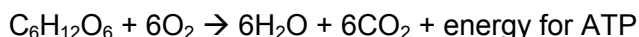


## Aerobic Cell Respiration and Fermentation

The processes that release energy from fuel molecules for the formation of **ATP** are known as **cell respiration**. The pathways of cell respiration are catabolic, and involve a series of oxidation-reduction reactions. Both autotrophs and heterotrophs must do cell respiration and the metabolic pathways of cell respiration are the same in virtually all eukaryotes.

Some of the energy released during cell respiration is heat energy; the rest is used to synthesize ATP. Many organic molecules can be oxidized in cell respiration, including fats and proteins, but glucose is the most common fuel molecule, and the one from which we obtain most energy.

During a complete aerobic glucose metabolism (which is needed to sustain life for most organisms) glucose is oxidized into water and carbon dioxide. This process requires oxygen.



Although most eukaryotic organisms are **aerobic** (oxygen requiring), the metabolic pathways of cell respiration are variable, depending on the type of organism, the enzymes the organism has, and what the **final electron acceptor** in the cell respiration pathway is. Electrons removed from glucose move down an electron transport system to a final electron acceptor. In aerobic cellular respiration, the final electron acceptor is **oxygen**, hence, the emphasis on oxygen in cell respiration. Aerobic respiration involves **glycolysis**, which occurs in the cytoplasm, and the **Krebs cycle** and **electron transport**, which occur in mitochondria.

Not all cell respiration is aerobic. Fuel molecules can be oxidized without oxygen to yield smaller amounts of ATP. The **fermentations** involve the partial breakdown of glucose without using oxygen. Fermentations include glycolysis, but not the oxidative reactions that occur in the mitochondria. Many prokaryotes have a variety of fermentation pathways, using a variety of different fuel molecules. Most eukaryotes have a fermentation pathway, used when oxygen is lacking. However, most eukaryotes are obligate aerobes. They cannot survive without aerobic respiration.

You will be investigating both fermentation and aerobic cell respiration in this laboratory.

### Exercise I Respiration Reactions in the Electron Transport System

The oxidation-reduction reactions occurring in the electron transport system in the mitochondria can be detected using dyes that change color when reduced. One such dye is tetrazolium (2,3,5-triphenyl tetrazolium chloride). Cytochromes will transfer electrons to tetrazolium in the electron transport system as well as to oxygen. Oxidized tetrazolium is colorless. Reduced tetrazolium is bright pink to red in color. If the electron transport system is functioning in viable mitochondria, a tetrazolium solution will turn pink or red. You will test viability in seeds by checking to see if the electron transport system in their mitochondria are doing cell respiration. You will be using two different types of seeds, corn seeds and bean seeds. One group of each type has been boiled to kill the seeds. The other group has been soaked for 24 hours to initiate the germination process. (Germinating seeds have a high rate of respiration. Why?)

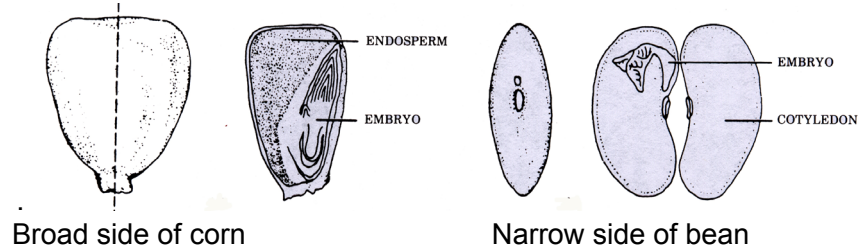
#### Materials Needed

Soaked lima beans  
Soaked corn grains  
Boiled soaked lima beans  
Boiled soaked corn grains

Petri dishes  
Tetrazolium  
Sharp razor blade  
Forceps

**Procedure**

1. Obtain 3 seeds of each of the four seed categories: boiled corn, boiled beans, soaked corn and soaked beans. Cut the corn seeds with a razor blade along the vertical axis, as shown in the diagram below. Remove the seed coats from the bean seeds. The two halves of the seeds should separate easily. The embryo within each seed should be exposed



2. Label two petri dishes, one “boiled seeds” and the second “soaked seeds”: and put several drops of tetrazolium in each. Place your seed halves cut side down in the petri dishes and then add enough tetrazolium to completely cover the seeds and soak the seeds for 20 minutes.
3. Using forceps, turn your seeds over and examine each for evidence of mitochondrial activity. Record your results in Table 3 below and make sketches of your corn and bean seeds indicating areas in which you found evidence of mitochondrial activity.

Corn seeds before and after treatment

Bean seeds before and after treatment

Table 3: Respiration the Electron Transport System in Seeds

Treatment	Results
Soaked Corn	
Soaked Beans	
Boiled Corn	
Boiled Beans	

**Discussion Question**

Why would beans and corn do cell respiration?

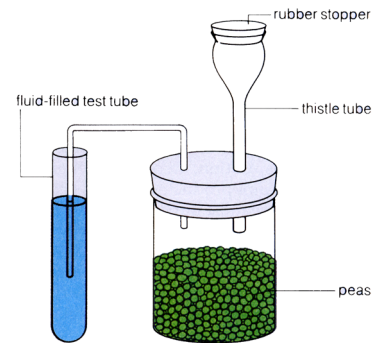
## Exercise II Production of CO<sub>2</sub> during cell respiration

The production of CO<sub>2</sub> during respiration can be measured a number of ways, including using gas pressure sensors, respirometers, or indicator dyes, such as bromothymol blue. CO<sub>2</sub> combines with H<sub>2</sub>O to form H<sub>2</sub>CO<sub>3</sub> which liberates H<sup>+</sup> ions, changing the pH of the solution. Bromothymol blue is blue in pH solutions higher than pH7.6. It is green in neutral solutions and yellow in pH solutions less than pH6.2.

In this exercise we will determine if CO<sub>2</sub> is being produced using the indicator dye, bromothymol blue.

### Materials Needed

- Germinating peas
- Dormant peas
- Boiled germinating peas
- 6 Test tubes
- 3 respirometer chamber apparatuses  
(See diagram at right. You might use a flask chamber rather than a jar.)
- Dilute bromothymol blue solution



Respirometer Chamber Apparatus

### Procedure

1. Put equal amounts of boiled peas, germinating peas and dormant peas into your three respirometer bottles.
2. Place the apparatus top into the bottle securely.
3. Fill the test tube with sufficient water so that the bent tubing is submerged. The test tube should stay balanced, but you might want to place it in a small test tube rack to be sure that it doesn't spill.
4. Be sure that the thistle tube rubber stopper is in place, so that no air can enter the respirometer chamber.
5. Set your three chambers aside for 75 minutes.
6. After 75 minutes, pour the water out of your test tube and replace it with an equal volume of bromothymol blue solution.
7. Remove the stopper from the thistle tube of each respirometer chamber and slowly pour water from the beaker down the thistle tube into the chamber. The water will displace gases in the chamber, which will be forced into the test tube. If CO<sub>2</sub> is one of the gases in the chamber, the bromothymol blue should change color.
8. Record your observations in Table 4 below

Table 4: CO<sub>2</sub> Production in Pea Seeds

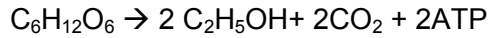
Pea Seeds	Color of Bromothymol blue	CO <sub>2</sub> present or absent
Germinating peas		
Boiled germinating peas		
Dormant peas		

### Discussion Question

What conclusions can you draw from this exercise with respect to peas and cellular respiration?

### Exercise III Fermentation in Yeast

The yeast, *Saccharomyces cerevisiae*, performs alcohol fermentation when oxygen is unavailable. One of the products alcohol fermentation is CO<sub>2</sub>. The chemical reaction for alcohol fermentation is:



The rate of CO<sub>2</sub> evolution can be used as an indication of the relative rate of fermentation of the yeast organisms. There are a number of ways to collect the CO<sub>2</sub> given off during fermentation, including respirometers, fermentation tubes or gas pressure sensors. Several factors can affect fermentation rate: concentration of yeast, concentration of the fuel molecules, type of fuel molecules, temperature, etc.

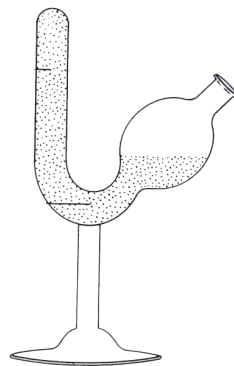
In this exercise you will observe how the concentration of yeast organisms affects fermentation rate.

#### Materials Needed

4 50ml beakers	10% Glucose solution
4 Fermentation Tubes	Yeast suspension
Ruler	30° C "Hot" water bath

#### Procedure

1. Label the four beakers and the four fermentation tubes 1 → 4.
2. Fill your 4 beakers following the instructions in Table 5 below.
3. Mix the contents of your beakers thoroughly and transfer the solutions to your 4 fermentation tubes.
4. Tilt the fermentation tubes so the tube portion of the fermentation tube is filled with your solution. See diagram below.



Fermentation Tube

5. Place your fermentation tubes in the 30° C water bath. As fermentation occurs, the CO<sub>2</sub> evolved will rise to the top of the fermentation tube, displacing the solution. Measure the level of gas (in mm) in the fermentation tubes at 10-minute intervals for 60 minutes in Table 6 below. If there is lots of activity, you might want to record at 5-minute intervals.

Table 5: Contents of Fermentation Solutions

Tube#	Distilled Water	Yeast Suspension	Glucose Solution
1	8ml	0	6ml
2	12ml	2ml	0
3	6ml	2ml	6ml
4	2ml	6ml	6ml

Table 6: CO<sub>2</sub> Production in Fermentation

Time (min)	CO <sub>2</sub> Evolved (mm) Tube#			
	1	2	3	4
0				
10				
20				
30				
40				
50				
60				

**Discussion Questions**

1. Did concentration of yeast organisms affect the rate of fermentation? Which tube had the greatest fermentation activity?
  
2. What was the function of tubes # 1? Was there and CO<sub>2</sub> production in tube # 2? If so, why might there be fermentation in a tube with no added glucose?
  
3. Could you detect evidence of any other fermentation product? If so, what product and how did your determine the presence of this product?
  
3. How would you design an experiment to test other variables the affect fermentation rate of yeast? What other variables might you test?