Biological Processes

Lab 4

Respiration

Lab 4: Respiration

Concepts covered

- ✓ **Cellular Energy**
- ✓ **Respiration**
- ✓ **Anaerobic Respiration** (does not require oxygen)
- ✓ **Aerobic Respiration** (requires oxygen)

ATP is the energy currency of the cell. It is produced through a process called **respiration**.

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 energy. As shown in the equation below, respiration is a controlled, multistep 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 of respiration, **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.

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

glucose oxygen carbon dioxide water

Yeast has been used to make leavened bread for centuries. When yeast undergoes fermentation, CO_2 is trapped and causes the bread to rise. Ethanol, another byproduct of yeast fermentation, generates the alcohol content in beer, and the CO_2 provides effervescence. What ingredients must be present in order for this process to occur?

The second stage, called an **oxidation** reaction or the Krebs Cycle, is always coupled with a **reduction** reaction. Both involve the manipulation of electrons. The process used at this point in respiration depends on the presence of oxygen (**aerobic respiration**) or without oxygen (**anaerobic respiration**).

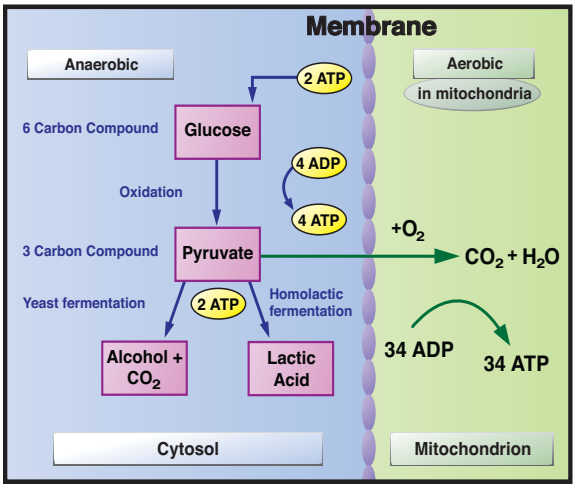

Figure 4.1: Aerobic Respiration

Aerobic respiration takes place in the mitochondria (a specialized organelle) of the cell and uses oxygen as the final electron transporter. In this process, 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 carbon atom), 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.

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!

Experiment 4.1: Fermentation in Yeast

Yeast cells produce ethanol and CO_2 during fermentation. We will measure the production of CO_2 to determine the rate of anaerobic respiration in the presence of different carbohydrates.

We will be using two different types of sugars in this lab:

- *Sucrose* (a disaccharide) is made up of glucose and fructose
- *Glucose* is a monosaccharide

Define for this experiment:

Hypothesis:

Control(s):

Independent Variable(s):

Dependent Variable(s):

Materials

5 Respirometers: (two test tubes that fit into each other) – small plastic and large glass
1% solutions of glucose, sucrose
Equal, Splenda and Sugar Packets
Yeast
(4) 250ml beaker
*Warm water
Pipettes
*Watch or timer
Permanent marker
Ruler
Measuring Spoon
*You must provide

Procedure

1. 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 (Figure 4.2). Invert the respirometer so that the larger tube is upright (there should be a small bubble at the top of the internal tube). 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.

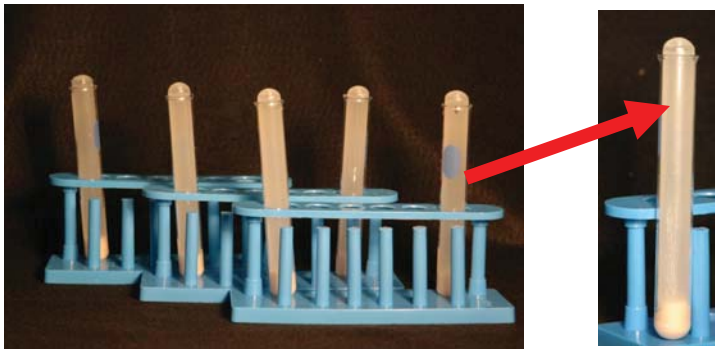

Figure 4.2: Respirometer

2. Mix 1/4 teaspoon of yeast into 175mL of warm (40-43°C) water in a 250mL beaker. Stir until dissolved.

Note: Make sure the yeast solution is stirred before each test tube is filled.

3. Label both the big and small test tubes 1-5 with a marker.

4. In a 250ml beaker, mix the packet of Equal with 100ml of water. In another 250ml beaker, mix the packet of Splenda with 100ml of water. In another 250ml beaker, mix the packet of sugar with 200ml of water. These are now 1% solutions.

5. Fill the smaller tubes with 15mLsolution 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: Make sure you rinse the graduated cylinder between each use.

6. Then, fill each tube to the top with the yeast solution.

7. Slide the corresponding larger tube over the small tube and invert it as practiced. This will mix the yeast and sugar solutions.

8. 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 4.1.

9. Allow the test tubes to sit in a warm place (~37˚C) for one hour. This may be a sunny windowsill, atop a warm oven, under a very bright (warm) light, etc.

10. At the end of the respiration period, measure the air space in the internal tubes, and record it in Table 4.1.

Table 4.1: Recording Respirometer Values

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 4.2: Aerobic Respiration in Soybeans

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 by-products of respiration is CO_2 (carbon dioxide):

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

We will use a carbon dioxide indicator (bromothymol blue) to show oxygen is being consumed and 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.

Define for this experiment:

Hypothesis:

Control(s):

Independent Variable(s):

Dependent Variable(s):

Materials

100 beans
(3) 250ml beakers
*Paper towels
3 measuring cups
Pipette
Bromothymol blue solution
Parafilm
3 Rubber bands
*Water

*You must provide

Figure 4.3: Beaker Set up

Procedure

1. Soak 50 beans for 24 hours in 200ml water.
2. Label three beakers: Beaker 1, Beaker 2, and Beaker 3.
3. Place several layers of moist paper towels at the bottom of the 250ml beakers.
4. Place 50 pre-soaked beans into Beaker 1, 50 control (dry) beans in Beaker 2, and zero beans in Beaker 3.
5. Dispense 4ml of bromothymol blue solution into the bottom of each measuring cup, and place one measuring cup inside each beaker on top of the beans.
6. Stretch the Parafilm across the top of each beaker. Secure with a rubber band to create an air-tight seal (Figure 4.3).

7. Place the beakers on a shelf or table, and let sit undisturbed at room temperature.
8. Observe the jars at 10 minute intervals for three hours, and record any color change of the bromothymol blue in Table 4.2.

Table 4.2: Color changes of the bromothymol blue over time

Time	Jar with pre-soaked beans	Jar with un-soaked beans	Jar with no beans
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?

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?