

# Biology for Science Majors



**Lab 10**

**Cell Respiration**





## AP BIOLOGY

### Lab 5: Cellular Respiration

#### Concepts covered

- ✓ Respirometers
- ✓ Metabolism
- ✓ Glycolysis
- ✓ Respiration
- ✓ Anaerobic vs. aerobic respiration
- ✓ Fermentation

**ATP** is the energy currency of the cell. It is produced through a process called **respiration**. Cell respiration refers to the process of converting the chemical energy of organic molecules into a form immediately usable by organisms.

All organisms, including plants and animals, oxidize glucose for energy. Often, this energy is used to convert **ADP**, and a free phosphate molecule, into **ATP**. The energy molecules (ATP), generated through respiration, are available to fuel the processes of the cell as needed. When ATP levels become too low a special protein signals the cell to begin respiration. As long as all the critical components for the reaction are available, this cycle provides a constant source of energy for the cell.

Respiration harvests biological energy from fuel molecules, such as carbohydrates, and stores it as ATP. Together with oxygen, the cell converts carbohydrates to carbon dioxide, water, and most importantly, energy. Respiration is a controlled, multi-step process which slowly releases the energy stored in glucose and converts it to ATP. If all of this energy from glucose were released at once, most would be lost as heat and light.

Carbohydrates contain high energy bonds that, when broken, release electrons. The first stage, **glycolysis**, breaks carbohydrates (glucose) into **pyruvate** molecules. Though the bonds holding pyruvate together contain a great deal of potential energy, this step yields little energy. Glycolysis occurs with or without oxygen and takes place in the cytoplasm outside the mitochondria.

Interestingly, it is a pathway found in all living things.



Yeast has been used to make leavened bread for centuries. When yeast undergoes fermentation, CO<sub>2</sub> is trapped between gluten and causes the bread to rise. Ethanol, another byproduct of yeast fermentation, generates the alcohol content in beer, and the CO<sub>2</sub> provides effervescence. What ingredients must be present in order for this process to occur?

The second stage, called an oxidation reaction, is always coupled with a **reduction** reaction. Both involve the manipulation of electrons. As shown in Figure 5.1, the process depends on if it takes place in the presence of oxygen (**aerobic respiration**) or without oxygen (**anaerobic respiration**).



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**Aerobic respiration** takes place in the mitochondria (a specialized organelle) of the cell and uses oxygen as the final electron transporter. Pyruvate is oxidized to generate energy. The key to efficiency in this step, is the use of oxygen to shuttle electrons. However, if the electrons released were transferred directly to oxygen, it would combust. To avoid this, special molecules shuttle electrons to the ATP production site. Since oxygen has a very high affinity for electrons, aerobic respiration is the most efficient means of producing ATP (36 per reaction).

**Anaerobic respiration** takes place in the cytoplasm of the cell and uses other, less efficient, molecules to transport electrons. If the final transfer molecule is organic (contains a C), the process is called **fermentation**. Fermentation is an anaerobic process that uses enzymes to reduce pyruvate into energy-rich molecules. Because it cannot fully break down the glucose molecule, fermentation is far less efficient than aerobic respiration, generating only two ATP molecules.

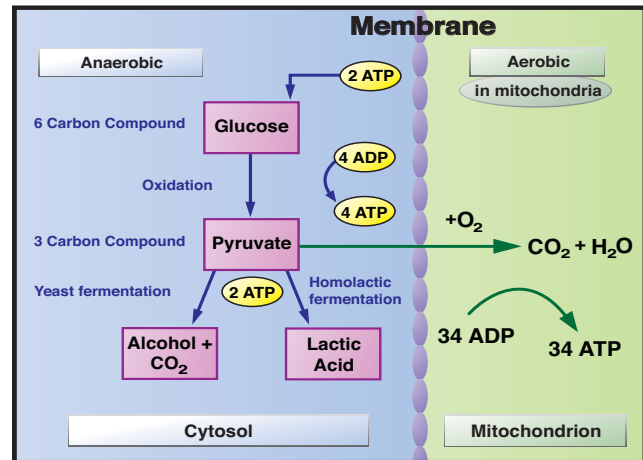


Figure 5.1

Scientists use a respirometer to measure the rate of respiration. This device capitalizes on the ideal gas law for its function. The ideal gas law applies to a gas in which all collisions between atoms or molecules are perfectly elastic and no intermolecular forces exist. The internal energy is completely kinetic, and any change in energy will be accompanied by a change in temperature, volume, and pressure. The relationship is defined by the ideal gas law equation:

$$PV = nRT$$

where P is the absolute pressure, V is the volume, R is the universal gas constant (8.3145 J/mol °K), and T is the absolute temperature (°Kelvin). Respirometers typically consist of a device to absorb carbon dioxide produced through respiration and measure oxygen uptake by displacement of fluid in a tube connected to the sealed chamber. Since the carbon dioxide is absorbed, air will be taken in through the tube (displacing the fluid) to maintain equal pressure within the chamber.

During physical activity, cells require more energy. As long as enough oxygen can be delivered to cells, aerobic respiration dominates. When energy consumption exceeds the oxygen supply, anaerobic respiration starts. Lactic acid is a byproduct, and is what causes muscle soreness after a hard workout!

## Definitions

**Aerobic respiration** – the oxidation of molecules to produce energy in the presence of oxygen

**Anaerobic respiration** – the oxidation of molecules to produce energy in the absence of oxygen

**ATP** – Adenosine triphosphate; the main energy source of the cell



**Fermentation** – respiration under anaerobic conditions in which there is no external electron acceptor

**Glycolysis** – the initial process of carbohydrate catabolism that produces ATP and pyruvate

**Oxidation** – the loss of electrons

**Oxidation state** – the degree of oxidation of an atom

**Pyruvate** – (pyruvic acid); a three carbon molecule generated through the oxidation of glucose through glycolysis

**Reduction** – the gain of electrons

## Pre-laboratory Questions

1. Why is cellular respiration necessary for living organisms?
2. Why is fermentation less effective than respiration?
3. How is this topic important to the brewing and baking industries?
4. Why does deep breathing continue after strenuous exercise?
5. What is the purpose of glycolysis?

## Experiment 5.1: Fermentation by Yeast

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Yeast cells produce ethanol and CO<sub>2</sub> during fermentation. We will measure the production of CO<sub>2</sub> to determine the rate of anaerobic respiration in the presence of different carbohydrates with the use of a simplified respirometer.

*Note: Sucrose (a disaccharide) is made up of glucose and fructose. Glucose is a monosaccharide.*

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Define for this experiment:

Hypothesis:



Control(s):

Independent Variable(s):

Dependent Variable(s):

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## Materials

5 Respirometers: (two test tubes that fit into each other – small plastic and large glass)

1% Glucose solution

1% Sucrose solution

1g Packets of Equal™, Splenda™, and sugar

Yeast

(4) 250mL Beakers

\*Warm water

3 Plastic pipettes

\*Watch or timer

Permanent marker

Ruler

\*You must provide

## Procedure

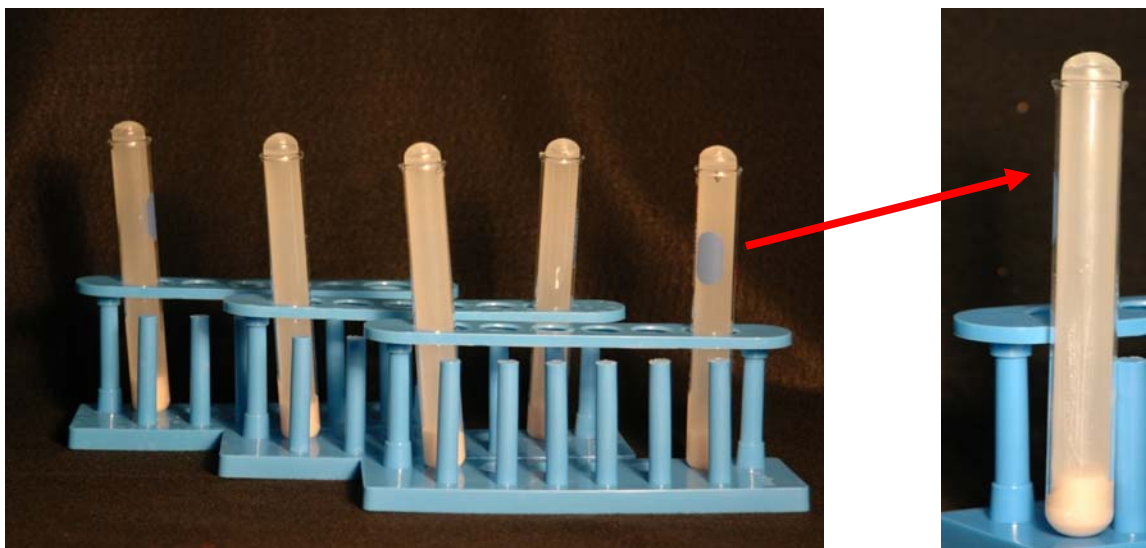
1. Label three 250ml beakers, Equal™, Splenda™, and sugar.
2. Empty the packets into the coordinating beakers, and fill to the 100ml mark with water in the Equal™ and Splenda™ beakers; add 200ml water to the beaker for the sugar solution. Mix thoroughly by pipetting. Each is now a 1% solution.
3. Completely fill the smallest tube with water and invert the larger tube over it. Push the small tube up (into the larger tube) until the top connects with the bottom of the inverted tube. Invert the respirometer so that the larger tube is upright (there should be a small bubble at the top of the internal tube).
4. Repeat this several times as practice – strive for the smallest bubble possible. When you feel comfortable with this technique, empty the test tube and continue with this experiment.
5. Mix 1teaspoon yeast into 175mL of warm (40-45°C) water in a 250mL beaker. Stir until dissolved.  
*Make sure the yeast solution is stirred before each test tube is filled.*
6. Label both the big and small test tubes 1-5.
7. Fill the smaller tubes with 15ml solution as follows:  
Tube 1: 1% glucose solution  
Tube 2: 1% sucrose solution  
Tube 3: 1% Equal™ solution



Tube 4: 1% Splenda™ solution  
 Tube 5: 1% sugar solution

*Note: Rinse the graduated cylinder between each use.*

8. Then, fill each tube to the top with the yeast solution.
9. Slide the corresponding larger tube over the small tube and invert it as practiced. This will mix the yeast and sugar solutions.



**Figure 5.2**

10. Place respirometers in the test tube rack, and measure the initial air space in the rounded bottom of the internal tube. Record these values in the Table 5.1.
11. Allow the test tubes to sit in a warm place (~37°C) for one hour. Placement suggestions include: a sunny windowsill, atop a warm oven, or under a very bright (warm) light.
12. At the end of the respiration period, measure the air space in the internal tubes, and record it in Table 5.1.

**Table 5.1**

Tube	Initial gas height (mm)	Final gas height (mm)	Net Change
1			
2			
3			
4			
5			



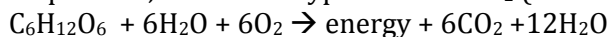
## Questions

1. Did you notice a difference in the rate of respiration between the various sugars? Did the “fake” sugar provide a good starting material for fermentation?
  
2. How do you know that anaerobic fermentation was occurring?

## Experiment 5.2: Aerobic Respiration in Soybeans

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We will evaluate respiration in beans by comparing carbon dioxide production between germinated and non-germinated beans. As shown in the balanced equation for cellular respiration, one of the byproducts is CO<sub>2</sub> (carbon dioxide):



We will use a carbon dioxide indicator to show carbon dioxide is being released by the beans. Bromothymol blue is an indicator that turns yellow in acidic conditions, green when it is neutral and blue when it is in basic conditions.

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Define for this experiment:

Hypothesis:

Control(s):

Independent Variable(s):

Dependent Variable(s):

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## Materials

200 Beans  
(5) 250 ml Beakers  
\*Paper towels  
5 Measuring cups  
Disposable pipette  
Bromothymol blue solution  
Parafilm





5 Rubber bands

\*Water

\*You must provide

## Procedure

1. Commence germination by soaking 150 beans for 24 hours in 200ml water.
2. Label five beakers: beaker 1, beaker 2, beaker 3, beaker 4, and beaker 5.
3. Place several layers of moist paper towels at the bottom of each of the 250ml beakers.
4. Place 50 germinating beans into beakers 1, 2, and 3. Place 50 non-germinating beans in beaker 4, and zero beans in beaker 5.
5. Dispense 4ml of bromothymol blue solution into each of the measuring cups, and place the measuring cup inside each beaker (Figure 5.3).
6. Stretch parafilm across the top of each beaker. Secure with a rubber band to create an air-tight seal.
7. Place beaker 1 in an ice water bath; beaker 2 in a hot water bath; and place the remaining beakers on a shelf or table, and let sit undisturbed at room temperature.
8. Observe the jars at 30 minute intervals for three hours, and record any color change of the bromothymol blue in Table 5.2 below. Be sure to maintain the water bath temperatures throughout the experiment.



Figure 5.3

Table 5.2

Time	Beaker 1	Beaker 2	Beaker 3	Beaker 4	Beaker 5
0 min					
30 min					
60 min					
90 min					
120 min					
150 min					
180 min					



## Questions

1. How did the color of the bromothymol blue solution in each beaker change over time in each condition?
2. What can be inferred from the color change of the bromothymol blue solution?
3. What is the mechanism driving the bromothymol blue solution color change?
4. What are the controls in this experiment, and what variables do they eliminate? Why is it important to have a control for this experiment?
5. What else could you incorporate into this experiment to verify that the gas is responsible for the color change? Design an experiment that shows the steps required.