knowledge to other alternative fuel possibilities. For information on the worldwide status of MFC research, consult the webpage of the MFC research community: www.microbialfuelcell.org.
Chapter 1: What is a Battery?

How does understanding batteries help students understand MFCs?

Since our microbial fuel cell effectively acts as a battery, it is beneficial to begin by demonstrating how it functions as a battery. When students conceptualize that the MFC is acting as a battery, they can internalize that all of the processes that occur within a typical dry cell battery are also occurring within the MFC. The topics begin with a review of the design and operation of a basic battery and then apply those concepts to the MFC. Parts of this topic may be unnecessary if the students have prior knowledge of batteries and circuits gained at the middle school or introductory physical science level.

BATTERY BASICS

What have the students give their definition of a battery. (NOTE: The public calls it a “battery,” but the industry refers to it as a “cell.”)

A battery is an electrochemical device that contains two or more power cells connected electrically so that chemical energy can be converted into electricity. Simply stated, a battery powers products that require electricity to work. They are useful because they allow us to transport electricity and use products in locations where there are no electrical outlets, such as beaches, sporting events, picnics, etc.

In a battery, each cell that stores the electrical energy in a chemical state has two electrodes that react with the chemical and each other to release energy. The battery’s two metal ends are called terminals. Usually one terminal is flat (negative end) and the other is button-shaped (positive end). In a typical Carbon-Zinc battery, the positive electrode is a carbon rod and the negative electrode is the zinc case. The case is important also because it keeps the chemicals from leaking out.

Look for the terminals on a battery. How are they marked?

The ends are marked with a + and -.

MFCs will also have positive and negative terminals. Our specific laboratory model MFC, instead of having just two terminals at opposite ends, will have three terminals (two negative and one positive) all coming out of the top of the MFC. The terminals will be the conductive carbon electrodes (rods) that emerge from the two anolytic (negative) chambers and the catholytic (positive) chamber. The two negative electrodes will be linked by alligator clips and copper wire, essentially creating one negative terminal from the two.
Each battery consists of four main parts:

- a) **Positive Electrode (Cathode)** - the electron acceptor.
- b) **Negative Electrode (Anode)** - the electron donor.
- c) **Electrolyte** - a paste-like substance or solution that contains charged particles that can move or conduct an electric current.
- d) **Separator** - material that provides separation of the (+) from the (–) charges and insulation.

The electrochemical reaction that fuels the battery occurs at the interface of positive and negative electrodes and the electrolytes. Electricity created by a battery consists of a stream of tiny invisible particles called **electrons** flowing from one metal end of the battery to the other metal end, just like a liquid. The path it follows is called a **circuit**. When electricity flows in a circuit, it is called **current**. Electricity only flows when it can go from one terminal to another. The electrons must have a pathway or circuit to follow from the positive to the negative electrode.

When the circuit is complete, electricity flows from an area of low (more negative) electrical potential to one of higher (less negative or even positive) potential. The difference in electrical potential makes the electricity move. Electricity can flow through some things, but not through others. Materials that allow electricity to flow through it are called **conductors**. Metals usually make good conductors. If electricity cannot flow through the material, it is called an **insulator**.

### What is electricity?

*Electricity or electric current is a movement or flow of electrically charged particles, called electrons.*

### Can you name some materials that make good conductors and insulators?

*Good conductors include aluminum, carbon, copper, salt water, and zinc. Insulators include dry salt, glass, plastic, and wood.*

The electrical symbol for a battery is:

```
+  I  –
```

The longer line indicates positive and the shorter line indicate negative. All batteries have a strength indicated by a number followed by the letter “V”. The “V” stands for volts. The **volt** is an electrical unit that measures the potential difference between two points in an electrical circuit. The **potential difference** between two points is the amount of work that would need to be done on a unit of electric charge to move it from one point to the other against an electric field.
The voltage number for a battery gives a relative measure of how hard the electrons are being pulled through the circuit from an area of low electrical potential (more electron rich) to one of higher potential. For instance, a 1.5 V battery contains a single cell whereas a 9 V battery contains six cells, with the current moving through all six cells.

What do the numbers on a battery mean? How do they relate to the strength of the battery? Can you suggest how voltage could be increased?

The numbers indicate voltage. Batteries with higher numbers have a higher strength. You can make a voltage stronger by linking several batteries.

Activity 1.1 (Demonstration) – Light It Up

Show the brightness of a bulb resulting from one and two batteries added in a series circuit. Then, use a multimeter or voltmeter to show how the voltage is additive.

PRIMARY AND SECONDARY CELLS

The most common household battery is a dry cell battery. A dry cell battery is characterized by a pasty, low moisture electrolyte. One kind of dry cell is a primary cell battery. These batteries automatically convert chemical energy into electrical energy. This kind of battery CANNOT be recharged because it has a definite fuel stock. It is designed to be used once.

After the chemicals in the electrolyte solution (that transmit the electric currents) have been used up, the energy is no longer available and the battery is said to be exhausted, used, or “dead,” and is then discarded. An example of such a primary cell battery would be the alkaline battery, used in most remote controls. The anode of this battery is a mixture of powdered zinc and a concentrated solution of potassium hydroxide. The cathode is a mixture of solid manganese (IV) oxide and graphite. The anode and cathode chambers are separated by a porous divider. The most common sizes of alkaline batteries are the “round cells”, such as AAA, AA, C, and D (Figure 1.1).

Figure 1.1. Typical alkaline batteries depicting D, C, AA, and AAA sizes.
A secondary cell battery CAN be recharged and used repeatedly. The discharged energy can be restored by forcing electrons to flow in the opposite direction by utilizing an external electrical energy source. An example of a secondary battery that can be used for a number of years is the lead-acid battery or lead storage cell found in automobiles (Figure 1.2). The main function of a car battery is to store electrical energy for starting the car and operating electrical devices when the car is not running. Both electrodes in the lead storage cell contain a lead grid. The anodes are impregnated with a spongy lead metal, while in the cathode, the grid is packed with red-brown, lead (IV) oxide. A dilute sulfuric acid solution serves as the electrolyte. The battery is constantly being discharged every time the engine is started. During this chemical reaction, sulfuric acid is turned into water and both electrodes are converted to lead sulfate. While the car is being driven, electrical energy generated by the alternator reverses the decomposition, and the battery is continuously recharged.

Figure 1.2. A typical automotive lead-acid battery

Cathode Reaction (reduction):

\[
PbSO_4(s) + 5 H_2O(l) \leftrightarrow PbO_2(s) + 3 H_3O^+(aq) + HSO_4^-(aq) + 2e^-\]

Anode Reaction (oxidation):

\[
PbSO_4(s) + H_3O^+(aq) + 2e^- \leftrightarrow Pb(s) + HSO_4^-(aq) + H_2O(l)\]

One of the most recently developed rechargeable batteries is the lithium ion battery commonly used in cell phones and laptop computers (Figure 1.3). The anode consists of Li\(^+\) ions that have been inserted reversibly into layers of graphite. The cathode contains lithium cobalt oxide (LiCoO\(_2\)). When the cell discharges, Li\(^+\) ions spontaneously migrate from the graphite anode to the cathode, and electrons flow through the external circuit. When the battery is recharged, cobalt ions are oxidized and Li\(^+\) ions move back into the graphite.
ELECTRICAL CIRCUITS

An electric circuit is an interconnecting path, external to the battery, which allows charge to flow between the positive and negative terminals of the battery. A simple circuit may consist of a single strand of metal wire linking the terminals. However, a more realistic circuit possesses multiple branch points so that charge can take many different paths between the two terminals. Although there can be many different paths through the external circuit that the charge could take, the electrical energy that the charge acquires in making this journey is always the same. Since, when analyzing electrical circuits, we are primarily interested in energy (i.e., in the transformation of the chemical energy of the battery into heat energy in some electric heating elements, or mechanical energy in some electric motors, etc.), it follows that the property of a battery, which primarily concerns us, is its voltage. Recall that the voltage number $V$ for a battery gives a relative measure of how hard the electrons are being pulled through the circuit.

The rate at which charge flows out of the positive terminal is termed the electric current flowing out of the battery. Likewise, the rate at which charge flows into the negative terminal is termed the current flowing into the battery. Of course, these two currents must be the same otherwise charge would build up in either the battery or the circuit. Electric current, represented by the letter “I”, is measured in units of amperes or amps, abbreviated “A”. 

MFCs cannot be “recharged” by reversing the flow of electrons, but since they are a type of fuel cell, they will run indefinitely as long as a fuel feed is constantly supplied. This ensures the bacteria are constantly metabolizing glucose to produce electricity. The potassium ferricyanide solution can be recirculated in the cathode chamber for an extended period of time as well, but should be replaced regularly.
Though, in actuality, the current is carried by negative charges (i.e., by electrons) flowing in the opposite direction, conventionally, the direction of the current is taken to be the direction positive charges would have to move to account for the flow of charge. The current at all points in the external circuit must remain constant over time. We call this type of circuit a **direct current** or **DC circuit** because the current always flows in the same direction. There is a second type of circuit, which is called an **alternating current** or **AC circuit**, in which the current periodically switches direction.

A simple circuit can be described as being somewhat analogous to a small ski resort. The charges flowing around the external circuit are like people skiing down the skislope. The charges flow down a gradient of electric potential just as the people ski down a gradient of gravitational potential. Note that the good skiers who ski directly down the slope acquire exactly the same gravitational energy as the poor skiers who ski from side to side. In both cases, the total acquired energy depends only on the difference in height between the top and bottom of the slope. Likewise, charges flowing around an external circuit acquire the same electrical energy no matter what route they take because the acquired energy only depends on the potential difference between the two terminals of the battery. Once the people in our ski resort reach the bottom of the slope, they must be lifted to the top in a skilift before they can ski down it again. Thus, the skilift in our resort plays an analogous role to the battery in our circuit. Of course, the skilift must expend non-gravitational energy to lift skiers to the top of the slope, in just the same manner as the battery must expend non-electrical energy to move charges up a potential gradient. If the skilift runs out of energy, then the circulation of skiers in the resort rapidly stops. Likewise, if the battery runs out of energy (i.e., if the battery "runs down"), then the current in the external circuit stops flowing.

**OHM'S LAW**

Consider, again, a simple circuit in which a steady current $I$ flows through a single conducting wire connecting the positive and negative terminals of a battery of voltage $V$. What is the relationship between the current $I$ flowing in the wire and the potential difference $V$ applied across the two ends of the wire by the battery? If we were to investigate this relationship experimentally, we would quickly conclude that the electrical current $I$ is **directly proportional** to the potential difference $V$. In other words,

$$V = I R$$

where the constant of proportionality "$R$" is termed the (electrical) **resistance** of the wire. The above formula is called **Ohm's law** after its originator, the early nineteenth century German physicist Georg Simon Ohm. The unit of electrical resistance is the **ohm** ($Ω$).

Good conductors of electricity (i.e., copper, silver, aluminium, and most other metals) possess non-zero electrical resistances. However, these resistances are generally so small that if we were to connect the terminals of a battery together using a wire fashioned out of a good conductor, then the current, which would flow in the wire, according to Ohm's law, would be so
large that it would damage both the wire and the battery. We usually call such a circuit a short circuit. To prevent excessively large currents from flowing, conventional electric circuits contain components called resistors, whose electrical resistance is many orders of magnitude greater than that of the conducting wires in the circuit. When we apply Ohm’s law, \( V = I R \), to a circuit, we usually only count the net resistance \( R \) of all the resistors in the circuit, and neglect the resistances of the interconnecting wires. This means that all of the major drops in electric potential, as we travel around the circuit from one terminal of the battery to the other, take place inside the resistors. The drop in potential in the conducting wires themselves is usually negligible. Thus, for all intents and purposes, good conductors, and wires made out of good conductors, act as if they have zero resistance.

Sample Ohm’s Law Problems

A nine-volt battery supplies power to a cordless curling iron with a resistance of 18 ohms. How much current is flowing through the curling iron? 0.5 amps

A 110-volt wall outlet supplies power to a strobe light with a resistance of 2200 ohms. How much current is flowing through the strobe light? 0.05 amps

MFCs commonly achieve a working voltage of 0.3 – 0.7 V. The voltage (or cell potential) is a function of the external resistance or load on the circuit and the current. Ohm’s law can also be used to calculate the cell potential for the MFC. The current produced from a single MFC is usually so small that when constructed in a laboratory, the current from the MFC is not measured, but is calculated from the measured voltage drop across the resistor as \( I = V/R \). The highest voltage produced in the MFC is called the open circuit voltage, OCV, which is measured with the circuit disconnected (infinite resistance, zero current). As the resistances are decreased, the voltage decreases. The power at any time is calculated as \( P = I V \), which will be described in more detail later (see Chapter 1 – Power in Electrical Circuits).

CIRCUIT DIAGRAMS

Electric circuits, whether simple or complex, can be described with mere words – saying something like, "A light bulb is connected to a D-cell" is a sufficient amount of words to describe a simple circuit. But, another means of describing a circuit is to simply draw it. Such drawings, called circuit diagrams, provide a quicker mental picture of the actual circuit.

Describing Circuits with Words

"A circuit contains a light bulb and a 1.5 Volt D-cell."
A final means of describing an electric circuit is by use of conventional circuit symbols to provide a schematic diagram of the circuit and its components. Some circuit symbols used in schematic diagrams are shown below.

- Single Cell
- Batteries
- Connecting Wire
- Resistor
- Switch (open)
- Switch (closed)

A single cell or other power source, as previously noted, is represented by a long and a short parallel line. A collection of cells, or batteries, is represented by a collection of long and short parallel lines. In both cases, the long line is representative of the positive terminal of the energy source and the short line represents the negative terminal. A straight line is used to represent a connecting wire between any two components of the circuit. An electrical device, which offers resistance to the flow of charge (a resistor), is represented by a zigzag line. An open switch is generally represented by providing a break in a straight line by lifting a portion of the line upward at a diagonal. As an illustration of the use of electrical symbols in schematic diagrams, consider the following two examples.

**Example 1:**
Description using words: Three D-cell batteries are used to power a circuit containing three light bulbs.

Using the verbal description, one can acquire a mental picture of the circuit being described. This verbal description can then be represented by a drawing of three cells and three light bulbs connected by wires. Finally, the circuit symbols presented above can be used to represent the same circuit. Note that three sets of long and short parallel lines have been used to represent the three D-cells. Also note that each light bulb is represented by its own individual resistor symbol. Straight lines have been used to connect the two terminals of the battery to the resistors and the resistors to each other.

The above circuits presumed that the three light bulbs were connected in such a way that the charge flowing through the circuit would pass through each one of the three light bulbs in consecutive fashion. The path of a positive charge leaving the positive terminal of the battery and traversing the external circuit would involve a passage through each one of the three connected light bulbs before returning to the negative terminal of the battery. But is this the only way that three light bulbs can be connected? Do they have to be connected in consecutive
fashion as shown above? Absolutely not! In fact, example 2 below contains the same verbal description with the drawing and the schematic diagrams being drawn differently.

**Example 2:**
Description using words: Three D-cell batteries are used to power a circuit containing three light bulbs.

Again, using the verbal description, one can acquire a mental picture of the circuit being described, however this time, the connections of light bulbs are done in a manner such that there is a point on the circuit where the wires branch off from each other. The branching location is referred to as a **node**. Each light bulb is placed in its own separate branch. These branch wires eventually connect to each other to form a second node. A single wire is used to connect this second node to the negative terminal of the battery.

These two examples illustrate the two common types of connections made in electric circuits. When two or more resistors are present in a circuit, they can be connected **in series** or **in parallel**.

**Activity 1.2 – Creating Electrical Circuits**

Students will sketch and construct several circuits and draw corresponding circuit diagrams to investigate parallel and series circuits.
### Activity 1.2 – Creating Electrical Circuits

<table>
<thead>
<tr>
<th>Problem A</th>
<th>Type of Circuit: _________________</th>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one D-type battery can light two bulbs as brightly as one bulb.</td>
<td></td>
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<table>
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<tr>
<th>Problem B</th>
<th>Type of Circuit: _________________</th>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one bulb is lit brighter with two D-type batteries than with one D-type battery.</td>
<td></td>
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<tr>
<th>Problem C</th>
<th>Type of Circuit: _________________</th>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one bulb is lighted no brighter with two D-type batteries than with one D-type battery.</td>
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<tr>
<th>Problem D</th>
<th>Type of Circuit: _________________</th>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one D-type battery lights two bulbs less brightly than one bulb.</td>
<td></td>
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</table>
### Activity 1.2 – Creating Electrical Circuits (with answers)

<table>
<thead>
<tr>
<th>Problem A</th>
<th>PARALLEL</th>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one D-type battery can light two bulbs equally as bright as one bulb.</td>
<td>Rationale: Two bulbs wired in parallel are equally bright and have the same brightness as the standard. The voltage is evenly distributed across the entire circuit.</td>
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<tr>
<th>Problem B</th>
<th>SERIES</th>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one bulb is lighted brighter with two D-type batteries than with one D-type battery.</td>
<td>Rationale: Two batteries wired in series have twice the voltage as a single cell. The voltage is additive.</td>
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<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one bulb is lighted no brighter with two D-type batteries than with one D-type battery.</td>
<td>Rationale: Two batteries wired in parallel have the same voltage as a single cell, but last twice as long. The voltage is evenly distributed across the entire circuit.</td>
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<tr>
<th>Problem D</th>
<th>SERIES</th>
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</thead>
<tbody>
<tr>
<td>Sketch and construct an electric circuit in which one D-type battery lights two bulbs less brightly than one bulb.</td>
<td>Rationale: Two bulbs wired in series are equally bright, but dimmer than the standard. The voltage is additive.</td>
</tr>
</tbody>
</table>
Activity 1.2 – Creating Electrical Circuits (Instructor Notes)

• Have the students observe both current and voltage with a multimeter as they construct the circuits.

• Ask them to make observations about the differences between parallel and series connections as they relate to Ohm’s Law (taking into account the resistance of the bulbs).

--Questions for Analysis--

? What are the basic components of an electrical circuit?
Two or more interconnected components consisting of a source of current or voltage and a resistor.

? What is the difference between series and parallel circuits?
A series circuit is a single path for electric current through all of its components. Several batteries connected in series can produce greater voltage than a single battery can. A parallel circuit is a different path for current through each of its components. A parallel circuit provides the same voltage across all its components.

? You may have noticed that an entire string of old Christmas lights goes out if one bulb burns out or is removed. Explain.
Christmas lights are connected in series, so when one goes out or is removed, an open circuit is created. An open circuit is an incomplete circuit due to a break in the continuous connection of conductors from one end of an electrical source to the other.
SERIES AND PARALLEL CIRCUITS

A series circuit is a circuit in which resistors are arranged in a chain so that the current has only one path to take. The current is the same through each resistor. The total resistance of the circuit is found by simply adding up the resistance (or voltage) values of the individual resistors:

\[
\text{equivalent resistance of resistors in series: } R = R_1 + R_2 + R_3 + \ldots
\]

A series circuit is shown in the diagram above. The current flows through each resistor in turn. If the values of the three resistors are:

\[R_1 = 8 \, \Omega, \quad R_2 = 8 \, \Omega, \quad \text{and} \quad R_3 = 4 \, \Omega.\]

Then the total resistance is \[8 + 8 + 4 = 20 \, \Omega.\]

With a 10 V battery, by \[V = I \, R,\] the total current in the circuit is:

\[I = V/R = 10/20 = 0.5 \, \text{A}.\] The current through each resistor would be 0.5 A.

A parallel circuit is a circuit in which the resistors are arranged with their heads connected together, and their tails connected together. The current in a parallel circuit breaks up, with some flowing along each parallel branch, and re-combining when the branches meet again. The voltage across each resistor in parallel is the same.

The total resistance of a set of resistors in parallel is found by adding up the reciprocals of the resistance values, and then taking the reciprocal of the total:

\[
\text{equivalent resistance of resistors in parallel: } 1/R = 1/R_1 + 1/R_2 + 1/R_3 + \ldots
\]
A parallel circuit is shown in the diagram above. In this case, the current supplied by the battery splits up, and the amount going through each resistor depends on the resistance. If the values of the three resistors are:

\[ R_1 = 8 \, \Omega, \quad R_2 = 8 \, \Omega, \quad \text{and} \quad R_3 = 4 \, \Omega, \]

then the total resistance is found by

\[ \frac{1}{R} = \frac{1}{8} + \frac{1}{8} + \frac{1}{4} = \frac{1}{2}. \]

This gives \( R = 2 \, \Omega \).

With a 10 V battery, by \( V = I \, R \), the total current in the circuit is: \( I = \frac{V}{R} = \frac{10}{2} = 5 \, A \).

The individual currents can also be found using \( I = \frac{V}{R} \). The voltage across each resistor is 10 V, and therefore:

\[ I_1 = 10/8 = 1.25 \, A \]
\[ I_2 = 10/8 = 1.25 \, A \]
\[ I_3 = 10/4 = 2.5 \, A \]

Note that the currents add together to 5 A, the total current.

In a manner analogous to resistors, batteries (or sources of voltage) can also be placed within circuits in series and in parallel. In each instance, batteries added in series increase voltage, while batteries added in parallel increase current.

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The reason our experimental MFC has two anode chambers is analogous to the connection of two batteries in series – more voltage is generated. To light the LED, approximately 0.45 V is necessary, and a single anode chamber often is not capable of supplying that much voltage. The reason the anode chambers are situated on either side of the cathode chamber is to provide increased surface area at the interface between the anode and cathode for the migration of positive ions through the ion-exchange membrane.

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**EMF AND INTERNAL RESISTANCE**

Real batteries are constructed from materials, which possess non-zero resistivities, so real batteries are not just pure voltage sources. They also possess internal resistances. Incidentally, a pure voltage source is usually referred to as an emf (which stands for electromotive force). Of course, emf is also measured in units of volts. A battery can be modeled as an emf \( \varepsilon \) connected in series with a resistor \( r \), which represents its internal resistance. Suppose that such a battery is used to drive a current \( I \) through an external load resistor \( R \) as shown in the circuit diagram below. Note that in circuit diagrams, an emf \( \varepsilon \) is represented as two closely spaced parallel lines of unequal length. A resistor is represented as a zig-zag line.
Consider the battery above. The voltage $V$ of the battery is defined as the difference in electric potential between its positive and negative terminals: i.e., the points A and B, respectively. As we move from B to A, the electric potential increases by $+\varepsilon$ volts as we cross the emf, but then decreases by $1/r$ volts as we cross the internal resistor. Thus, the voltage $V$ of the battery is related to its emf $\varepsilon$ and internal resistance $r$ via:

$$V = \varepsilon - IR$$

Now, we usually think of the emf of a battery as being essentially constant (since it only depends on the chemical reaction going on inside the battery, which converts chemical energy into electrical energy). Therefore, we must conclude that the voltage of a battery actually decreases as the current drawn from it increases. In fact, the voltage only equals the emf when the current is negligibly small.

If we short-circuit a battery, by connecting its positive and negative terminals together using a conducting wire of negligible resistance, the current drawn from the battery is limited only by its internal resistance. In fact, in this case, the current is equal to the maximum possible current. A real battery is usually characterized in terms of its emf $\varepsilon$ (i.e., its voltage at zero current) and the maximum current that it can supply. For instance, a standard dry cell (i.e., the sort of battery used to power remote controls) is usually rated at 1.5 V and (around) 0.1 A. Thus, nothing really catastrophic is going to happen if we short-circuit a dry cell. We will run the battery down in a comparatively short period of time, but no dangerously large current is going to flow. On the other hand, a car battery is usually rated at 12 V and something like 200 A (this is the sort of current needed to operate the starter motor). It is clear that a car battery must have a much lower internal resistance than a dry cell. It follows that if we were foolish enough to short-circuit a car battery, the result would be fairly catastrophic (imagine all of the
energy needed to turn over the engine of a car going into a thin wire connecting the battery terminals together).

**Problem:** What is the terminal (final) voltage supplied by a cell of emf 2.0 V with an internal resistance of 1.0 Ω when it is connected to a 9.0 Ω resistor?

Begin by using Ohm’s law to find the current supplied by the battery.

\[ I = \frac{V}{R} = \frac{2.0}{9.0} = 0.22 \, \text{A} \]

Then, use the internal resistance formula to solve the rest of the problem.

\[ V = \varepsilon - IR \]
\[ V = 2.0 - (0.22)(1) = 1.8 \, \text{V} \]

It is not unusual to note significant differences in voltage output between differing MFC assemblies based on specific system architecture. The main reason is because of the internal resistance of the reactor compared to the maximum possible potential due to the chemical reactions at the anode and cathode, or the cell potential, \( E_{\text{emf}} \) (see Chapter 1 – Standard Reduction Potentials). To determine the total voltage capable of being produced by the cell, we must view the MFC as having current through two resistors linked in series, with one being the external load and the other the internal resistance.

Being that the operational conditions within the MFC are defined by the requirements of the microbes for optimal growth and metabolism and are not necessarily compatible with the most favorable electrochemical conditions, a key challenge for those laboratories and scientists working to improve MFC voltage (and resulting power) outputs is to identify possible sources of internal resistance within the specific MFC design and significantly reduce these resistances.

**POWER IN ELECTRICAL CIRCUITS**

In addition to voltage and current, another measure of electron activity in a circuit is power. In electric circuits, power is a function of both voltage and current. Not surprisingly, this relationship bears a striking resemblance to Ohm’s law:

\[ P = I \, V \]

In this equation, power \( P \) is exactly equal to the current \( I \) multiplied by the voltage \( V \). When using this formula, the unit of measurement for power is the watt, abbreviated with the letter "W."

It must be understood that neither voltage nor current by them selves constitute power. Rather, power is the combination of both voltage and current in a circuit. Remember that voltage is the specific work (or potential energy) per unit charge, while current is the rate at
which electric charges move through a conductor. Voltage is analogous to the work done in lifting a weight against the pull of gravity. Current is analogous to the speed at which that weight is lifted. Thus, the rule is *the power in a circuit is the product of the voltage and the current.*

According to the formula, a circuit with high voltage and low current may be dissipating the same amount of power as a circuit with low voltage and high current. Neither the amount of voltage alone, nor the amount of current alone, indicates the amount of power in an electric circuit.

In an open circuit, a circuit contains no external resistors, voltage is present between the terminals of the battery, and there is zero current. As a result, there is zero power dissipated, no matter how great that voltage may be. Since $P = I \times V$ and $I = 0$ and anything multiplied by zero is zero, the power dissipated in any open circuit must be zero. Likewise, if we were to have a short circuit constructed of a loop of conducting wire (essentially zero resistance), we could have a condition of current in the loop with zero voltage and, likewise, no power would be dissipated.

The power formula does not just apply to batteries. It could also apply to a resistor external to the battery. Such a resistor is referred to as a load resistor. It could be either an electric light, an electric heating element, or, maybe, an electric motor. The basic purpose of the circuit is to transfer energy from the battery to the load, where it actually does something useful for us (e.g., lighting a light bulb or lifting a weight).

The power transfer between a voltage source and an external load (or load resistor) is most efficient when the resistance of the load matches the internal resistance of the voltage source. If the load resistance is too low, then most of the power output of the voltage source is dissipated as heat inside the source itself. Electrical energy is converted into heat (*i.e.*, random motion of the atoms that make up the voltage source) as the electrically accelerated free electrons inside the source collide with the atoms and, thereby, transfer all of their kinetic energy to the atoms. If the load resistance is too high, then the current, which flows in the circuit, is too low to transfer energy to the load at an appreciable rate. The optimal case exists when the load resistance is equal to the internal resistance of the voltage source, however, only half of the power of the voltage source is transferred to the load. The other half is dissipated as heat inside the source.
To make MFCs useful as a method to generate power, it is necessary to first optimize the system for power production. Power is calculated from the voltage and the current as \( P = I E \), where “E” stands for the cell potential (in some instances, “V” is not used for voltage because the symbol and the units \( V = \text{Volts} \) can lead to confusion).

The power generated by an MFC is calculated from the measured voltage, \( E_{\text{MFC}} \), across the load and the current using \( P = I E_{\text{MFC}} \). The current produced by a laboratory-scale MFC is commonly calculated, as previous stated, by measuring the potential across the load (i.e., the external resistor, \( R_{\text{ext}} \)), and using \( I = E_{\text{MFC}} / R_{\text{ext}} \). Thus, power output can be found using

\[
P = \frac{E_{\text{MFC}}^2}{R_{\text{ext}}}
\]

Based on the relationship \( I = E_{\text{MFC}} / R_{\text{ext}} \), we can alternatively express power output in terms of the calculated current as:

\[
P = I^2 R_{\text{ext}}
\]

Activity 1.3.A (Assessment) – The Shoebox Room
Activity 1.3.B (Assessment) – Can You Light Your Way?

Students will demonstrate an understanding of the requirements of making an electrical circuit; will be able to build a working series circuit and parallel circuit; will be able to describe how both types of circuits work; will be able to describe the advantages and disadvantages of parallel and series circuits; will understand that electrical energy can be converted to heat, light, sound and motion; and will wire a shoebox house with series and parallel circuits to operate lights and other resistors that are controlled by switches.
Activity 1.3.A – The Shoebox Room

Hello, my name is Stuart. I live in a very small house, and you won’t find me complaining about that. I’m sure my little house is much more comfortable than your average cave or mud hut. Houses, as I’m sure you’ve noticed, seem to be getting smaller and smaller and smaller until eventually, I imagine, we will all be living in shoeboxes. Not just me.

Yes, although the entire house has not yet shrunk into something you could easily walk around with, my bedroom is ridiculously tiny. It is by far the smallest in the house.

I am looking to do some redecorating and remodeling in my bedroom and would like to include a few new amenities and upgrades to its current design:

• An alarm that sounds when the door opens
• A ceiling fan that can run on more than one speed with a light
• A reading lamp with more than one level of brightness

Being that most interior decorating businesses and electrical engineering firms don’t deal with rooms as small as shoeboxes, Stuart has sought you out to design and construct his new bedroom.

You should begin by sketching out a floor plan for the room, including the location of all the furniture and fixtures, then drawing out circuit diagrams for the necessary electrical work, and, finally, completing the electrical work within the room.

In addition to creating a fully functioning shoebox room for Stuart, he has also asked that you prepare a report detailing how the electrical energy is being transferred and transformed throughout his room. The report should also include a discussion of wiring choices, resistor arrangements, and energy flow.

The following materials will be provided for you to use in the design of Stuart’s shoebox room:

For the teacher to develop:
Need to develop materials list

Need to develop scoring rubric
Activity 1.3.B – Can You Light Your Way?
(after Prof. G. Watson, University of Delaware, www.physics.udel.edu)

"What's wrong with the carbide lantern?"

Before Chris could utter a reply to Pat's question, darkness suddenly descended.

"Great! I've been begging my folks for a new lamp since the last time we went caving. Too late now! Let's fire up one of our candles."

It took a bit of rummaging through the pack to find one in the shroud of darkness. Finally ready, Pat struck a match but it immediately flickered out. This happened again with the second match, and again with the third. The reason suddenly occurred to Chris.

"You've gotta be kidding me -- I think the air is bad down here! The guidebook didn't say anything about that, did it?"

"Not that I remember. You know, my breath does seem a little short right now that you mention it. Think we made a wrong turn?"

"Don't know, but we need to get out of here right now. Shine your flashlight over here while I take another look at the map."

A minute passed while Pat rifled through the pack again.

"Bad news! I must have left the flashlight lying on the ground where we ate our lunch."

"You'd better be joking! We have walked for four hours since we ate. We can't make it back there without a light."

"I'm serious. I remember taking it out to get the sandwiches. Guess I forgot to put it back."

"Never mind, I keep a small flashlight in my jacket. Hey, where is it? Darn it, my little brother is always stealing my stuff!"

"Calm down! Getting upset won't help anything!"

"OK. OK. You're right. What do we have for a light?"

"Well, I do have a spare bulb for my lantern in my pocket. But I didn't bring a spare battery; they're so heavy."
"Do you have anything else with batteries in it? I've got two extra AAA batteries for the flashlight that little thief took."

"I've got nothing else with batteries. I don’t carry a little bit of everything like you do."

"Good thing I do! But the bulb is for a 6 V battery. Do you think it will work with two AAA cells?"

"Even if it does, do you think it will burn long enough for us to get out? I'm starting to get worried..."

"Maybe we can use one at a time to make them last longer. It won't be as bright and we’ll have to walk slower, but maybe one at a time would be better. Hey, you've been learning about electrical circuit in school. What should we do?"

**The Exercise**

Your lab exercise comes in two parts.

1. Your group will have available two AAA cells and the bulb described above. In the lab you will have available two multimeters, lengths of wire, and a choice of resistors. Make the measurements necessary to answer the questions in the preceding story. You may wish to consult additional online resources for supporting data.

2. Design a flashlight using materials that you wish you had in your pockets. Be creative - assume that you are not carrying a length of wire! Also, a switching mechanism would be a desirable feature. In the next class period, your group will be given five minutes to assemble a working flashlight. And we may even turn out the lights!!!
ELECTROCHEMICAL CELLS

An electrochemical cell generates an electromotive force or emf (voltage) and electric current from chemical reactions. The current is caused by the chemical reactions releasing and accepting electrons at the different ends of a conductor. Electrochemistry is the branch of chemistry involved with the study of electricity produced by chemical reactions.

There are two different kinds of electrochemical cells. Electrolytic cells are those in which electrical energy causes nonspontaneous chemical reactions to occur, and voltaic cells are those in which spontaneous chemical reactions produce electricity and pass it through an external circuit. In a voltaic cell, electrical current can be conducted through metal wires or along metal surfaces and through pure liquid electrolytes or solutions that contain electrolytes. When a metallic conductor is employed, current is conducted through the metal without causing any chemical change. Electrolytic conduction occurs via the motion of ions through the solution or pure liquid.

The chemical reactions in batteries occur when two dissimilar materials, such as aluminum and copper (called electrodes) react together when inserted into a chemical conduction solution called an electrolyte. The electrolyte solution begins to slowly dissolve the aluminum electrodes, yielding positive aluminum ions and electrons. These electrons must remain bound to an electron conducting material and cannot move into the electrolyte. The positive ions accumulate in the electrolyte and flow (via concentration gradient) towards the cathode. The electrons travel around, from the anode, through the conducting wires, and complete the circuit at the cathode. At the cathode, the positive aluminum ions in combination with the electrons cause aluminum to be deposited on the copper electrode.

Activity 1.4 – A Homemade “Lemony” Battery

Students will construct a functional battery from several common household items.
Activity 1.4 – A Homemade “Lemony” Battery

Materials:

lemons, coins (such as copper pennies), paper towels, aluminum foil (or coins, such as dimes), bowls, scissors, lemon juicer, wire strippers, plastic tape, paper tube (toilet paper or paper towel tube will work), plastic-covered electrical wire

Procedure:

1. Wrap foil over one end of the paper tube and then secure it by taping it down.

2. With the wire cutters, strip 1-2” of the plastic from the wire. Tape one end to the foil. Then, set up the paper tube with the foil down and the opening on top.

3. Squeeze the juice from the lemons into a dish. Soak the paper towels in the juice. Then, start filling the paper tube with a small rolled piece of toweling, then a coin, followed by a piece of foil. Continue to layer these three materials, filling the tube and ending with a coin. Tape a second stripped wire to the coin.

4. Moisten a finger tip on each hand and touch the ends of the two wires. Students will experience a small shock or tingle but it will be very harmless. Students may also try attaching an LED to each end of the battery.

--Questions for Analysis--

? If you are not generating enough electricity to light the LED, what could you do to generate more voltage?

Connect several homemade batteries together in series.
The homemade battery is an example of a wet cell. The lemon juice acts as the electrolyte that conducts the electricity created by the coins and the foil. Wet cells are characterized by liquid electrolytes.

While lemon juice acted as the sole electrolyte in this homemade battery, our MFC will be using two different electrolytes, one in the anode (negative) chamber and one in the cathode (positive) chamber. The anode will contain a mixture of soil bacteria in a glucose nutrient growth media with an added redox mediator (methylene blue). The cathode will contain a solution of a soluble chemical electron acceptor (oxidizer) called potassium ferricyanide. The MFC must utilize two different electrolytes because of the nature of the reactions occurring in each chamber. In the anode chamber, there is no oxygen present (anaerobic conditions), while there typically is oxygen present in the cathode chamber. Some MFC designs can also have anaerobic cathode chambers, e.g. with chemical electron acceptors, such as ferricyanide.

Activity 1.5 – The Hand Battery

Students will observe that voltage output (i.e., batteries) can result from many different materials as long as the principles of electron flow are observed.

Activity 1.5 – The Hand Battery

Materials:

- multimeter* or voltmeter (for instructions on operations and settings consult [www.extech.com/instrument/products/400_450/manuals/EX410_UM.pdf](http://www.extech.com/instrument/products/400_450/manuals/EX410_UM.pdf) or your instruction manual), copper plate and aluminum plate (each about the size of your hand), two electrical lead wires with alligator clips, a piece of wood or other nonmetallic surface, plates of other metals, such as tin or zinc (optional)

* The multimeter must be capable of measuring low range current. Industrial gauges cannot always read in milliamp ranges. Multimeters sensitive enough for this activity can be purchased at electronics stores, such as Radio Shack.

Procedure:

1. Mount both metal plates on a piece of wood or simply clamp them to a nonmetallic surface. (If you prefer, you don’t even have to mount the two plates. You can attach the wires as described below and then simply hold one plate in each hand. This has the benefit of allowing you to substitute other metals easily.)
2. Using the clip leads, connect one plate to one of the meter's terminals and connect the other plate to the other terminal. At this point, it doesn't matter which plate attaches to which terminal.

3. Place one hand on each plate. You should notice a reading on the meter. If the meter doesn't show an electrical current, simply reverse your connections, attaching the copper plate to the terminal that the aluminum was connected to and vice versa. If there is still no current, check the connections and the wiring. If that doesn't produce current, try cleaning the plates with a pencil eraser or steel wool to remove oxidation.

4. Experiment with different metals to find out what combination produces the most current (amps). Try pressing harder on the plates. Get your hands wet and try again.

5. Have one person put a hand on the copper plate and another person put a hand on the aluminum plate, and then have them join their free hands.

6. Also, experiment with measuring the resistance (ohms) of wet vs. dry hands.

7. Vigorously exercise (30 push ups or 30 jumping jacks), and once again measure the resistance.

--Questions for Analysis--

? Is there a difference between dry and wet hands in the voltage or current? Why or why not?
   Wet hands (less resistance) should yield a higher current and lower voltage due to Ohm’s law.

? When did you obtain the highest and the lowest resistance?
   Wet (low resistance), Dry (higher resistance)

? Is the resistance of your skin a function of moisture in your skin?
   Yes

? Suggest how the hand battery could be utilized as a simple type of lie detector.
   One could assume that a person who is lying may be nervous, causing their hands to perspire.

Discussion:

When you touch the two metal plates, the thin film of sweat on your hands acts as the electrolyte, reacting with the copper plate and with the aluminum plate. In one of these reactions, your hand takes negatively charged electrons away from the copper plate, leaving positive charges behind. In the other reaction, your hand gives electrons to the aluminum plate, causing it to become negatively charged.

This difference in charge between the two plates creates a flow of electrical charge, or electrical current. Since electrons can move freely through metals, the excess electrons on the aluminum plate flow through the meter on their way to the copper plate. (In metals, positive charges
cannot move.) In your body, both positive and negative ions move. Negative electrons move through your body from the hand touching the copper to the hand touching aluminum. At the same time, positive ions move in the opposite direction. As long as the reactions continue, the charges will continue to flow and the meter will show a small current.

Your body resists the flow of current. Most of this resistance is in your skin. By wetting your skin, you can decrease your resistance and increase the current through the meter. Since two people holding hands have more resistance than one person, the flow of current will be less.

If you disconnect one of the wires to the multimeter, the aluminum becomes negatively charged. Electrons pile up on the aluminum side because they cannot cross the gap in the wire. The copper becomes positively charged as your hand removes electrons from the metal. These piles of charge create a voltage, which is measured when the multimeter is set to read volts.

Most batteries use two different metals and an electrolyte solution to create a potential difference and, thus, a voltage. When the terminals of the battery are connected with a wire, this voltage produces a current.

You can use other pairs of different metals in a circuit to produce a current. The success you have using various metals will depend on a metal's electric potential, that is, its ability to gain or lose charges. Try various metals to see which produces the highest current reading. An electromotive series table (found in most chemistry textbooks) shows the electric potentials of metals and allows you to predict which metals will work well in making a hand battery (see table, page 33).

You can sometimes get a small current even between two plates made of the same metal. Each plate has a slightly different coating of oxides, salts, and oils on its surface. These coatings create slight differences in the surfaces of the metals, and these differences can produce an electrical current.
OXIDATION – REDUCTION REACTIONS

Batteries rely on chemical reactions to generate voltage and current. The current is caused by the reactions releasing and accepting electrons at the different ends of a conductor. The reaction is always an oxidation-reduction (or redox) reaction involving a transfer of electrons. Redox reactions can be broken down into two half-reactions: (1) oxidation at the anode as electrons are transferred from an electron donor to the electrode, and (2) reduction at cathode as an electron acceptor gains electrons from the electrode. Thus, in a spontaneous redox reaction, electrons flow from the oxidized reactant (electron donor) to the reduced reactant (electron acceptor). The electrodes are simply the ordinary metallic surfaces upon which the oxidation or reduction half-reactions occur.

Redox processes occur in single displacement or substitution reactions. The redox component of this type of reaction is the change of oxidation state (charge) on certain atoms, not the actual exchange of atoms in the compounds.

In electrochemical cells, spontaneous oxidation-reduction reactions generate electrical energy. For example, in the reaction between iron and copper(II) sulfate solution:

$$\text{Fe} + \text{CuSO}_4 \rightarrow \text{FeSO}_4 + \text{Cu}$$

The ionic equation for this reaction is:

$$\text{Fe} + \text{Cu}^{2+} \rightarrow \text{Fe}^{2+} + \text{Cu}$$

As two half-equations, it is seen that the iron is oxidized (electron donor):

$$\text{Fe} \rightarrow \text{Fe}^{2+} + 2e^-$$

And the copper is reduced (electron acceptor):

$$\text{Cu}^{2+} + 2e^- \rightarrow \text{Cu}$$

If we dip an iron strip into a copper(II) sulfate solution, the iron will be eaten away, a spongy layer of metallic copper will plate out on the iron strip, and the deep blue color of copper(II) sulfate will gradually fade. [Iron(II) sulfate, which is formed, is colorless.] In contrast, if we immerse a copper strip in an iron(II) sulfate solution, no reaction will occur because the reverse reaction is highly nonspontaneous.

In this arrangement, the spontaneous transfer of electrons from iron to copper is not useful for generating electrical energy because the energy released is dissipated as heat. It is analogous to burning a spoonful of sugar with a match instead of eating it and converting the free energy of oxidation into useful muscle work (see Chapter 2 – Metabolism as a Redox Process). If some means could be found to separate the removal of electrons from iron (oxidation) from the
donation of electrons to copper ions (reduction), then the electrons may be made to do something useful along the way.

When the two half-reactions are separated, this flow of electrons, instead of occurring at the surface of the metal, occurs through an external circuit and electric current is generated. This is called a voltaic cell, and is exactly how a battery works. The two halves of the redox reaction are referred to as half-cells.

A battery, like the ones found in a flashlight or calculator, contain oxidizing and reducing substances. As the electrons are transferred from anode to cathode, they are “tapped” to provide the voltage necessary to power the electrical device.

VOLTAIC CELLS

Voltaic cells consist of two half-cells connected by a salt bridge. Each half cell is typically a metal electrode in a 1.0 molar (1 mol/L or M) aqueous solution of the metal’s salt. The half cells are connected by a metal wire and a voltmeter can be placed into the circuit to measure the potential difference between the two electrodes. The salt bridge is often employed to provide electrical contact between two half-cells with very different electrolytes – to prevent the solutions from mixing – and is made of some medium through which ions can slowly pass and maintain charge balance in the two half-cell solutions. A simple salt bridge consists of filter paper soaked with a relatively inert electrolyte, usually potassium chloride or sodium chloride. Another type of salt bridge consists of U-shaped glass tubes- filled potassium chloride or sodium chloride. Agar is often used for gelification within the tube.

The two electrodes, connecting wire, and salt bridge form a closed circuit, with electrons moving from negative electrode to positive electrode through the wire, and positive and negative ions moving through the salt bridge. Thus, electrons will flow spontaneously from the anode, where oxidation is occurring, to the cathode, where the reduction is occurring. Electricity in the voltaic cell is generated due to electric potential difference between two electrodes. The potential difference between the electrodes provides the driving force that pushes the electrons through the external circuit. This potential, or EMF (electromotive force), of a cell is a measure of the tendency of the electrons to flow, and is dependant on the difference in oxidizing or reducing strength of the two half-cells.

A good analogy for the flow of electrons is the flow of water. Water flows spontaneously downhill. Dams and waterwheels are examples of ways that the energy of flowing water is tapped to generate power. Cell potential is roughly analogous to a height difference between two reservoirs of water that are connected. Water will have a tendency (potential) to flow between the reservoirs, and that flow can be tapped to do work.

To maintain electrical neutrality and complete the circuit in the iron-copper cell described above, two Cl\(^-\) ions from the salt bridge migrate into the anode solution for every Fe\(^{2+}\) ion
formed. Simultaneously, two K\(^+\) ions migrate into the cathode solution to replace every Cu\(^{2+}\) ion reduced.

In the MFC, electrons are generated at the anode as glucose is oxidized through a process called glycolysis (see Chapter 2 – *Step 1 – Glycolysis*). The electrons are then shuttled to the anode with the help of a chemical redox mediator called methylene blue. Copper wires, connected to the electrodes by alligator clips, conduct the electrons, potentially through a resistor (LED), to the cathode. In the cathode chamber, electrons are discharged from the cathode to potassium ferricyanide, or, more specifically, the ferricyanide ion, Fe(CN)\(_6\)\(^{-3}\), is reduced to the ferrous cyanide ion, Fe(CN)\(_6\)\(^{−4}\). In our MFC, the circuit is closed, not by a salt bridge, but rather by semi-porous, ion-exchange membranes that separate the anode and cathode chambers. If the potential difference between the anode and cathode chambers is large enough (over 0.45 V required), the MFC can power the LED.

Voltaic cells can be represented in shorthand form:

\[
\text{Fe} | \text{Fe}^{2+} (1.0 \text{ M}) | | \text{Cu}^{2+} (1.0 \text{ M}) | \text{Cu}
\]

The anode (Fe) is shown on the left side and the cathode (Cu) on the right side. The electrode surfaces and the species (and their concentrations) in contact with the electrode surfaces are separated by single vertical lines. Double vertical lines represent the salt bridge.

**STANDARD REDUCTION POTENTIALS**

There is competition between Fe\(^{2+}\) and Cu\(^{2+}\) in the iron-copper voltaic cell for electrons. It turns out that Cu\(^{2+}\) is easier to reduce than Fe\(^{2+}\) and wins the battle for the electrons. This is known because each half-cell has a characteristic voltage or potential.

It would be desirable to separate the individual contributions each half-cell reaction makes to the total cell potential. This would allow us to determine the relative tendencies of the particular oxidation or reduction half-reactions to occur. However, it is not possible to determine experimentally the potential of a single electrode since every oxidation must be accompanied by a reduction; that is, the electrons must have somewhere to go. As a consequence, it is necessary to establish some arbitrary standard.

By international agreement, the reference electrode was selected to be the **standard hydrogen electrode (SHE)**. This standard hydrogen electrode consists of a piece of metal electrolytically coated with a grainy, black surface of inert platinum metal immersed in a 1.0 M H\(^+\) solution. Hydrogen gas, H\(_2\), is then bubbled over the electrode at one atmosphere (atm) pressure. By convention, the SHE is assigned a potential of 0.00 V, and then all other half-cell potentials are measured relative to that assigned value.
H₂(g) → 2H⁺(aq) + 2e⁻  E° = 0.00 V (SHE as anode)
2H⁺(aq) + 2e⁻ → H₂(g)  E° = 0.00 V (SHE as cathode)

The Copper – SHE Cell

To determine the half-cell potential for the reduction of copper, a voltaic cell is constructed using a copper electrode and the standard hydrogen electrode. As the half-cell reactions ensue, the copper electrode decreases in mass and the concentration of Cu²⁺ ions decreases in the solution around the copper electrode. Simultaneously, gaseous hydrogen decreases in mass and the H⁺ ion concentration increases in the solution of the SHE. The total electrical potential for this cell has been experimentally determined to be +0.34 V at the start of the reaction.

| (anode) | H₂ → 2H⁺ + 2e⁻ | E° = 0.00 V |
| (cathode) | Cu²⁺ → 2e⁻ + Cu | E° = ? |
| (cell reaction) | H₂ + Cu²⁺ → 2H⁺ + Cu | E° = 0.34 V |

Thus, the half-cell potential for the reduction of copper is

Cu²⁺ + 2e⁻ → Cu  E° = +0.34 V

The Iron – Copper Cell

As described earlier, in this cell, copper deposits on one electrode as the iron electrode decreases in mass. The total electrical potential of this cell has been calculated to be +0.78 V.

Fe → Fe²⁺ + 2e⁻  E° = ?
Cu²⁺ + 2e⁻ → Cu  E° = +0.34 V
Fe + Cu²⁺ → Fe²⁺ + Cu  E° = +0.78 V

Thus, the half-cell potential for the oxidation of iron is

Fe → Fe²⁺ + 2e⁻  E° = +0.44 V

The reaction for the half-cell is always written as a reduction (gain of electrons)

Zn²⁺ + 2e⁻ → Zn  E° = −0.76 V
By measuring the potentials of other standard electrodes versus the SHE or some other standard half-cell whose electrode potential is known, a series of standard electrode potentials can be established. When the electrodes involve metals or nonmetals that are in contact with their ions, the resulting series is called the electromotive series or activity series of the elements (see the table below). Those species at the top of the series and on the right side of the reduction half-reaction are the most active and possess the greatest tendency to be oxidized or lose electrons (the best reducing agents). The species at the bottom of the series on the left side of the equation undergo reduction easily to gain electrons (the best oxidizing agents). Any species on the left side of a given half-reaction will react spontaneously with a substance that is on the right side in half-reaction above it, and the voltaic cell will have a positive potential.

<table>
<thead>
<tr>
<th>Electrode Reaction</th>
<th>$E^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li$^+(aq) + e^- \rightarrow$ Li(s)</td>
<td>$-3.05$ V</td>
</tr>
<tr>
<td>K$^+(aq) + e^- \rightarrow$ K(s)</td>
<td>$-2.93$ V</td>
</tr>
<tr>
<td>Na$^+(aq) + e^- \rightarrow$ Na(s)</td>
<td>$-2.71$ V</td>
</tr>
<tr>
<td>Mg$^{2+}(aq) + 2e^- \rightarrow$ Mg(s)</td>
<td>$-2.38$ V</td>
</tr>
<tr>
<td>Al$^{3+}(aq) + 3e^- \rightarrow$ Al(s)</td>
<td>$-1.66$ V</td>
</tr>
<tr>
<td>Zn$^{2+}(aq) + 2e^- \rightarrow$ Zn(s)</td>
<td>$-0.76$ V</td>
</tr>
<tr>
<td>Cr$^{3+}(aq) + 3e^- \rightarrow$ Cr(s)</td>
<td>$-0.74$ V</td>
</tr>
<tr>
<td>Fe$^{2+}(aq) + 2e^- \rightarrow$ Fe(s)</td>
<td>$-0.44$ V</td>
</tr>
<tr>
<td>Cd$^{2+}(aq) + 2e^- \rightarrow$ Cd(s)</td>
<td>$-0.40$ V</td>
</tr>
<tr>
<td>Ni$^{2+}(aq) + 2e^- \rightarrow$ Ni(s)</td>
<td>$-0.25$ V</td>
</tr>
<tr>
<td>Sn$^{2+}(aq) + 2e^- \rightarrow$ Sn(s)</td>
<td>$-0.14$ V</td>
</tr>
<tr>
<td>Pb$^{2+}(aq) + 2e^- \rightarrow$ Pb(s)</td>
<td>$-0.13$ V</td>
</tr>
<tr>
<td>$2H^+(aq) + 2e^- \rightarrow$ H$_2$(s)</td>
<td>$0.00$ V</td>
</tr>
<tr>
<td>Cu$^{2+}(aq) + 2e^- \rightarrow$ Cu(s)</td>
<td>$0.34$ V</td>
</tr>
<tr>
<td>I$_2$(s) + $2e^- \rightarrow$ 2I$^-(aq)$</td>
<td>$0.54$ V</td>
</tr>
<tr>
<td>Fe$^{3+}(aq) + e^- \rightarrow$ Fe$^{2+}(aq)$</td>
<td>$0.77$ V</td>
</tr>
<tr>
<td>Hg$^{2+}(aq) + 2e^- \rightarrow$ Hg(l)</td>
<td>$0.79$ V</td>
</tr>
<tr>
<td>Ag$^+(aq) + e^- \rightarrow$ Ag(s)</td>
<td>$0.80$ V</td>
</tr>
<tr>
<td>Br$_2$(l) + $2e^- \rightarrow$ 2Br$^-(aq)$</td>
<td>$1.07$ V</td>
</tr>
<tr>
<td>Cl$_2$(g) + $2e^- \rightarrow$ 2Cl$^-(aq)$</td>
<td>$1.36$ V</td>
</tr>
<tr>
<td>Au$^{3+}(aq) + 3e^- \rightarrow$ Au(s)</td>
<td>$1.50$ V</td>
</tr>
<tr>
<td>F$_2$(g) + $2e^- \rightarrow$ 2F$^-(aq)$</td>
<td>$2.87$ V</td>
</tr>
</tbody>
</table>
Problem: What is the spontaneous electrochemical reaction that occurs when a standard copper half-cell is combined with a standard silver half-cell and what is the value of $E^0$ for this voltaic cell?

From the table we know that

\[
\begin{align*}
Cu^{2+} + 2e^- & \rightarrow Cu \quad E^0 = +0.337 \, V \\
Ag^+ + e^- & \rightarrow Ag \quad E^0 = +0.800 \, V \\
\end{align*}
\]

Combining both half-cells, we have the following equation to give the answer

\[
\begin{align*}
Cu & \rightarrow Cu^{2+} + 2e^- \quad E^0 = -0.337 \, V \\
2Ag^+ + 2e^- & \rightarrow 2Ag \quad E^0 = +0.800 \, V \\
Cu + 2Ag^+ & \rightarrow Cu^{2+} + 2Ag \quad E^0 = +0.463 \, V \\
\end{align*}
\]

Note that doubling the silver half-cell reaction balances the electrons, but does not affect the value of $E^0$ for the half-reaction.

NON-STANDARD STATE REDUCTION POTENTIALS

The Nernst equation was derived to obtain electrode potentials for concentrations and partial pressures other than the standard state values.

\[
E_{cell} = E^0 - 2.303 \left( \frac{RT}{nF} \right) \log Q
\]

- $E_{cell}$ = the electrode potential under nonstandard conditions
- $E^0$ = the standard electrode potential
- $R$ = the gas constant, 8.314 J/mole K
- $T$ = the absolute temperature, in K
- $n$ = the number of moles of electrons transferred per mole of cell reaction
- $F$ = the Faraday constant, 96485 J/V mole e
- $Q$ = the reaction quotient (concentration of products divided by concentration of reagents)

Putting the values given above into the Nernst equation at 298 K (25°C) simplifies the equation to $E_{cell} = E^0 - (0.0592/n) \log Q$.

Problem: Calculate the reduction potential for the $Fe^{3+}/Fe^{2+}$ electrode if the concentration of $Fe^{2+}$ is five times larger than that of $Fe^{3+}$.

From a table of standard reduction potentials, we know that

\[
Fe^{3+} + e^- \rightarrow Fe^{2+} \quad E^0 = +0.771 \, V
\]

Using the Nernst equation, we can solve the algorithm to find the answer

\[
E_{cell} = E^0 - (0.0592/n) \log Q
\]

\[
E_{cell} = +0.771 \, V - (0.0592/1) \log 5 = +0.771 \, V - 0.0592 (0.699)
\]

\[
E_{cell} = +0.771 \, V - 0.041 \, V = +0.730 \, V
\]
**Problem:** A voltaic cell is prepared by combining the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple with the $\text{MnO}_4^-/\text{Mn}^{2+}$ couple. In one compartment of the cell, $[\text{Fe}^{3+}] = 1.00 \, M$ and $[\text{Fe}^{2+}] = 0.100 \, M$. In the other compartment, $[\text{MnO}_4^-] = 1.00 \times 10^{-2} \, M$, $[\text{Mn}^{2+}] = 1.00 \times 10^{-4} \, M$, and $[\text{H}^+] = 1.00 \times 10^{-3} \, M$. The cell reaction is $\text{MnO}_4^- + 8\text{H}^+ + 5\text{Fe}^{2+} \rightarrow \text{Mn}^{2+} + 4\text{H}_2\text{O} + 5\text{Fe}^{3+}$, with $E^0 = +0.74 \, V$. What is the cell potential, $E$, for the cell?

\[
E_{\text{cell}} = E^0 - (0.0592/n) \log \frac{[\text{Mn}^{2+}][\text{Fe}^{3+}]^5}{[\text{MnO}_4^-][\text{H}^+]^8[\text{Fe}^{2+}]^5} \\
E_{\text{cell}} = +0.74 - (0.0592/5) \log (1.00 \times 10^{-4})(1.00)^5/(1.00 \times 10^{-2})(1.00 \times 10^{-3})^8(1.00 \times 10^{-1})^5 \\
E_{\text{cell}} = +0.74 - (0.0592/5) \log (1.00 \times 10^{27}) = +0.74 \, V - 0.32 \, V \\
E_{\text{cell}} = +0.42 \, V
\]

**Problem:** The nickel-lead voltaic cell undergoes the electrochemical reaction $\text{Ni} + \text{Pb}^{2+} \rightarrow \text{Ni}^{2+} + \text{Pb}$ with $E^0 = +0.124 \, V$. If the concentration of $\text{Pb}^{2+}$ in the cell is $1.00 \, M$ and the cell potential, $E$, is $0.183 \, V$, what is the concentration of $\text{Ni}^{2+}$ in the cell?

\[
E_{\text{cell}} = E^0 - (0.0592/n) \log \frac{[\text{Ni}^{2+}]}{[\text{Pb}^{2+}]} \\
0.183 = 0.124 - (0.0592/2) \log \frac{[\text{Ni}^{2+}]}{1.00} \\
0.059 = -0.0296 \log \frac{[\text{Ni}^{2+}]}{1.00} \\
0.059/(-0.0296) = -2.0 = \log \frac{[\text{Ni}^{2+}]}{1.00} \\
[\text{Ni}^{2+}]/(1.00) = 1.0 \times 10^{-2} \\
[\text{Ni}^{2+}] = 1.0 \times 10^{-2} \, M
\]

The voltage generated by an MFC (and the resulting power output) is far more complicated to predict or calculate than that of a standard voltaic cell or even a chemical fuel cell. In an MFC, the bacteria colonizing the anode chamber must grow and manufacture enzymes or structures capable of transferring electrons outside the cell (see Chapter 3 – Mediatorless MFCs). In a mixed culture, different bacteria can grow at different rates, setting different potentials. Additionally, the maximum voltages that can be generated by the MFC are based on the specific electrochemical relationships between the electron donors (substrates) and acceptors (oxidizers).

As noted previously, the open circuit voltage, OCV, measured for an MFC represents the maximum voltage that can be obtained with the system. The OCV is subject to the limitations imposed by the specific bacterial community and the inherent electrochemical restrictions defined by the electron donor and acceptors. For an MFC, as with any power source, the objective is to maximize power output and, therefore, obtain the highest current possible under the conditions of maximum voltage. The OCV is only achieved under a condition of infinite resistance. When the resistance is reduced, the voltage drops proportionately. Thus, to maximize the power production, the key is to determine the smallest possible drop in voltage as the current is increased over a specific range of current interest.

To achieve this, a polarization curve is generated to characterize current as a function of voltage (Figure 1.4). By changing the circuit external resistance (load), a new voltage is obtained, and consequently, a new current at that resistance. Therefore, to obtain a polarization curve, a series of different resistance on the circuit is used and the voltage
measured at each resistance. The current is calculated using \( I = \frac{E}{R_{\text{ext}}} \) and the voltage versus the current plotted to generate the curve. The polarization curve indicates how well the MFC maintains its voltage as a function of the current production.

It should be noted that a polarization curve is very easily generated using simple resistors that can be purchased at a retail electronics supply store, such as Radio Shack, and then connecting the resistors to the circuit of the MFC assembly.

A power density curve is then calculated from the measured voltage as \( P = \frac{E_{\text{MFC}}^2}{R_{\text{ext}}} \) or, alternatively, as \( P = I^2 R_{\text{ext}} \). MFC researchers typically use the top of the power curve to report the “maximum power” capable of being generated by an MFC.

Figure 1.4. (A) The cell potential (voltage) was plotted versus current density to give the polarization curve, and then multiplied with each other (B) to obtain the power density curve (\( P = E*I \)). Maximum power is indicated as 1.0 mWcm\(^{-2}\).
Activity 1.6 – Demonstration of a Voltaic Cell:

Option 1.6.A (simulation) – After viewing a voltaic cell animation, students will use a flash applet to simulate various metal / electrolyte combinations to create a voltaic cell with the greatest cell potential.

Option 1.6.B (wet lab) – Students will construct and evaluate the potential of several voltaic cells and compare their values to the accepted values found in a chemistry textbook.

Activity 1.6.A – The Virtual Voltaic Cell

Materials:

copies of student worksheet (downloadable from site), one PC per pair of students

Procedure:

1. If you have the capability, the tutorial can be shown to the entire class and time given for class discussion.
2. Direct students to the following website:
   www.blackgold.ab.ca/ict/Division4/Science/Div.%204/Voltaic%20Cells/Voltaic.htm
3. Upon completion of the simulation, students should complete the student worksheet included in the website.

Activity 1.6.B – Voltaic Cells Lab

Introduction

An electrochemical reaction is a chemical reaction that involves reduction and oxidation (a redox reaction). The energy released in a spontaneous redox reaction can be used to do electrical work. This is accomplished using a voltaic (or galvanic) cell, a device in which the transfer of electrons occurs through an external pathway rather than directly between reactants. A spontaneous redox reaction takes place when a strip of zinc is put into a solution containing copper sulfate. The reaction is Zn(s) + Cu^{2+}(aq) → Zn^{2+}(aq) + Cu(s). Carrying out the reaction in this way, however, will not permit the obtaining of useful electrical energy. Instead, a zinc strip is placed into a solution of zinc sulfate in one container. A copper strip is placed into a solution of copper sulfate in another container. An electrical conducting wire is connected
between the zinc and copper strips. A salt bridge containing an electrolyte capable of maintaining charge balance in each solution is placed between the two solutions. A voltmeter can be placed in the circuit to measure the potential generated in the reaction. Zinc is oxidized to \( \text{Zn}^{2+} \) in one compartment. The zinc strip is called the anode. \( \text{Cu}^{2+} \) is reduced to copper in the other compartment. The copper strip is called the cathode. The electrons lost by the zinc to form \( \text{Zn}^{2+} \) travel from the anode through the external circuit to the cathode and are picked up by \( \text{Cu}^{2+} \) ions as they form copper atoms on the electrode. A diagram of this voltaic cell is shown in the figure below:

![Diagram of voltaic cell](image)

The electrochemical reaction can be represented as two half-reactions:

\[
\begin{align*}
\text{Zn(s)} & \rightarrow \text{Zn}^{2+}(\text{aq}) + 2e^- & \text{anode} \\
\text{Cu}^{2+}(\text{aq}) + 2e^- & \rightarrow \text{Cu(s)} & \text{cathode} \\
\text{Zn(s)} + \text{Cu}^{2+}(\text{aq}) & \rightarrow \text{Zn}^{2+}(\text{aq}) + \text{Cu(s)} & \text{overall reaction}
\end{align*}
\]

If all the components of the cell are in their standard states (with \([\text{Zn}^{2+}] = 1.0 \text{ M} \) and \([\text{Cu}^{2+}] = 1.0 \text{ M}\)), the cell is called a standard cell. The voltage generated by the standard cell is called the standard cell potential (\(E^o\)).

It is convenient to have a shorthand mechanism for designating voltaic cells. The standard zinc-copper cell can be written as:

\[
\text{Zn(s)} | \text{Zn}^{2+} (1.0 \text{M}) | | \text{Cu}^{2+} (1.0 \text{M}) | \text{Cu(s)}
\]

In this designation, the anode, or oxidation half-cell, is represented on the left side and the cathode, or reduction half-cell, is represented on the right side. The electrodes in the two half-reactions are electrically connected using a salt bridge represented by two vertical lines. The
cell electrodes are represented at the two ends in this notation. A single vertical line shows the phase boundary between the solid electrode and the electrolyte solution in each half-cell.

**Pre-Lab Problem:** What is the cell potential in a Zn/Cu cell that has a \([Zn^{2+}]\) of 0.10 M in the Zn half-cell and a \([Cu^{2+}]\) of 0.010 M in the Cu half-cell?

**Materials:**
- Filter paper disks
- Dropper bottles
- Voltmeter
- Strips of the following metals: copper, lead, silver, and zinc
- 1.0 M solutions of the following salts: \(\text{CuSO}_4\), \(\text{Pb(NO}_3\)_2\), \(\text{AgNO}_3\), \(\text{ZnSO}_4\), \(\text{NaNO}_3\)
- 0.010 M solutions of the following salts: \(\text{CuSO}_4\), \(\text{Pb(NO}_3\)_2\), \(\text{AgNO}_3\)

**I. Standard Cells**

Six standard voltaic cells will be set up and the potential generated by each will be measured. However, instead of setting up the half-cells in beakers, they will be set up on a piece of filter paper. The half-cells are established by placing three drops of 1.0 M solutions containing the required metal ions at four spots around the filter paper, and then setting a piece of the appropriate metal in contact with the solution. Two drops of 1.0 M \(\text{NaNO}_3\) are placed on the center of the filter paper to serve as a salt bridge.

**Procedure:**

Obtain a disk of filter paper. Place three drops of 1.0 M \(\text{CuSO}_4\) solution at the top of the filter paper (at 12 o’clock). Put a strip of copper in contact with the \(\text{CuSO}_4\) solution. Place three drops of 1.0 M \(\text{Pb(NO}_3\)_2\) solution on the right side of the filter paper (at 3 o’clock). Put a piece of lead in contact with the \(\text{Pb(NO}_3\)_2\) solution. Transfer three drops of 1.0 M \(\text{AgNO}_3\) solution to the bottom of the filter paper (at 6 o’clock). Put a piece of silver in contact with the \(\text{AgNO}_3\) solution. Transfer three drops of 1.0 M \(\text{ZnSO}_4\) solution to the left side of the filter paper (at 9 o’clock). Put a zinc strip in contact with the \(\text{ZnSO}_4\) solution. Put two drops of 1.0 M \(\text{NaNO}_3\) in the center of the filter paper to serve as the salt bridge. The arrangement will be as represented in the following figure:
Turn the voltmeter on to the 2 volts DC range. Place the leads of the voltmeter successively on the Cu and Pb strips, Cu and Ag strips, Cu and Zn strips, Pb and Ag strips, Pb and Zn strips, and, finally, Ag and Zn strips to measure the potentials of the Cu/Pb, Cu/Ag, Cu/Zn, Pb/Ag, Pb/Zn, and Ag/Zn standard voltaic cells, respectively. If you get a negative potential, switch the leads on the metals so that you get a positive potential. The metal that you connect to the black lead of the voltmeter to get a positive reading is the anode of the cell. Record the potentials for each of the cells on the data sheet, and compare them to the literature values.

II. Nonstandard Cells

If one or more of the components of a voltaic cell is not in its standard state, the cell is not a standard cell. The potential that is expected to be generated in such a cell can be calculated from the Nernst equation, $E = E^\circ - \frac{0.0592}{n} \log Q$.

In this equation, $E^\circ$ is the standard cell potential, $E$ is the cell potential, $n$ is the number of electrons transferred in the reaction, and $Q$ is the reaction quotient.

Procedure:

Obtain a filter paper disk and put three drops of 1.0 M AgNO$_3$ at the top (at 12 o’clock). Put a piece of silver in contact with the AgNO$_3$ solution. Transfer three drops of 0.010 M CuSO$_4$ to the right side of the filter paper (at 3 o’clock). Set a strip of copper on the CuSO$_4$ solution. Place three drops of 0.010 M Pb(NO$_3$)$_2$ solution at the bottom of the filter paper (at 6 o’clock). Put a piece of lead in contact with the Pb(NO$_3$)$_2$ solution. Put two drops of 1.0 M NaNO$_3$ solution in the center of the filter paper. Press the leads of the voltmeter onto the metal strips and measure the potentials of the Ag/Cu and Ag/Pb nonstandard voltaic cells. Record the values of the potentials on the data sheet. Calculate values for the potentials expected using the Nernst equation.

III. Concentration Cell

A concentration cell is a voltaic cell in which the anode and cathode compartments are the same except that the concentrations of species present are different. For example, a silver concentration cell contains a silver electrode in contact with a silver nitrate solution of a certain concentration in one compartment, and a silver electrode in contact with a silver nitrate solution of a different concentration in the other compartment. The half-cell potential generated in each of the half-cells is given by the Nernst equation, $E = E^\circ_{\text{Ag}} - \frac{0.0592}{n} \log \left[\text{Ag}^+\right]$. 
The potential of the concentration cell is the difference between the two half-cell potentials,

\[ E_{cell} = E_{Ag}^\circ - (0.0592/1) \log [Ag^+]_{concentrated} - (E_{Ag}^\circ - (0.0592/1) \log [Ag^+]_{diluted}), \]
or

\[ E_{cell} = 0.0592 \log [Ag^+]_{concentrated}/[Ag^+]_{diluted} \]

**Procedure:**

Use a disk of filter paper, and put three drops of 1.0 M AgNO₃ on it. Place a piece of silver in contact with the 1.0 M AgNO₃ solution. Transfer three drops of 0.010 M AgNO₃ to a different spot on the filter paper. Put a piece of silver on the 0.010 M AgNO₃ solution. Add two drops of 1.0 M NaNO₃ between the other two solutions. Press the leads of the voltmeter against the two silver strips, and measure the potential of the cell. Record the value of the potential on the data sheet. Calculate the expected values of the potential from the Nernst equation.
### Activity 1.6.B – Student Data Sheet

#### I. Standard Cells

<table>
<thead>
<tr>
<th>Voltaic Cell</th>
<th>Experimental Potential (Volts)</th>
<th>Literature Value (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb(s)</td>
<td>Pb$^{2+}$(1.0 M)</td>
<td>Cu$^{2+}$(1.0 M)</td>
</tr>
<tr>
<td>Cu(s)</td>
<td>Cu$^{2+}$(1.0 M)</td>
<td>Ag$^+$ (1.0 M)</td>
</tr>
<tr>
<td>Zn(s)</td>
<td>Zn$^{2+}$(1.0 M)</td>
<td>Cu$^{2+}$(1.0 M)</td>
</tr>
<tr>
<td>Pb(s)</td>
<td>Pb$^{2+}$(1.0 M)</td>
<td>Ag$^+$ (1.0 M)</td>
</tr>
<tr>
<td>Zn(s)</td>
<td>Zn$^{2+}$(1.0 M)</td>
<td>Pb$^{2+}$(1.0 M)</td>
</tr>
<tr>
<td>Zn(s)</td>
<td>Zn$^{2+}$(1.0 M)</td>
<td>Ag$^+$ (1.0 M)</td>
</tr>
</tbody>
</table>

#### II. Nonstandard Cells

<table>
<thead>
<tr>
<th>Voltaic Cell</th>
<th>Experimental Potential (Volts)</th>
<th>Literature Value (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(s)</td>
<td>Cu$^{2+}$(0.010 M)</td>
<td>Ag$^+$ (1.0 M)</td>
</tr>
<tr>
<td>Pb(s)</td>
<td>Pb$^{2+}$(0.010 M)</td>
<td>Ag$^+$ (1.0 M)</td>
</tr>
</tbody>
</table>

#### III. Concentration Cell

<table>
<thead>
<tr>
<th>Voltaic Cell</th>
<th>Experimental Potential (Volts)</th>
<th>Literature Value (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag(s)</td>
<td>Ag$^+$ (1.0 M)</td>
<td>Ag$^+$ (0.010 M)</td>
</tr>
</tbody>
</table>

**Post-Lab Questions:**

1. Why would you expect no potential to be generated between two half-cells in this experiment until the NaNO$_3$ solution migrates to a point where it overlaps both of the half-cell solutions?

2. Discuss some possible sources of error that could account for the differences in the experimental values of the cell potentials you obtained and the literature values.
Chapter 2: How do organisms obtain energy? or Why don’t I run on batteries?

METABOLISM AS A REDOX PROCESS

Burning a fuel is an oxidative process and the formation of a fuel is a reductive process. These processes are perpetually linked. There is always a donor and an acceptor. As one substance becomes oxidized, the other substance is reduced and vice versa. The common feature between the two processes is the transfer of electrons. (In biology classes, the transfer of electrons, as it occurs in living cells, is almost always referred to as electron transport.) The substance that is being reduced accepts electrons donated by the substance that is being oxidized. Although the act of gaining an electron, which is called a reduction, may be confusing at first, the terminology starts to make more sense when we remember that electrons are negatively charged. Reduction implies becoming less and, obviously, accepting something negative can be interpreted as a decrease.

Respiration, whether carried out by humans or plants, is essentially the same process of “burning” or oxidizing organic compounds. The carbon containing compounds burned in respiration are most likely to be sugars. The combustion is more controlled (by enzymes that keep the reactions going at nondestructive temperatures), but energy is produced and carbon dioxide is liberated just as it would be if sugar were burned on a fire.

Some biological oxidations are similar to traditional combustion reactions in that they involve the direct addition of oxygen, but more often in living organisms, oxidation occurs by the removal of hydrogens. Because hydrogen is composed of a proton (i.e., a hydrogen ion, H+) and an electron, e–, the transfer of hydrogens always involves the transfer of electrons.

\[ H \rightarrow H^+ + e^- \]

In metabolism, there are often long sequences, or chains, of hydrogen (or electron) acceptors. This is the reason why burning sugar in respiration, although it eventually involves the addition of oxygen, does not liberate energy quite as dramatically as it does if sugar is burned on a fire. Instead, energy is released in a trickle rather than a flood. In addition, much of the energy is not released at all, immediately, but is conserved, as chemical energy, in “high-energy” substances, such as adenosine triphosphate or ATP.

The characteristics of ATP make it exceptionally useful as the basic energy source of all cells. The three phosphate groups of ATP are the key to its ability to store and release energy. Whenever the chemical bond between the second and third phosphate group is broken, energy is released, fueling a variety of cellular activities. The resulting adenosine diphosphate (ADP) is
identical to ATP except it has two phosphate groups instead of three. When the pathways of cellular metabolism create those small amounts of energy, a little at a time, it can be stored by adding a phosphate group to ADP molecules, producing ATP.

Activity 2.1 (Demonstration) – Fruit Snack Terminator

Students will observe the action of a strong oxidizer, KClO₃, on sugars and gain a very visual appreciation for the amount of energy that can be liberated from sugars.

Activity 2.1 – Fruit Snack Terminator

MATERIALS

One small package of fruit snacks (any variety will work), ring stand and clamp, one medium sized pyrex test tube, around 2.5 g of solid potassium chlorate (KClO₃), safety shield and goggles, Bunsen burner & striker, tongs or long forceps

PROCEDURE

Fill the test tube to a depth of about one inch with potassium chlorate. Clamp the test tube in place at an approximately 45° angle, directed away from any person. Set up the ring stand-clamp-test tube assembly behind a safety shield in front of the class. Connect the burner so that the test tube can be heated easily.

Light the burner and heat the test tube at the bottom until the solid melts (m.p. is around 350° C). Stand behind the safety shield and carefully drop one fruit snack into the test tube using tongs or forceps. A violent flame-shooting reaction ensues and lasts for about one minute.

DISCUSSION

The fruit snack is mostly sugar, which is easily oxidized by something like molten potassium chlorate. Ideally, a balanced equation would show sucrose (C₁₂H₂₂O₁₁) being converted to carbon dioxide and water while the KClO₃ becomes KCl. The actual reaction does not seem to go to total completion since there is usually a little gunky residue left behind from gelatin or agar additives.
HAZARDS

Molten KClO₃ can cause very severe burns. Exercise good safety technique while presenting this demonstration. There is also a lot of smoke produced during the oxidation, so this experiment should only be done in a room with good ventilation (be careful in rooms with smoke detectors).

THE ROLE OF GLUCOSE IN METABOLISM

We, and all other living creatures, require a continuous source of chemical energy to remain alive. This is the reason for eating: We take in highly ordered molecules that have high free energy, and eject disordered molecules with low free energy.

All nutrition is based on one molecule, glucose (C₆H₁₂O₆). Even more remarkable, all life on Earth uses the same metabolic machinery to extract free energy from glucose - not just the same overall reactions, but the same steps, the same intermediates, and the same controlling enzymes. The only difference is that not every organism uses the entire scheme.

It cannot be emphasized too strongly that this chapter is not intended to be an exercise in memorization. The focus is the pathways of energy flow that living organisms (and specifically microorganisms) use to stay alive. It is far less important that students remember how to write the conversion of one molecule into another, than for them to look at the two molecules, understand what happened between one and other, and recognize how energy was liberated. Our purpose is to indicate how a series of chemical reactions can be said to have a strategy. The specific strategy utilized specifies the direction in which electrons will travel. In the MFC, we can exploit these strategies and direct the flow of electrons (energy) for our purposes.

THE STAGES OF METABOLISM

Metabolism is always initiated by the pathway called glycolysis. Glycolysis releases only a small amount of energy. If oxygen is present, glycolysis leads to two other pathways that release a great deal more energy. If oxygen is not present, however, glycolysis is followed by different pathways.

METABOLISM WITH OXYGEN = CELLULAR RESPIRATION

In the presence of oxygen, glycolysis is followed by the Kreb’s cycle and the electron transport chain. When linked together, these pathways make up a process called cellular respiration.
Cellular respiration is the oxidation of glucose \((C_6H_{12}O_6)\) to \(CO_2\) and the reduction of oxygen to water. The summary equation for cell respiration is:

\[
C_6H_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O + \text{energy}
\]

Do not be misled by the simplicity of this equation. If cellular respiration took place in just one step, all of the energy from glucose would be released at once, and most of it would be in the form of light and heat (just like in the fruit snack demo). The key for a living cell is to control that energy. It cannot simply start a fire, but must release the explosive chemical energy in sugars a little bit at a time. The cell then needs to trap those little bits of energy by using them to produce ATP, the "molecular currency" of intracellular energy transfer.

--Questions for Review--

What is cellular respiration?

Cellular respiration is the breakdown of food molecules, such as sugars, into simpler compounds with a release of energy.

How often does cellular respiration occur in cells?

Cellular respiration occurs continuously in living cells. If cellular respiration stops, a cell dies.

Create a picture or poster that shows (a) the word equation for the cellular respiration, (b) the chemical formula, and (c) a non-linguistic representation of the process.

\[
C_6H_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O + \text{energy}
\]

\(glucose + oxygen \rightarrow carbon\ dioxide + water + energy\)

STEP 1 – GLYCOLYSIS

The first step in the overall scheme of energy extraction in most organisms is glycolysis. In the first step, one glucose molecule is degraded (essentially broken in half) to two molecules of pyruvic acid \((CH_3-CO-COOH)\), a 3-carbon compound, with the production of relatively little ATP.

Although glycolysis is an energy releasing process, the cell needs to input a small amount of energy to get the process started. At the beginning of the pathway, two molecules of ATP are used up. In a way, the process of glycolysis is analogous to a savings account at a bank. A person has to deposit money into the account to earn interest, just as a cell must put two ATP into the “account” to earn the interest of additional molecules of ATP. When glycolysis is complete, four ATP molecules have been produced from each molecule of glucose. This gives the cell a net gain of two ATP molecules.

In more critical reactions of the glycolysis pathway, four high-energy electrons are removed and then passed to an electron carrier called \(NAD^+\), or nicotinamide adenine dinucleotide. Each
molecule of NAD\(^+\) can acquire two electrons; that is, be reduced by two electrons. However, only one proton accompanies the reduction. The other proton produced as two hydrogen atoms are removed from the molecule being oxidized is liberated into the surrounding medium. For NAD\(^+\), the reaction is thus:

\[
\text{NAD}^+ + 2\text{H} \rightarrow \text{NADH} + \text{H}^+
\]

Consequently, the overall reaction that occurs during the process of glycolysis is as follows:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{NAD}^+ \rightarrow 2\text{C}_3\text{H}_4\text{O}_3 + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP}
\]

---Questions for Review---

¿ What is the source of the energy used by the cell to split the glucose molecule into pyruvate?

*Two ATP molecules provide the energy.*

¿ How many pyruvate molecules are produced from each glucose molecule?

*Two*

¿ What is added when NAD\(^+\) becomes NADH?

*Electrons*

¿ What are the end products of glycolysis?

*Pyruvate and energy*
STEPS 2 & 3 - KREB’S CYCLE AND THE ELECTRON TRANSPORT CHAIN

The second step in the machinery of cellular respiration is much more efficient in extracting energy. Pyruvic acid (or pyruvate) enters the **Kreb’s cycle**, or **citric acid cycle** (because citric acid is the product of the first reaction), where it is broken down to CO$_2$, with hydrogen atoms being used to reduce NAD$^+$ to NADH. Some additional ATP also is made along the way. The NADH from the citric acid cycle flows into the third process, the **electron transport chain**. Here NADH is reoxidized to NAD$^+$ and is recycled. The hydrogen atoms are translocated over the cell membrane from "inside" to "outside", establishing a concentration gradient across the membrane, which temporarily stores the energy released in the chemical reactions. This potential energy (the free energy that is liberated) is stored in the form of ATP. The hydrogen atoms ultimately are added to O$_2$ to make water. The overall process - the combustion of glucose with oxygen - carried out in these series of small steps so that the maximum amount of energy from the reaction can be saved – yields 38 molecules of ATP per molecule of glucose.

AEROBIC VS. ANAEROBIC METABOLISM

At this point it becomes necessary to clear up a few misconceptions and terminology errors that frequently occur. It is not uncommon for the terms **anaerobic respiration** and **fermentation** to be used interchangeably, but this is incorrect. Fermentation is the metabolic process that occurs in microorganisms (and occasionally in human muscle tissue) in the absence of oxygen. During fermentation, the pyruvate generated by glycolysis goes through a series of enzymatic transformations to form reduced metabolites, such as lactic acid or ethanol. During anaerobic respiration, glycolysis is followed by the Kreb’s cycle and the electron transport chain, just as in normal aerobic respiration, however, the final electron acceptor is a molecule other than oxygen.

**Anaerobic respiration is a key element to the understanding and the functioning of practical application** microbial fuel cells (see Chapter 2 – Anaerobic Respiration). Fermentation is a critical component of our simple experimental MFC.

Under nonstrenuous conditions, when the oxygen supply is ample, glucose is degraded to pyruvate during glycolysis, and pyruvate is broken down to CO$_2$ in the Kreb’s cycle and electrons transferred to oxygen in the respiration chain, with a yield of 38 molecules of ATP per molecule of glucose. However, during vigorous exercise where O$_2$ demand is very high in the muscles, an interesting reaction called **fermentation** is responsible for allowing our muscles to keep working hard even when oxygen starts to run low.

As the cell supplies large amounts of ATP (energy) from glycolysis to muscle cells during exercise, it runs into a problem. In just a few seconds, all of the cell’s available NAD$^+$ molecules are filled up with electrons (becoming NADH). The NADH cannot be re-oxidized back to NAD$^+$ fast enough since the oxygen, which functions as the final electron acceptor in the electron transport chain, is limited. Without NAD$^+$, the cell cannot keep glycolysis going, and ATP production stops. During fermentation in human muscle, instead of entering the Kreb’s cycle,
the pyruvate generated by glycolysis is reduced to lactate. This action converts all the NADH back into the electron carrier NAD⁺, allowing glycolysis to continue:

\[
\begin{align*}
\text{O} & \quad 2\text{CH}_3\text{C} \quad \text{COOH} + 2\text{NAD}^+ + 2\text{H}^+ \\
& \quad \text{pyruvic acid} \\
\quad \text{OH} & \quad \text{2CH}_3\text{CH} \quad \text{COOH} + 2\text{NAD}^+ \\
& \quad \text{lactic acid}
\end{align*}
\]

This is a redox reaction that consists of two half reactions:

1. \[\text{pyruvate} + 2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{lactate} \quad \text{pyruvate gains e}^- \text{ (is reduced)}\]
2. \[\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ + 2\text{H}^+ + 2\text{e}^- \quad \text{NADH loses e}^- \text{ (is oxidized)}\]

The NAD⁺ that is generated is then available to be used in glycolysis again to generate more ATP for the muscles. The lactate (lactic acid) generated by this reaction causes acidification of the blood (lowered pH), which is believed to be responsible for the "burn" that you feel in muscles that you worked too hard. Because this fermentation process does not require oxygen, it is said to be anaerobic.

**A DETAILED LOOK AT FERMENTATION**

It is possible (or even routine) for other organisms to also produce energy without using oxygen to “burn” glucose. In 1861, Louis Pasteur discovered that yeast cells are capable of converting glucose to carbon dioxide and ethyl alcohol (ethanol). This reaction yields only about 1/20 the amount of energy released when glucose is metabolized all the way to carbon dioxide and water through the use of oxygen (aerobic respiration). This is not a significant problem, however, as yeast do not have as high an energy requirement as more complex organisms. The food industry makes use of this (most delightful) reaction to produce a variety of consumable (and quite tasty) products.

**Ethanol fermentation** (performed by yeast and some types of bacteria) breaks the pyruvate down into ethanol and carbon dioxide. It is important in bread-making, brewing, and wine-making. Usually only one of the products is desired; in bread the alcohol is baked out, and in alcohol production the carbon dioxide is released into the atmosphere.
**Lactic acid fermentation** breaks down the pyruvate into lactic acid. As previously discussed, it occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. However, it also occurs in some bacteria and some fungi. It is this type of bacteria that convert lactose into lactic acid in yogurt, giving it its sour taste.

Ethanol and lactic acid are typical examples of fermentation products. However, more exotic compounds can be produced by fermentation, such as molecular hydrogen, butyric acid, and acetone. For living cells, the products produced by fermentation are actually waste products created during the reduction of pyruvate to regenerate NAD$^+$ in the absence of oxygen.

<table>
<thead>
<tr>
<th>In our experimental MFC, we will use the “waste” products of anaerobic bacterial fermentation as a source of electrons. Molecular hydrogen will be oxidized to generate electrons and protons (H$^+$) in the following reaction:</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ H_2 \rightarrow 2 \text{H}^+ + 2 \text{e}^- ]</td>
</tr>
<tr>
<td>The electrons are subsequently transported to the anode and dropped into the electrical circuit by the action of a soluble electron transport mediator called methylene blue.</td>
</tr>
</tbody>
</table>

Humans can use anaerobic respiration and fermentation in much the same way yeast do, including using the same pathways. However, unlike yeast that can excrete alcohol into the surrounding medium, our cells (if we possessed the same enzymes) would have to excrete alcohol into our bloodstream. Then we would have to eliminate it or metabolize it quickly to avoid being continuously drunk. Instead, our bodies, lacking the necessary enzymes to produce alcohol, follow an alternative pathway, which yields a substance called lactic acid. While lactic acid is less toxic than alcohol, it nonetheless cannot be tolerated at too high a concentration.

As muscle activity increases, we need more energy, so more glucose is metabolized. As we begin to use oxygen (for aerobic respiration) at a rate that exceeds what our lungs can provide, locally in the muscle cells a switch to the fermentation pathway occurs and lactic acid quickly builds up. We rapidly become fatigued and our muscles start to ache and burn as the blood pH lowers in response to the increasing concentration of lactic acid. If the production of lactic acid would continue, we would soon be unable to move at all. As we lie in a heap on the ground panting for air, our heart and lungs work especially hard to deliver as much oxygen as possible to our starved muscle cells. At this point, the lactic acid can be metabolized further by the oxygen through the remainder of the cellular respiration pathway. This means we get the full benefit of all the energy released when glucose goes to lactic acid and then to carbon dioxide and water.

To summarize, glycolysis carried out in both aerobic and anaerobic conditions produces pyruvate. The difference is that without oxygen, the pyruvate is turned into lactic acid or
ethanol, and, when oxygen is present, the pyruvate continues on (through the Kreb’s cycle and electron transport) to eventually give carbon dioxide and water.

**Activity 2.2 – Lactic Acid Sit-Out Challenge**

Find a long wall. Have the students line up against the wall and place their backs against the wall. They then slide down the wall into a sitting position, with their thighs parallel to the floor and their shins parallel to the wall. The objective of the game is to see who can maintain that position for the longest time. Soon after being in the position, the students feel the burn of the lactic acid accumulation.

Analyze the results of who had the longest wall time, and analyze what the burning feeling is and why a burning feeling became a problem. The winners get a candy bar, and then there can be more discussion about what will happen upon eating the candy bar.

---Questions for Review---

**?** Describe the role of NAD$^+$ (nicotinamide adenine dinucleotide) in the cell. Write a chemical equation for the reduction of NAD$^+$. 

*The main function of NAD$^+$ is to act as an electron carrier in the redox reactions that occur in the cell during metabolism. It is found in two forms: NAD$^+$ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced, this reaction forms NADH, which can then be used as a reducing agent to donate electrons.*

$$\text{NAD}^+ + 2H \rightarrow \text{NADH} + H^+$$

**?** Summarize what happens during fermentation, and then explain the importance of the process (especially in terms of NAD$^+$).

*During strenuous exercise (in humans) or in organism that exist under anaerobic conditions, the oxygen debt makes it impossible for the pyruvate generated by glycolysis to be fully metabolized to carbon dioxide, water, and ATP. Subsequently, all NAD$^+$ molecules in the cell become saturated with electrons and are converted to NADH. Without the aerobic cellular respiration processes of the Kreb’s cycle and electron transport to recycle NADH to NAD$^+$, glycolysis and the production of ATP would cease. Instead, under anaerobic conditions, organisms make a switch and follow-up glycolysis with fermentation. In fermentation, which does not require oxygen, the pyruvic acid is reduced to lactic acid (in humans) or ethanol (in yeasts and some bacteria), and the electrons generated are used to oxidize NADH back to NAD$^+$. Thus, NAD$^+$ is again available for glycolysis to continue.*
Activity 2.3.A – Fermentation by Yeast

During the fermentation process by yeast cells, ethanol is produced and carbon dioxide is released. The chemical formula for fermentation in yeast is:

**Chemical equation**

\[ C_6H_{12}O_6 \rightarrow 2 \text{CH}_3\text{CH}_2\text{OH} + 2 \text{CO}_2 + 2 \text{ATP} \]

**Word equation**

Sugar (glucose) \( \rightarrow \) alcohol (ethanol) + carbon dioxide + energy (ATP)

Many of the biochemical reactions in the fermentation of glucose require magnesium ions. Magnesium ions activate several enzymes involved in fermentation. To test the effect of magnesium ions on the breakdown of glucose, extra magnesium ions may be added to the reaction. Magnesium ions may be removed from the reaction solution by adding a compound containing fluoride. Fluoride causes magnesium ions to precipitate from solutions.

Students should formulate a hypothesis for the lab before conducting the experiment. *If extra magnesium ions are added to the reaction solution, then fermentation should occur at a faster rate; if removed, fermentation will slow down.* Review all safety precautions associated with this lab. Be careful when using sodium fluoride. It can be toxic if ingested or inhaled, and may irritate the skin. If any spill occurs on skin or clothes, it should be washed off immediately with large amounts of water. Develop a plan to dispose of waste materials and inform students of the plan before executing the experiment.

Organize the class into groups of four students. Work with the class to design the experimental procedures. Inform the groups that they will have available the following materials: five 50-mL beakers, grease pencils or sharpies, five fermentation tubes (Figure 2.1 or similar gas collection apparatus), six 10-mL graduated cylinders, yeast suspension*, distilled water, 10% glucose solution.
0.01 M magnesium sulfate solution, 0.06 M sodium fluoride solution, 0.20 M sodium fluoride solution, and a metric ruler.

* To prepare the yeast suspension, mix the entire contents of one fresh package of baker’s yeast in 90 mL of diH₂O and 10 mL glucose solution. Then, incubate the suspension at 37°C for 12 hours to develop the yeast colonies.

Guide the students so that each beaker contains the following materials:
- Beaker #1 - 10 mL yeast suspension, 20 mL distilled water
- Beaker #2 - 10 mL yeast suspension, 10 mL distilled water, 10 mL glucose
- Beaker #3 - 10 mL yeast suspension, 10 mL glucose, 10 mL of 0.10 M magnesium sulfate
- Beaker #4 - 10 mL yeast suspension, 10 mL glucose, 10 mL of 0.06 M sodium fluoride
- Beaker #5 - 10 mL yeast suspension, 10 mL glucose, 10 mL of 0.20 M sodium fluoride

Have students pour the contents of each beaker into a fermentation tube numbered the same as the beaker. After the groups have set up the experiment, allow it to run overnight. The next day (Day 2), write the following class data chart on the chalkboard:

<table>
<thead>
<tr>
<th>Group #1</th>
<th>Group #2</th>
<th>Group #3</th>
<th>Group #4</th>
<th>Group #5</th>
<th>Group #6</th>
<th>Group #7, etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. mL of CO₂ produced</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranking</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**Special Note:** The results for ranking are based on the expected outcome if the tubes contain the materials as listed above in each numbered beaker and transferred to the tube with the corresponding number.

On Day 2, have students measure the amount of carbon dioxide collected using a metric ruler or read the volume of carbon dioxide (in mL) produced if the fermentation tube is graduated. Have students use a rating scale for each of the tubes. Use #1 for the tube that produced the greatest average amount of carbon dioxide and #2 for the tube with the 2nd largest average amount of carbon dioxide. Continue this ranking method until the tube with no carbon dioxide produced is rated #5. The expected outcome based on the contents of the fermentation tubes as listed above is: Tube three should be #1 with the greatest amount of carbon dioxide produced, Tube two should be #2 with the 2nd greatest carbon dioxide produced, Tube four
should be #3, Tube five should be #4, and Tube one should be #5 with no carbon dioxide produced.

Assign each group a number and have a member of each group write their results in the class data chart. Have students compare their results with those of other groups using the class data chart. Look for common responses. If the class data do not agree, ask students to account for any differences. Come to an agreement on the ranking for each tube.

Another application to this lab would be to use TES-TAPE (available at most pharmacies for $3 - $5) to measure the relative amount of glucose in the tubes before and after incubation. TES-TAPE is a glucose testing strip used by diabetics to monitor the amount of blood sugar in urine, but it will also work to visually confirm that the amount of glucose in the tubes has been reduced overnight as the yeast metabolize it into CO₂.

### Activity 2.3.B (Extention) – Other Variables in Fermentation

Have students design and carry out an inquiry experiment to test a hypothesis about the effects of sweeteners (instead of, and/or in addition to, glucose) on carbon dioxide production by yeast. Students should propose a hypothesis about how different types and amounts of sweeteners will affect the amount of carbon dioxide production by yeast. Have available various sweeteners, such as table sugar, corn syrup, molasses, honey, fruit juice concentrate, saccharine, aspartame, etc. Remind students to set up a control. Have students analyze the data and draw conclusions. Discuss the outcome and results.

### ANAEROBIC RESPIRATION

Certain bacteria, which thrive in chemically-rich but nearly oxygen-free environments, are adapted to use substances other than oxygen as terminal electron acceptors during respiration. In anaerobic respiration, as the electrons from the electron donor (e.g., sugars and other organic compounds) are transported down the electron transport chain to the terminal electron acceptor, protons are translocated over the cell membrane from "inside" to "outside", establishing a concentration gradient across the membrane, which temporarily stores the energy released in the chemical reactions. This potential energy is then converted into ATP by the same enzyme used during aerobic respiration. If given a choice, oxygen is always the preferred final electron acceptor, however, other possible electron acceptors (electron sinks) for anaerobic respiration include NO₃⁻, SO₄²⁻, Fe⁴⁺, and MnO₂. Anaerobic respiration is a common occurrence in nature but is restricted to prokaryotic organisms (e.g., bacteria).

The property of specific bacterial genera (e.g., Geobacter and Shewanella) that makes the microbial fuel cell without artificial mediators, such as methylene blue, possible is their ability to perform anaerobic respiration and use an external electron receptor, as opposed to fermentation, which utilizes the internally generated electron acceptor, pyruvate. When no dissolved electron acceptors are available, these bacteria are able to transport electrons to extracellular undissolved electron acceptors, such as some metals and electrodes (i.e., anodes), and thus create an external
voltage potential between the solution and the undissolved electron acceptors (i.e., the anode). Another critical adaptation of these bacteria is that in the absence of soluble electron acceptors, they automatically colonize and grow to cover the electrode that is acting as the electron acceptor (see Chapter 3 – *Bacterial Biofilms*).
Chapter 3: What is a Microbial Fuel Cell?

PRINCIPLES OF FUEL CELLS

A fuel cell is an electrochemical device capable of the direct conversion of chemical energy into electrical energy. It produces electricity from an external fuel (on the anode side) from which electrons are withdrawn, and then transferred to an oxidant (on the cathode side). The circuit is closed by an ion or proton exchange connection between the fuel and oxidant chamber. Fuel cells are different from batteries in that they consume reactant, which must be constantly fed into the device, whereas, batteries store electrical energy chemically in a closed system. Fuel cells can operate virtually continuously as long as the necessary flows are maintained. Many combinations of fuel and oxidant are possible in a fuel cell. A hydrogen fuel cell uses hydrogen as fuel and oxygen as oxidant (Figure 3.1).

![Figure 3.1. A basic chemical (or hydrogen) fuel cell](image)

In essence, a fuel cell works by catalytically separating the component electrons and protons of the reactant fuel, and then by forcing the electrons to travel through a circuit, converting them to electrical power. The protons move through a separator (ion conductive membrane) to the cathode to maintain electroneutrality. The catalyst is typically comprised of a platinum group metal, or alloy, that has been coated onto a carbon or graphite electrode. Another catalytic process takes the electrons back in, combining them with the protons and the oxidant to form waste products (typically simple compounds like water and carbon dioxide).
The effectiveness of the electron transfer at the electrode surface, and subsequent fuel oxidation at the anode and the reduction of the oxidant at the cathode surface, are determined by the effectiveness of the catalyst. The differences in the standard electrode potentials for the anodic oxidation of the fuel and for the cathodic reduction of the electron acceptor determine the content of stored chemical energy in the fuel cell. The larger the difference between the individual electrode potentials, the more electrical energy can be generated (see Chapter 1 – Standard Reduction Potentials).

To deliver the desired amount of energy, the fuel cells can be combined in series and parallel circuits, where series yields higher voltage, and parallel allows a stronger current to be drawn (see Chapter 1 – Electrical Circuits). Such a design is called a fuel cell stack.

What is the chief difference between a battery and a fuel cell?

Batteries have a definite fuel stock, which will eventually become depleted and the battery will be “dead”. Fuel cells can run indefinitely because fuel is constantly fed into the device.

PRINCIPLES OF BIOFUEL CELLS

In a biofuel cell, electrons are made accessible from a non-electroactive fuel by the use of biocatalysts. Biofuel cells can be characterized as either enzymatic fuel cells or microbial fuel cells (MFCs), depending on the kind of biocatalyst used – enzymes or bacteria, respectively.

Enzymatic Fuel Cells

Enzymatic fuel cells usually employ immobilized enzymes (commonly redox enzymes) as catalysts to accelerate highly specific reactions. Purely enzymatic fuel cells have the great advantage of being very small in scale. Due to the small size of the enzymes and the high specificity of the anode and cathode reactions, they have high turnover rates and, thus, high power densities (and a membrane separation of the electrodes is often not necessary). Therefore, they give the possibility to construct low energy power supply units for small electrical devices as well as possible in-vivo applications (e.g., medical implantations).
Microbial Fuel Cells

Microbial fuel cells (MFCs) are as diverse chemically as the bacteria that power them. In an MFC, the oxidation reactions occur inside the bacteria, and electrons must then be transferred to the extracellular anode. Though they operate on the same principles, the design goals of the MFC are different from those of a chemical fuel cell. Rather than a non-renewable source, such as hydrogen gas, MFCs use biomass as the substrate (metabolic oxidation-reduction reactions) and microorganisms as the catalyst, exploiting whole living cells in an aim to gain energy.

Describe the oxidation and reduction reactions that occur in bacteria.

Bacteria oxidize glucose during the process of glycolysis, producing two, three-carbon molecules of pyruvate. Under aerobic conditions, the pyruvate continues through the pathways of the Kreb’s cycle and electron transport to eventually yield H₂O as molecular oxygen (O₂) is reduced. Under anaerobic conditions (no O₂), the pyruvate undergoes fermentation to yield either ethanol or lactic acid or anaerobic respiration (see Chapter 2 – Aerobic Respiration vs. Fermentation).

BIOLOGICAL PRINCIPLES OF MFCs

In terms of the chemistry, every biological degradation of organic matter is an oxidation process. However, when we keep the degradation anaerobic, we get the chance to exploit this process for electron recovery (power production). Anaerobic conversion of sugars is realized either by bacterial fermentation leading to the formation of small, reduced energy-rich metabolic products, such as ethanol, acetate, or hydrogen or by anaerobic respiration using another terminal electron acceptor instead of oxygen to take up the electrons coming from the sugar. Different MFC techniques allow us to utilize both of these anaerobic metabolic processes.

Activity 3.1 – Can a Fuel Cell Run on Coke?

Have the students read the articles found at:

http://blog.wired.com/gadgets/2007/03/like_programmer.html
http://www.slu.edu/x14605.xml
http://www.slu.edu/readstory/more/4479
http://www.slu.edu/readstory/newsinfo/2474

Have students discuss how an enzymatic fuel cell functions. After reading the articles, discuss the advantages and disadvantages of enzymatic fuel cell technology. Do you think this technology has any practical applications in the near future? Why or why not?
What must be done to ensure an anaerobic environment in the anode chamber of the MFC?

We must not allow oxygen into the chamber. (Note: Students should be informed that it will be nearly impossible to keep the chamber completely anaerobic due to the openings (ports) in the MFC bodies.) The bacteria will be grown in a sealed container, so no oxygen can get in. When the bacterial solution is added to the MFC, it must be done quickly to limit oxygen exposure.

A typical MFC consists of two separate chambers, which can be inoculated with liquid media (Figure 3.2). These chambers, an anaerobic anode chamber and an aerobic cathode chamber, are generally separated by an ion-exchange membrane. A MFC such as this can be classified into two types. One type generates electricity from the addition of artificial electron shuttles (mediators) to accomplish electron transfer from bacterial cytoplasm to the anode. The other type does not require these additions of exogenous chemicals and can be loosely defined as a mediatorless MFC. Mediatorless MFCs can be considered to have more commercial potential than MFCs that require mediators because the typical mediators are expensive. One major challenge is that, if oxygen will be used as the final electron acceptor, the cathode chamber needs to be filled with a solution and aerated to provide ample oxygen to the cathode.

![Figure 3.2. A basic microbial fuel cell](reprinted with permission of Cell Press)
ELECTRICITY GENERATION IN THE MFC

In normal microbial metabolism (see Chapter 2), a carbohydrate (glucose) is oxidized initially without the participation of oxygen when its electrons are released by enzymatic reactions. The electrons are stored as intermediates, which become reduced, and, in this state, they are used to fuel the reactions, which provide the living cell with energy for maintenance and growth. The ultimate “electron sink” (or endpoint of the redox reaction) is molecular oxygen (dioxygen, O₂). To an electrochemist, a simplified representation of the anode half-cell reaction involved in the oxidation of glucose by a whole bacterial cell would be as follows:

\[
C_6H_{12}O_6 + 6 H_2O \rightarrow 6 CO_2 + 24 H^+ + 24 e^-
\]

The large harvest of electrons is stored as reduced intermediates, but the eventual terminus the respiratory chain is oxygen, as demonstrated in the cathode half-cell reaction:

\[
6 O_2 + 24 H^+ + 24 e^- \rightarrow 12 H_2O
\]

pH EFFECTS IN THE MFC

The term pH refers to the concentration of hydrogen ions (H⁺) in a solution. An acidic environment is enriched in hydrogen ions, whereas, a basic environment is relatively depleted of hydrogen ions. The pH of biological systems is an important factor that determines which microorganisms are able to survive and operate in a particular environment, such as the anode chamber of a microbial fuel cell. Most microorganisms prefer pH values that approximate that of distilled water, a neutral solution.

The hydrogen ion concentration can be determined empirically and expressed as the pH. The pH scale ranges from 0 to 14, with 1 being the most acidic and 14 being the most basic. The pH scale is a logarithmic scale. That is, each division is different from the adjacent divisions by a factor of 10. For example, a solution that has a pH of 5 is 10 times as acidic as a solution with a pH of 6.

The range of the 14-point pH scale is enormous. Distilled water has a pH of 7 (neutral). A pH of 0 corresponds to 10 million more hydrogen ions per unit volume and is the pH of battery acid. A pH of 14 corresponds to 1 10-millionth as many hydrogen ions per unit volume, compared to distilled water, and is the pH of liquid drain cleaner.

Compounds that contribute hydrogen ions to a solution are called acids. For example, hydrochloric acid (HCl) is a strong acid. This means that the compounds dissociate easily in solution to produce the ions that comprise the compound (H⁺ and Cl⁻). The hydrogen ion is also
a proton. The more protons there are in a solution, the greater the acidity of the solution, and the lower the pH.

Mathematically, pH is calculated as the negative logarithm of the hydrogen ion concentration. For example, the hydrogen ion concentration of distilled water is $10^{-7}$ and, hence, pure water has a pH of 7.

$$\text{pH} = -\log [H^+] = -\log 10^{-7} = 7$$

In the MFC, maintaining the pH of microbial anode chamber and the anode feed solution is important to ensure that growth of the target microbes occurs. Also, pH needs to be monitored as the anode and cathode reactions are occurring. If the pH varies too widely, the growth and metabolism of the microorganism can be halted. This inhibition is due to a numbers of reasons, such as the change in shape of proteins due to the presence of more hydrogen ions. If the altered protein ceases to perform a vital function, the normal functioning and even the survival of the microorganism can be threatened.

Ion exchange membranes pose an important challenge in pH maintenance in the MFC. Ion exchange membranes separate the biological anode from the cathode reactions, while, at the same time, facilitating the transport of ions through the membrane to maintain electroneutrality in the system and proper bacterial respiration. Ion transfer between the anode and cathode is necessary because of the movement of negatively charged electrons from the anode to the cathode. To achieve a counterbalance, either negative charge equivalents (anions/hydroxide ions) travel from the cathode to the anode, or positive charge equivalents (cations/protons) move from the anode to the cathode, depending on the selection of the ion-exchange membrane material.

Since the cathode reactions of MFCs consume protons in equal amounts as electrons, ideally only protons are transported through the ion exchange membrane. In this way, electroneutrality is observed without pH changes taking place at the cathode. However, because MFCs operate near neutral pH in the anode and cathode chambers, the concentration of cations other than protons (e.g., $\text{Na}^+$, $\text{K}^+$, $\text{NH}_4^+$) are typically $10^5$ times higher than $\text{H}^+$ ions in solution. This competitive transport of cations other than protons significantly effects MFC performance.

When the substrate is degraded (metabolized), protons are produced at the anode and consumed at the cathode. However, if due to competition and concentrations gradients, protons cannot migrate at a sufficient rate from the anode to the cathode, the pH will decrease at the anode and increase at the cathode while charge balance is maintained by the migration of other cations. The pH decrease at the anode affects bacterial respiration and, thus, current generation. Utilizing well-buffered solutions in the MFC can offset these pH changes, but the addition of chemical buffer solutions is not sustainable and it is not clear to what extent localized pH changes in the MFC may affect power generation.
At this point, it may be necessary to explain how certain bacteria can transfer electrons directly to an electrode and why, in some instances, mediators are necessary. Since electrodes are solid entities that cannot penetrate the bacterial cells, a major requirement is that electrons are to be transferred from the inside of the microbial cell membrane to its outside – either via the physical transfer of reduced compounds, or via electron hopping across the membrane using membrane-bound redox enzymes. An analogy of eating a cookie can be used to explore how the process works. When we eat a cookie, the sugars in the cookie are acting as electron donors as they are oxidized. The electron acceptor, then, is oxygen. Students should understand that the source of oxygen is the air around them as they respire. As they breathe in oxygen, it is available to be used as an electron acceptor. It is more difficult, though, to use the same analogy for bacteria that are transmitting electrons directly to an electrode. The electron donor is again sugar (glucose), but how can an electrode be “breathed in”? How can the electrons being generated by the reactions inside the cell be transported across cell membranes to the electrode? These questions will be answered to an extent in the following sections, but it should be noted that this is a budding area of MFC research and not all of the mechanisms of direct electron transfer to the electrode are known or fully understood.

MEDIATORLESS MFCs

(1) The use of bacterial biofilms

Bacterial biofilm MFCs are based on the direct physical and electronic interaction between the microorganisms and the anode surface (Figure 3.3). A biofilm is a community of microorganisms adhering to a surface. In the case of MFC technology, the surface is the anode (i.e., electron-accepting electrode - see Chapter 2 - Anaerobic Respiration). There are currently three main theories to which most MFC researchers subscribe: (1) The bacteria adhering to the anode have electrochemically active redox enzymes on their outer membranes that contain iron molecules. The iron molecules orient themselves in such a way as to traverse the outer membrane of the bacterium, allowing the direct transfer of electrons to external materials and, therefore, do not require any chemical assistance to accomplish electron transfer to the electrode; (2) Some evidence suggests that electrons may be transferred directly to the electrode via conductive, hair-like protein appendages (or pili) found on the surface of certain bacterial genera; and (3) Certain bacterial species can synthesize micromolar amounts of their own redox mediators that can then be used by a whole range of bacteria in a mixed culture.

The methodology notwithstanding, when these bacteria oxidize the organic matter present in the substrate, the electrons are shuttled to the anode. Oxygen, the hydrogen protons, and the electrons that are connected by a circuit from the anode to the cathode, are then catalytically combined with (routinely) a platinum catalyst to form water (Figure 3.3). (Platinum is a critical
catalyst at the cathode and no alternative metal has been proven to catalyze the combination of oxygen, the hydrogen proton, and the electron in a more efficient manner.)

In MFC research, microbial biofilms are quite common, however the start-up phase of such biofilm-based MFC anodes normally takes days or weeks and, therefore, cannot be realized in short-term investigations (but may be suitable for a long-term school project).

![Diagram of a biofilm anode](image)

**Figure 3.3. Bacteria fixed in a biofilm at an MFC anode**

(2) **Direct oxidation of secondary fuels at the anode**

A second, highly promising MFC approach is based on the preparation and investigation of electrocatalytic anodes, capable of an efficient and direct oxidation of bacterial end products under the diverse and complex microbial growth conditions (see Chapter 2 – *Aerobic Respiration vs. Fermentation*). Again, platinum can be used as a catalyst for the utilization of molecular hydrogen by fermentative microorganisms at the anode. However, at this stage, the low stability and high costs of platinum prevent large-scale applications.
Figure 3.4. Transformation of a primary substrate into a secondary fuel for direct oxidation at the anode (Pt or other catalyst required)

Platinum-coated carbon electrodes could be used as both the anode and cathode in our MFC (Figure 3.4). At the anode, platinum would function as an excellent electron acceptor during the oxidation of molecular hydrogen, \( \text{H}_2 \), present as a byproduct of the initial anaerobic processing of glucose. In the cathode, platinum is capable of donating electrons during oxygen reduction to produce water. There are several reasons we have chosen not to use platinum electrodes in our experimental MFC. (1) Platinum electrodes are very easily poisoned by the products of microbial metabolism if they are not coated with a protective polymer prior to use in the MFC. Sulfides, carbon monoxide, and, to a lesser extent, even carbon dioxide present in the anode chamber can cause the molecules to bind tightly to the surface of the platinum electrode, rendering it useless for electron transport. The platinum and polymer coating is quite expensive and the preparation of the electrodes is a very detailed process requiring specific equipment and computer software that does not lend itself to simple application in a high school setting. (2) The reduction of oxygen at a platinum-coated cathode, though not unworkable, does further complicate the setup of the MFC. An aquarium pump (or similar apparatus) must be used to provide a constant source of oxygen into the cathode chamber. This can be accomplished by attaching a thin needle to the tubing of the pump and then inserting the needle into the top port of the cathode chamber.
THE ROLE OF MEDIATORS

Direct electron transfer from most bacteria to an electrode is hampered by overpotentials, which can be described as transfer resistances. To reduce these resistances, the surface area of the electrodes needs to be increased, and/or redox mediators need to be added to the solution. A redox mediator is a compound that can be reversibly oxidized or reduced. Bacteria can use redox mediators to deposit their electrons onto an electron acceptor they cannot directly reduce (Figure 3.5).

In the absence of oxygen, electrons may be diverted from the respiratory chain by a redox mediator, which enters the outer cell membrane, becomes reduced, and leaves again in the reduced state. The reduced mediator then shuttles the “stolen” electrons to the anode. To complete the circuit, a second (oxidizing) electrode (the cathode) is required, again functioning as the electron sink. The oxidizing material can again be oxygen gas, but is more convenient for school purposes to use a simple soluble oxidizing agent, such as potassium ferricyanide.

In early MFC research, chemical redox-mediators (often dyes, such as methylene blue or neutral red) were used to shuttle metabolic energy (in the form of electrons) from the cytoplasm of bacteria to an anode. Today, these expensive and toxic dyes no longer play a role for the development of practical MFCs, mainly because in continuous systems, these mediators would have to be added and recycled permanently. However, these mediator-assisted MFCs are still a very good academic device to study electron transfer processes of microorganisms.

![Diagram of a MFC using methylene blue mediator](image)

**Figure 3.5.** Our experimental MFC design using a methylene blue redox mediator in the anode and ferricyanide in the cathode
Activity 3.2 – Methylene Blue Dye Reduction Test

Students will conduct a simple experiment to show that anaerobically active bacteria collected from the soil have the capability of reducing methylene blue.

Activity 3.2 – Methylene Blue Dye Reduction Test

INTRODUCTION

Though it has lost popularity with the advent of new technologies, the methylene blue dye reduction test remains a fairly accurate indicator of the bacterial content of milk. The test is based on the fact that the color imparted to milk by the addition of a dye, such as methylene blue will disappear more or less quickly depending on the number of bacterial cells present. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the color to disappear. The agents responsible for the oxygen consumption are the bacteria. Though certain species of bacteria have considerably more influence than others, it is generally assumed that the greater the number of bacteria in milk, the quicker the oxygen will be consumed, and in turn, the sooner the blue color will disappear. Thus, the time of reduction is taken as a measure of the number of organisms in milk.

The dairy industry classifies (grades) the milk based on bacterial content:

- Class 1. Excellent, not decolorized in 8 hours.
- Class 2. Good, decolorized in less than 8 hours, but not less than 6 hours.
- Class 3. Fair, decolorized in less than 6 hours, but not less than 2 hours.
- Class 4. Poor, decolorized in less than 2 hours.

In this activity, we will use the methylene blue reduction test to qualitatively assess our anaerobic bacterial cultures’ (which will later be used in our experimental MFC) ability to reduce methylene blue. This will visually confirm that methylene blue, when introduced into the anode chamber of our MFC, will be capable of mediating the transfer of electrons from inside of the bacterial cell membrane to the anode.
MATERIALS

Fertile soil (free of chemicals), anaerobic bacterial growth medium, 10 mM methylene blue solution, solution bottles with caps, glass Petri dish, oven, 15 mL conical tubes, 30°C (optimal growth temperature for Clostridium) water bath apparatus or incubator, pipettes, pipettors and tips

PROCEDURE

Preparations to be done at least one day in advance of experiment:

• Preparation of required solutions
  o Anaerobic bacterial growth medium
    For 1 L of diH₂O add:
    - 2.0 g NH₄HCO₃
    - 3.6 g KH₂PO₄
    - 0.1 g MgSO₄ x 7 H₂O
    - 0.01 g NaCl
    - 0.01 g Na₂MoO₄ x 2 H₂O
    - 0.01 g CaCl₂ x 2 H₂O
    - 0.015 g MnSO₄ x 7 H₂O
    - 0.00278 g FeCl₂
    - 2.0 g yeast extract
    - 5.0 g glucose

    Adjust pH to 6.5 ± 0.5 and autoclave (alternatively, for school lab, use freshly boiled, cooled down water for preparation – to maintain sterility until use, do not let water stand open.).

    Note: Soil culture must be grown overnight (or longer) to be active during experiments!

  o Methylene Blue (synthetic electron mediator)
    - Prepare a 10 mM solution (0.032 g / 10 mL)

• Preparation of anaerobic soil culture
  1. Put some fertile soil in a glass Petri dish and heat uncovered in an oven at approximately 120°C for one hour.
  2. Fill a tightly sealable bottle with bacterial growth media (about 150 mL) and add a small amount (about 1 tsp) of heat pre-treated soil.
  3. Grow the bacterial culture overnight at 30°C (or longer at room temperature). The solution should turn cloudy and start to foam. Caution: If growing longer than one day, build-up of gas pressure could cause bottle to rupture!
Note: We are working with an environmental sample of microorganisms, some of which may also be pathogenic. Thus, use gloves when handling the microbial solution and wash your hands carefully when you are finished. Clean up spills with bleach and decontaminate solution with bleach at the end of your experiment.

Day of experiment:

1. Measure increasing amounts of methylene blue into a series of 15 mL conical tubes:

   Tube 1 – no methylene blue, MB (control)
   Tube 2 – 0.25 mL MB
   Tube 3 – 0.50 mL MB
   Tube 4 – 1.0 mL MB
   Tube 5 – 2.0 mL MB
   Tube 6 – 1.0 mL MB + H$_2$O (negative control)

2. Add 12 – 13 mL (fill tube completely) of bacterial suspension to each tube 1 – 5 and 12 mL water to tube 6; tightly screw on cap. Gently invert tube once to ensure mixing of bacteria with the methylene blue. Do not shake tubes! Shaking introduces oxygen into the anaerobic cultures.

3. Immediately place tubes in 30°C incubator or water bath. Record this time as the beginning of the incubation period. Cover tubes to keep out light.

4. Check samples for decolorization after 15 minutes of incubation. Make subsequent readings at 30 minute intervals thereafter. Decolorization is determined by comparison of the the methylene blue containing tubes to the control (no MB).

5. After each reading, remove decolorized tubes and then slowly make one complete inversion of remaining tubes. Again, no shaking.

6. Record reduction (decolorization) time in minutes for each sample. For example, if the sample was still blue after 15 minutes but was decolorized at the 45-minute reading, the reduction time should be recorded as the average time between the two observations, or 30 minutes.

Factors affecting the test. Many factors affect the methylene blue reduction test, and therefore the steps of operation should be uniform.

Since the oxygen content must be used up before the color disappears, any manipulation that increases the oxygen affects the test. The tubes should be completely filled with the bacterial solution (even to the point of overflowing). Do not shake the tubes, and mix only by gentle inversion.

Light hastens reduction, and therefore the tests should be kept covered. The initial concentration of the dye (10 mM) should be uniform as an increased concentration lengthens
the time of reduction. Increasing the incubation temperature augments the activity of the bacteria, and therefore shortens the reduction time.

Over time the bacteria will settle to the bottom of the tube. This factor causes variations in the reduction time, since the bacteria are not evenly distributed. The accuracy of the test is increased, reduction time shortened, and decolorization more uniform if the samples are periodically inverted during incubation.

❓ How does the methylene blue reduction test show that our soil culture bacteria are “active”?

If the bacteria are capable of decolorizing the methylene blue, it means they are actively undergoing anaerobic metabolism. The bacteria are oxidizing the glucose in the nutrient broth and generating electrons. When the bacterial cells take up the methylene blue into their outer cell membrane, those electrons are used for reducing the dye to the colorless form of the methylene blue molecule.

❓ How can the methylene blue reduction test show that we have an anaerobic bacterial culture capable of generating electricity in the MFC?

The reduction of the methylene blue we are witnessing is exactly the same reaction the bacteria will be carrying out in the anode compartment of our MFC. Only in the MFC, the electrons generated by bacterial metabolism do not stay with the methylene blue molecules. Molecules of methylene blue are back-oxidized at the anode and the electrons are subsequently dropped into the electrical circuit of the MFC.

ADDITIONAL DEMONSTRATION

At the conclusion of the dye reduction test, pool all of the decolorized tubes into a larger solution bottle (large enough that the bottle is less than half full). By swirling the contents of the bottle, oxygen trapped in container will become dissolved in the bacterial solution and will back-oxidize the methylene blue, returning it to the blue (oxidized form). This is a great visual demonstration to use with the students so they can understand why the methylene blue retains its color in the MFC. In the MFC; the methylene blue is only an electron carrier and does not remain in the reduced (colorless) form. As the methylene blue is reduced by electrons generated by fermentation, the electrons are subsequently dropped into (donated to) the electrical circuit at the anode.
ELECTRON FLOW IN THE MFC

Remember that during glycolysis, glucose is broken down into carbon dioxide in an oxidation reaction (see Chapter 2 – Step 1 – Glycolysis). Oxidation reactions are always characterized by a loss of electrons.

\[
C_6H_{12}O_6 + 6 H_2O \rightarrow 6 CO_2 + 24 H^+ + 24 e^- 
\]

The electrons released have to be deposited in some sort of electron sink and expelled as waste. If the cell could not get rid of its electrons, it would build up a huge negative charge. Frequently, due to its availability, oxygen functions as the electron sink. A molecule of oxygen takes up electrons and combines with protons (H\(^+\)) to make water.

\[
6 O_2 + 24 H^+ + 24 e^- \rightarrow 12 H_2O 
\]

The electron sink, however, is not limited to oxygen. During anaerobic respiration, the final electron acceptor could be any molecule capable of accepting electrons provided the proper enzymatic pathway is available. So, how do we know in which direction (and to what molecules) the electrons will flow in our MFC? The answer is related to the reduction potential of the various electron carriers involved in the metabolic pathways of the bacteria and the mediators we have introduced into our MFC.

The table below gives the apparent biological standard reduction potentials (E\(^\circ\) at pH 7, instead of E\(^\circ\) at standard conditions, see Chapter I – Standard Reduction potentials) of some important biological half-reactions. Electrons flow spontaneously from the more readily oxidized substance (the one with the more negative reduction potential) to the more readily reduced substance (the one with the more positive reduction potential). Therefore, more negative potentials are assigned to reactions that have a greater tendency to donate electrons (i.e., reactions that tend to oxidize most easily).

<table>
<thead>
<tr>
<th>Standard Reduction Potentials of Interest in MFCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half Cell Reactions</strong></td>
</tr>
<tr>
<td>2H(^+) + 2e(^-) → H(_2)</td>
</tr>
<tr>
<td>NAD(^+) + 2H(^+) + 2e(^-) → NADH + H(^+)</td>
</tr>
<tr>
<td>Acetaldehyde + 2H(^+) + 2e(^-) → Ethanol</td>
</tr>
<tr>
<td>Pyruvate + 2H(^+) + 2e(^-) → Lactate</td>
</tr>
<tr>
<td>MB(<em>{ox}) + 2H(^+) + 2e(^-) → MB(</em>{red})</td>
</tr>
<tr>
<td>Fe(CN)(_6)(^{3-}) + e(^-) → Fe(CN)(_6)(^{4-})</td>
</tr>
<tr>
<td>NO(_3)(^-) + 2e(^-) + 2H(^+) → NO(_2)(^-) + H(_2)O</td>
</tr>
<tr>
<td>MnO(_2) + 4e(^-) + 4H(^+) → Mn(^{2+}) + 2H(_2)O</td>
</tr>
<tr>
<td>Fe(^{3+}) + e(^-) → Fe(^{2+})</td>
</tr>
<tr>
<td>(\frac{1}{2}) O(_2) + 2H(^+) + 2e(^-) → H(_2)O</td>
</tr>
</tbody>
</table>
It is important to note the direction of all these reactions is always written in the form of a reduction or gain of electrons. That's not important when it comes to determining the direction of electron flow. For example, note that the reduction of protons to hydrogen is at the top of the list \( E^0 = -0.43 \text{ V} \). Electrons released by the oxidation of molecular hydrogen will flow to any half reaction that has a higher (less negative) standard reduction potential. In this case, the electrons end up in NADH \( E^0 = -0.32 \text{ V} \). Notice the reduction of oxygen is way down at the bottom of the list. That's why it's an effective electron sink for getting rid of electrons. Other natural electron sinks include nitrate \((\text{NO}_3^-)\), iron \((\text{Fe}^{3+})\), and manganese \((\text{MnO}_2)\) (see Chapter 2 – Anaerobic Respiration).

In our experimental MFC, fermentative bacteria will convert the glucose-rich media into mainly butyrate, acetate, and molecular hydrogen. When this bacterial solution, in its redox state, is filled into the anode chamber together with the methylene blue synthetic redox mediator, the mediator itself will be reduced by the bacterial cells, as well as by molecular hydrogen. If the electric circuit to the cathode is closed (that means both electrodes are connected by a wire), the reduced mediator is reversibly oxidized at the anode and its electrons are dropped into the electric circuit. By making methylene blue, which has a higher standard reduction potential, available to the bacteria, we can divert the electrons away from the normal pathways of ethanol or lactate fermentation. The electrons then flow through the closed circuit from anode to cathode due, again, to the differences in electrical potential. The potassium ferricyanide introduced into the cathode chamber can then act as the electron sink as the ferricyanide ion is reduced to the ferrocyanide ion. Again, oxygen (at a platinum coated electrode) could be used as the final electron acceptor in our experimental MFC, but an apparatus for the continuous delivery of oxygen into the cathode chamber (aquarium pump) is necessary.

What effect would using oxygen as the final electron acceptor at the cathode have on the electricity generating abilities of the MFC?

Using oxygen would (theoretically) result in more power production in the MFC. Since the oxygen half-reaction has a more positive reduction potential, the potential difference between the oxidation and reduction half reactions would be greater. A larger potential difference means larger voltage across the circuit (see Chapter 1 – Reduction Potentials).

Activity 3.3 – The MFC in Action

Students will construct the experimental MFC, load the anode and cathode chambers with the appropriate oxidant and reductant, and harvest the electricity generated to power an LED.

Note: Students frequently have difficulty assembling the MFC. Some time should be given prior to the day of experimentation for practice. A good idea may be to review the step-by-step procedure one day prior to the actual date of experimentation, and have the students assemble a “dry” MFC using the Figures 3.6, 3.7, 3.8, and 3.9 provided as guides.
Activity 3.3 – The Microbial Fuel Cell in Action

MATERIALS

The experiment will be conducted in a modified model MFC cell commercially available at the NCBE, University of Reading, UK (http://www.ncbe.reading.ac.uk/).

The model MFC sets contain the acrylic MFC bodies, screws to assemble them, rubber gaskets, and ion exchange membrane and carbon cloth material for the preparation of electrodes.

Note: To set up the MFC as described in the following protocol, two complete MFC kits are needed. Assembling the MFC with a single anode and cathode chamber will demonstrate cell potential, but it is not significant enough to light the LED. If the desired outcome is to allow students the opportunity to power the LED, two anode chambers must be assembled (Figure 3.6 and Figure 3.7).

Figure 3.6. The 3-chambered MFC in explosion view
Preparations done in advance of session:

- Presoak the ion exchange membrane in distilled water for 24 hours prior to use.

- All electrode preparation and modification
  - Carbon cloth cut to right size for cell compartments
  - Pieces of carbon cloth glued to graphite rods* for external connection of electrodes using conductive carbon cement**
  - For the preparation of electrodes in the high school (no graphite rods or conductive cement needed), the carbon cloth could be cut in such a way that a thin strip of cloth can be led through the connection hole in the acrylic body, providing an external connection (Figure 3.7).

![Figure 3.7. The assembled MFC](image)

* Graphite rods are high carbon content pencil rods (very soft) purchased from the art supply section of the campus book store.


- Preparation of required solutions
  - Anolyte – bacterial growth medium
    For 1 L of diH₂O add:
    - 2.0 g NH₄HCO₃
    - 3.6 g KH₂PO₄
    - 0.1 g MgSO₄ x 7 H₂O
    - 0.01 g NaCl
- 0.01 g Na₂MoO₄ x 2 H₂O
- 0.01 g CaCl₂ x 2 H₂O
- 0.015 g MnSO₄ x 7 H₂O
- 0.00278 g FeCl₂
- 2.0 g yeast extract
- 5.0 g glucose

Adjust pH to 6.5 ± 0.5 and autoclave (Alternatively, for school lab, use freshly boiled, cooled-down water for preparation – to maintain sterility until use, do not let water stand open.).

**Note:** Soil culture must be grown overnight (24 – 36 hours) to be active during experiments! Bacteria tend to slow metabolism (and their resulting electroactivity) after 48 hours as glucose is depleted.

- Methylene Blue (synthetic electron mediator)
  - Prepare a 10 mM solution (0.032 g / 10 mL)

- Catholyte – ferricyanide:
  - Prepare a 100 mM solution of potassium ferricyanide (8.23 g / 250 mL)

  **CAUTION:** Potassium ferricyanide is hazardous and should not come in contact with the eyes. Eye protection is necessary when handling this material. If the solution does come in contact with the eyes, flood them with water and seek medical attention. Do not let Potassium ferricyanide react with strong acids (poison formation). Follow disposal guidelines at the end of this protocol.

**Additional materials needed:**

- Two – 30 mL syringes for delivery of solutions into anode and cathode chambers (Figure 3.9)
- Glassware for preparing and storing solutions
- Tube connectors and matching tubes to connect syringes to MFC cells
- Fertile soil (without chemical fertilizers) as a source of anaerobic bacteria
- Glass Petri dish for the heat pre-treatment of soil
- Handheld multimeters for the observation of cell potential and current
- Large tray to prevent spills of solutions
- “Real” loads, e.g. LED light or small fans
- Alligator clips and copper wire for making connections between chambers and to LED
- Gloves and safety goggles
PROCEDURE

**At least one day prior to investigation:**

1. Put some fertile soil in a glass Petri dish or clay crucible and heat uncovered in an oven at approximately 120°C for one hour.
2. Fill a tightly sealable bottle with bacterial growth media (about 150 mL) and add a small amount (about 1 tsp) of heat pre-treated soil.
3. Grow the bacterial culture overnight at 30°C (or longer at room temperature). The solution should turn cloudy and start to foam. *Caution: If growing longer than one day, build-up of gas pressure could cause bottle to rupture!*

**Day of investigation:**

1. Assemble the MFC cells according to the explosion Figure 3.8 – we will create three compartments (anode/cathode/anode). Separate the chambers with ion exchange membrane and use gaskets on both sides of the membrane to prevent leaking. *Several fuel cells may be joined together to give greater voltage.*

![Figure 3.8. The 3-chambered MFC (top view)]
2. Attach the tubing via tube connectors to the influent port (side, bottom) of the MFC chambers.
3. Using alligator clips and copper wire, connect the two anodes in parallel (so that the two chambers become electrically one).
4. Connect the handheld multimeter in parallel to the MFC to investigate the development of cell potential over time. Attach one lead to the cathode and one lead to either anode.
5. Add 0.3 mL (300 μL) of methylene blue to each anode chamber through the top effluent port.
6. Fill the two 30 mL syringes with catholyte and anolyte solution (as fast as possible to limit aeration) and connect them to the respective tubings at the influent ports (Figure 3.9).

Note: To eliminate spacing issues, the influent ports for both anode chambers should be one and the same side, and the influent port for the cathode chamber should be on the opposite side. Additionally, both anode chambers should be connected by tubing and a T-shaped connector so they can be filled simultaneously from the same syringe.

7. Fill the cathode cell first (limits the exposure to oxygen in the anode chambers) followed by both anode cells (from the same syringe) taking care not to overfill them.
8. Observe the cell potential on the multimeter and if it is significant (>450 mV), directly connect the MFC to the LED in short circuit.
9. The LED will flash for a short time until the potential drops below the required voltage necessary to power the LED.
10. Disconnect the LED, wait for recovery of the cell potential, and try it again.

Disposal and clean-up:

- To store the MFC set-ups and keep the electrodes reusable and active, it is necessary to clean and disinfect the whole cell and especially the electrodes intensively with ethanol after each experiment.
- After adding a small amount of bleach and a short incubation, spent bacterial broth solutions can be drained down the sink.
- Cathodic ferricyanide solution is hazardous and may produce toxic fumes in contact with strong acids! It may be used several times and gets lighter to colorless if the electron acceptor is spent. Local regulations should be observed when disposing of the used solution.
DISCUSSION

The heat pre-treatment of the soil killed all living cells and just the spores of bacteria strains survived. Fermentative Clostridium species represent a large group of these bacteria in soil. If the soil is inoculated and incubated anaerobically in a rich bacterial medium, the spores of only the anaerobic bacteria germinate and form a mixed bacterial culture. As they grow overnight, the fermentative Clostridium convert the glucose in the nutrient media mainly into butyrate, acetate, and molecular hydrogen. When this bacterial solution is introduced into the anode chamber, the methylene blue mediator is reduced by the bacterial cells as well as by molecular hydrogen. When the electrical circuit is closed, the methylene blue is back-oxidized at the anode by dropping its electrons into the electrical circuit. The electrons flow through the circuit to the cathode chamber where the ferricyanide acts as the final electron acceptor. If the potential difference between the reduced anode side and the ferricyanide on the cathode side is large enough, we can power the LED.
An alternative protocol that also may assist with students understanding of the functional MFC would be to add methylene blue to the growing bacterial solution prior to the day of investigation, as opposed to adding it directly into the anode chamber of the MFC during the actual investigation. As the bacteria grow and metabolize, they will reduce the methylene blue to its colorless form. When the bacterial solution (plus methylene blue) is then introduced into the MFC, the methylene blue would become blue again when it is back oxidized at the anode. The students would then have a visual representation (a color change) of the electrons being mediated to the anode by the methylene blue.

FURTHER INVESTIGATIONS

If time allows, a bacterial biofilm could be developed within the anode chamber to compare the function of the methylene blue mediated MFC to a mediatorless MFC.

• Prepare the anodic feed solution
  o Anolyte – bacterial feed solution for biofilm MFC
    For 1 L of diH₂O add:
    - 0.8 g sucrose
    - 3 g KH₂PO₄
    - 0.1 g yeast extract
    - 0.033 g NH₄Cl
    - 0.06 g K₂SO₄
    - 0.033 g FeCl₂ x 4 H₂O
    - 0.011 g iron (III) citrate
    - 0.5 g NaCl
    - 0.1 g KCl
    - 0.1 g CaCl₂
    - 0.1 g MgCl₂ x 6 H₂O

    Adjust pH to 6.5 ± 0.5 and autoclave (alternatively, for school lab, use freshly boiled, cooled-down water for preparation – to maintain sterility until use, do not let water stand open.).

• Assemble the MFC kit as in the previous experiment, but in addition, seal the hole around the external anode connections (influent and effluent ports) to prevent the leaking of liquid and oxygenation of the anode solution. (Tip: Aquarium silicone sealant works well.) Also, tubing should be connected (and sealed) to the top (effluent) port to allow the anode solution to be flushed out daily. To maintain anaerobic conditions in the anode chamber, be sure the tubing in the top (effluent) port remains closed (by clamp) except during the time of solution replacement.
• Mix very different samples of anaerobic soils, sludges, and water from stagnant puddles to get an anaerobic bacterial inoculum. Inject some of the inoculum into the anode chamber of the MFC (through influent port).

• As in the previous experiment, fill the two syringes with anolyte and catholyte solution, connect them to their respective tubings, and fill both cells with solution (through influent port).

• Add fresh anode solution once per day. By initially connecting (and sealing) tubing to the top (effluent) port of the anode chambers, (the spent solution can easily be forced out as fresh solution is added daily).

• Connect the multimeter in parallel to the MFC as before and periodically measure cell potential. If significant cell potential develops, connect the MFC to an external resistor (e.g., 100 Ohms, available at Radio Shack or similar electronics store). Wait until a stable potential is formed at this resistance (could take days or weeks). If the potential is stable over time, the resistor can be disconnected for 24 hours to “collect” potential between the two poles. If the potential goes over 0.45 V, the LED device will flash. It is also possible to perform a polarization test with a series of different external resistors as it was described in Chapter 1, page 37.

The anaerobic bacteria from the sludge mixture will grow in the anode compartment by utilizing the offered sucrose (sugar) in the feed solution. If the anodic chamber is kept air/oxygen free, some of the bacteria will begin anaerobic respiration by choosing the anode as the terminal electron acceptor (see Chapter 2 – Anaerobic Respiration). A biofilm will then be slowly established at the anode that delivers electrons into the electrical circuit.

MFC protocol for school experiments was adapted from Bioscience Explained by D. Madden and J. Schollar (http://www.bioscience-explained.org/ENvol1_1/pdf/FulcellEN.pdf)
Chapter 4: So Now What? – MFC Challenges and Applications

TECHNICAL CHALLENGES

System architecture. The idea of using bacteria in MFCs to capture electricity has been around for some time, but in early systems, power production was very low and required the addition of extracellular mediators to shuttle electrons from inside to outside of the bacterial cell. In newer systems (e.g., mediatorless MFCs that employ biofilms); mediators are not needed, and power production from MFCs has increased dramatically in just the past few years, in part because of designs that lower internal resistance (see Chapter 3 – Mediatorless MFCs).

Resistance can be described as anything that opposes the flow of electric current. Often the system components of the MFC themselves are regarded as the principle (internal) resistors (e.g., transfer resistance between electron mediator and anode or electrolyte and cathode). For example, just a 0.1 Ohm resistance occurring at a current of 2 amps can yield a 0.2 V drop in the electrical potential of the MFC (see Chapter 1 – Electrical Circuits). The theoretical power (or EMF) generated by MFCs is a function of the individual electrode potentials when the circuit is open, but the actual power generation of the MFC is often less than what is theoretically possible.

The anode potential is set by the respiratory enzymes of the bacteria and does not appear to vary substantially in different systems or with different substrates (fuels). On the other hand, the cathode potential varies depending on the catholyte and oxidant. Experimentally, lower than expected voltages are usually obtained when oxygen is used as the final electron acceptor. With ferricyanide as the final electron acceptor instead of oxygen, less internal resistance and higher cell voltages have been documented, however, power generation with ferricyanide is not sustainable – the ferricyanide must be externally regenerated over time.

With oxygen as the electron acceptor at the cathode, the main technical challenge in improving power generation is to create a system architecture that minimizes the internal resistance of the MFC but, at the same time, allows for continuous flow through the system. One possible remedy could be the use of an air cathode. Exposing the cathode directly to air (an air cathode), instead of to dissolved air (oxygen) in water, has resulted in substantial increases in system performance (Figure 4.1). Clearly, though, maximizing power generation in MFCs will require innovative flow patterns and increased electrode – electrolyte interactions that minimize internal resistance. Additionally, finding methods to increase the cathode potential, with oxygen as the electron acceptor, could have a substantial impact on power generation.

Materials. The cost of materials used to construct MFCs will also be a key factor for the successful application of the technology on a larger scale. Very large surface areas are needed
for supporting bacterial biofilms, and the structure must be able to bear the weight of the water and biofilm. Electrode materials range from carbon cloth and carbon paper, to graphite rods, plates, and granules. Cathodes are made from the same materials, but they also contain precious metals, such as platinum, when oxygen is used as the electron acceptor.

Some materials are not expected to be suitable for scale-up because of their inherent lack of durability or structural strength (e.g., carbon paper), or cost (e.g., platinum catalysts, ion exchange membranes). Future designers will need to consider conductive coatings on structurally strong supporting materials. System scale-up will also require that the design and application of these materials be adaptable to mass-manufacturing approaches.

**Microbiology.** Our understanding of electrochemically active microbes is still in its infancy, but clearly a whole new field of microbial ecology is emerging that is based on anodophilic bacteria (bacteria that will adhere to an anode) and possible interspecies electron transfer. These bacteria may be referred to as *electricigens* based on their ability to exocellularly release electrons. The initial understanding of electron transfer by bacteria to electrodes came from studies of metal-reducing bacteria, such as *Geobacter* and *Shewanella* species, which can produce electricity in MFCs. Biochemical and genetic characterizations indicated that for some bacterial species, outer-membrane *cytochromes* (membrane-bound proteins containing iron that carry out electron transport) can be involved in extracellular electron transfer. Also, some bacteria produce and use soluble electron shuttles that eliminate the need for direct contact between the bacterial cell and the electron acceptor (Figure 4.1).

The recent discovery of nanowires introduces a whole new dimension to the study of extracellular electron transfer. These conductive, pilli-like structures identified, thus far, in certain *Geobacter* species appear to be directly involved in extracellular electron transfer (see Chapter 3 – *Mediatorless MFCs*). These nanowire structures allow the direct reduction of a distant electron acceptor. This removes the need for soluble mediators that would be lost in a continuous-flow MFC and may allow for direct interspecies electron transfers.
Glucose serves as an example fuel. a | An indirect microbial fuel cell. A fermentative microorganism converts glucose to an end product, hydrogen, which can react with the anode to produce electrons and protons. This process only partially recovers the electrons available in the organic fuel as electricity, and results in the accumulation of organic products in the anode chamber. b | A mediator-driven microbial fuel cell. An electron-shuttling mediator accepts electrons from reduced cell constituents and transfers the electrons to the anode. The reoxidized mediator can then undergo repeated cycles of reduction and oxidation. In most instances, the cells that have been used in such fuel cells only incompletely oxidize their organic fuels as shown. c | The oxidation of glucose to carbon dioxide with direct electron transfer to the electrode surface. Glucose is taken into the cell and oxidized to carbon dioxide by typical metabolic pathways. Electrons derived from glucose oxidation are transferred across the inner membrane and outer membrane through electron transport proteins, such as iron-containing cytochromes. In this example, the system is illustrated with an air cathode rather than a cathode submerged in water. d | A two-chambered microbial fuel cell. This system is not optimized for maximum power production but is convenient for microbiological studies.

Figure 4.1. Summary of methods for electron transfer to the anode
(with permission from the Nature Publishing Group, License #2342510598123)
Activity 4.1 – Electricigens: Microbial Energizers

Students should read the article found at:


After reading, have students answer the questions that follow. This reading assignment will also provide some background information on current MFC applications and technology that will assist with the poster project.

¿ What are electricigens?

*Microorganisms with the ability to oxidize organic compounds to carbon dioxide while transferring electrons to electrodes with high efficiency.*

¿ How are the actions of electricigens similar to the functioning of a traditional hydrogen fuel cell?

*Both are capable of converting a fuel into electricity without losing substantial amounts of energy as heat. A hydrogen fuel cell oxidizes hydrogen and reduces oxygen to water while producing electricity. Electricigens utilize their own cellular metabolic pathways to oxidize sugars and reduce oxygen (or another final electron acceptor).*

¿ What are the differences between a hydrogen fuel cell and MFCs that employ electricigens?

*Hydrogen fuel cells require a very pure source of a highly explosive gas that is difficult to store and distribute, and hydrogen is derived mainly from fossil fuel rather than renewable sources. In contrast, the energy sources for microbial fuel cells that utilize electricigens are renewable organics, which are stable and very cheap. In terms of power production, MFCs will never be able to compete (in terms of electricity production) with the traditional fuel cell because the biomass fuel of the MFC will always be in lower concentrations than what is fed into a hydrogen fuel cell. Thus, the power capable of being generated by MFCs will always be orders of magnitude lower than hydrogen fuel cells.*

Activity 4.2 – MFC Applications Poster

Individual students or student groups will research the various applications of MFC technology currently in use and then present their findings in a poster session.
Activity 4.2 – MFC Applications Poster

MATERIALS

Internet / college library access, poster board, markers, colored pencils

PROCEDURE

Individually or in cooperative groups the students should research the applications of MFC (or BES) technology that are currently in use or that have been proposed. Have students perform some initial research into possible applications, or alternatively, present the information on applications that follow this activity during class lecture. Then, each student (or group) should select their topic. After a pre-determined period of time, the students should present their findings to the class.

Have the students focus their research to answering these questions:

- What is the specific application of the MFC, or what does the MFC power or produce?
- Where (location) is this technology in use?
- How long has it been used and how successful is the venture?
- Based on our study of the MFC, describe how the MFC is being used in this technology?
  For example:
  - Is it mediatorless or is a mediator used?
  - What reactions are occurring at the anode and cathode of the MFC?

A suggested rubric for grading the poster is provided at the end of this chapter.
WASTEWATER AND OTHER APPLICATIONS

When a new technology is introduced into the marketplace, the greatest likelihood for success occurs when the most immediately profitable application is targeted first. As the technology develops, becomes better understood, and improves, more difficult applications can be undertaken. The most immediate and useful applications of MFCs appear to be for wastewater treatment and other, similar niche applications. Renewable energy production is a longer-term prospect that will require substantial technical and manufacturing advances. But research advances during the last three years also showed promising other applications, besides microbiologically induced power generation, as in the case of an MFC. To name all new technologies that are based on the same functional principles, but can be used for different applications, the term Bioelectrochemical System (BES) is getting promoted.

Wastewater treatment. Worldwide, more than 2 billion people do not have adequate sanitation, in large part because of a lack of start-up capital as well as operating costs. In the U.S., approximately $25 billion is spent annually for water and wastewater treatment. Over the next 20 years, water and wastewater infrastructure demands will require over $2 trillion for building, maintaining, and operating these systems. In the U.S., close to 4% of the total electricity produced is used for the operation of the whole water and wastewater infrastructure. A treatment system based on an MFC provides a great opportunity to develop the technology because the substrate is “free” and wastewater must be treated.

Current methods for wastewater treatment utilize a filtration/straining procedure to remove large objects from primary wastewater and then employ an aerobic treatment of secondary wastewater to degrade the biological content of the sewage. Large amounts of oxygen are pumped into secondary wastewater, at an immense economical and electrical cost, creating what the industry refers to as activated sludge. Essentially, bacteria undergo aerobic respiration and metabolize the waste components of the sewage, using it as a food source.

MFCs could be used in a treatment system as a replacement for the existing energy-demanding aerobic treatment; however, we do not yet know how to economically scale up an MFC or what the costs would be to replace a conventional system with an MFC-based design. Scale-up and materials issues are the greatest challenges in the application of MFCs for wastewater treatment, but potentially, energy recovery at wastewater treatment plants could lead not only to a sustainable system based on energy requirements (an energy neutral wastewater treatment facility), but possibly to the production of a net excess of energy.

Environmental sensors. Data on the natural environment can be helpful in understanding and modeling ecosystem responses, but sensors distributed in the natural environment require power for operation. MFCs can possibly be used to power such devices, particularly in river and deep-water environments where it is difficult to routinely access the system to replace batteries. Sediment fuel cells are being developed to monitor environmental systems, such as creeks, rivers, and oceans. Power for these devices can be provided by organic matter in the sediments. Power densities are low in sediment fuel cells because of both the low organic
matter concentrations and their high intrinsic internal resistance; however, the low power density can be offset by energy storage systems that release data in bursts to central sensors (Figure 4.2).

![Image of sediment microbial fuel cell](https://example.com/image)

**Figure 4.2.** A sediment microbial fuel cell for use as an environmental sensor  
(with permission from the Nature Publishing Group, License #2342510598123)

**a** | A schematic of a sediment microbial fuel cell. Organisms in the family *Geobacter* can oxidize acetate and other fermentation products, and transfer the electrons to graphite electrodes in the sediment. These electrons flow to the cathode in the overlying aerobic water where they react with oxygen.  
**b** | An actual sediment fuel cell before deployment.

**Bioremediation.** An MFC can be modified in interesting and useful ways, and this can lead to new types of fuel cell-based technologies. With such modifications, however, these systems may no longer be true fuel cells because they do not produce electricity. One such application is the modification of the basic two-electrode system for bioremediation. The MFC is not used to produce electricity; instead, power can be put into the system to drive desired reactions to remove or degrade dangerous or toxic chemicals to safer or useful forms.

**Renewable electricity production from biomass.** Because of uncertainty about the materials needed and their costs, combined with comparatively low costs for oil, the application of MFCs for renewable energy production from crops, such as corn, is not likely in the immediate future. In the near term, MFCs will have to compete with more mature renewable-energy technologies, such as wind and solar power. The operating costs needed for electricity production with MFCs will probably be too great if the substrate for the MFC is grown as a crop in a manner similar to that for ethanol production from corn.
**Microbial electrolysis cells (MEC).** In addition to generating electrical energy, MFCs could also be used in a BES to generate industrial chemicals from waste products with external input of small amounts of energy. MECs are very versatile systems in which at least one of the anodic or cathodic oxidation-reduction reactions are microbially catalyzed. They are capable of generating products, such as hydrogen, methane, and ethanol. Likewise, they have the potential to catalytically degrade less desired reactants and contaminants into more economically profitable or ecologically sound products.

**BESs** have also been used successfully to remove sulfur components, promote denitrification, and reduce perchlorates and chlorinated organic compounds.

**EDUCATION**

Some of the most immediate and useful applications of MFCs are in the classroom: Students find electricity generation by bacteria both fascinating and fun! MFCs have been found to be an effective educational tool to capture student interest. These small and portable systems serve as a wonderful platform for motivating students to study and understand complex concepts of cell respiration, microbial ecology, electrochemistry, and materials science. Additionally, processes that can couple sustainable energy production with waste treatment have innate appeal to environmentally minded students. The MFC is a model system for science instruction that dissolves disciplinary boundaries and shows how technology can help solve significant social and environmental issues. Direct evidence for the appeal of this technology can be seen through recent science-fair projects on MFCs by students in middle and high schools around the world. At university level, the MFC has been used in undergraduate and graduate laboratory courses in environmental microbiology to teach students methods of microbial-community analysis.
# Rubric for MFC Applications Poster

<table>
<thead>
<tr>
<th>Criteria</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td><strong>Organization</strong></td>
<td>• Cluttered, no definitive sections, all over the place</td>
<td>• No headings, but sectioned</td>
<td>• Headings present but unclear</td>
<td>• Defined sections</td>
</tr>
<tr>
<td></td>
<td>• Hard to follow, requires assistance</td>
<td>• Must reread for clarity</td>
<td>• Some evidence of refinement</td>
<td>• Clear headings</td>
</tr>
<tr>
<td></td>
<td>• Obvious refinement required</td>
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<td>• Flows nicely to assist the reader without help</td>
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<td>• Finished product</td>
</tr>
<tr>
<td><strong>Creativity</strong></td>
<td>• Bland, no variability</td>
<td>• Very little use of color or pictures but enough to engage and hold attention</td>
<td>• Some use of color, diagrams, etc.</td>
<td>• Interesting, engaging, visually stimulating</td>
</tr>
<tr>
<td></td>
<td>• No use of color or diagrams</td>
<td></td>
<td>• Will engage but will not stimulate</td>
<td>• Aesthetically appealing use of color, diagrams and text</td>
</tr>
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<td></td>
<td>• Boring to look at, does not catch your attention</td>
<td></td>
<td></td>
<td>• Interest, motivation, effort and time obviously present</td>
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<tr>
<td></td>
<td>• Interest, motivation, effort and time obviously absent</td>
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<tr>
<td><strong>Content and Literacy</strong></td>
<td>• No analysis of topic</td>
<td>• Poor explanations</td>
<td>• Adequate explanations</td>
<td>• Concept fully and properly explained</td>
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<tr>
<td></td>
<td>• No explanations</td>
<td>• Inaccurate connections to MFC unit</td>
<td>• MFC unit connections present but could be developed further</td>
<td>• Insight present</td>
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<tr>
<td></td>
<td>• No specific connections to MFC unit</td>
<td>• Misinterprets the technology</td>
<td>• More than one resource present</td>
<td>• MFC unit specific connections made</td>
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<tr>
<td></td>
<td>• No use of resources</td>
<td>• One resource for sure</td>
<td></td>
<td>• Content is accurate, comprehensive and well supported</td>
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<tr>
<td><strong>Level and Difficulty of Understanding</strong></td>
<td><strong>(Depth of Thought)</strong></td>
<td></td>
<td></td>
<td>• Excellent use of resources</td>
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<tr>
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<td>• Difficulty (depth of thought) not suitable for grade level/not related to class discussions (too easy)</td>
<td>• Explanation describes minimal level of validity</td>
<td>• Difficulty could be increased or developed</td>
<td>• Difficulty appropriate for grade level</td>
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<tr>
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<td>• Superficial/irrelevant</td>
<td>• Needs serious refinement</td>
<td>• Some level of understanding shown</td>
<td>• Understanding present and apparent</td>
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