

EXERCISE 7

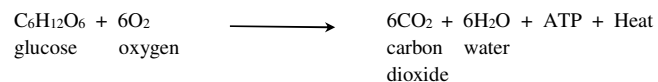
Cellular Respiration

Learning objectives

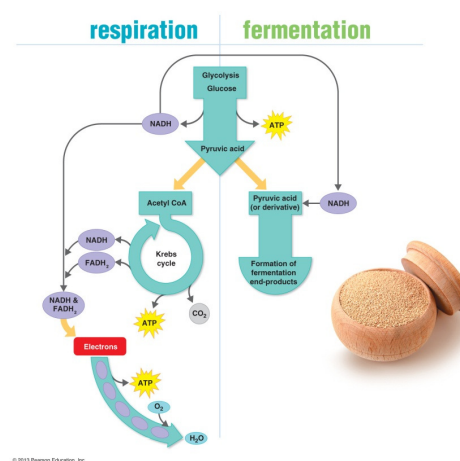
- Observe the process of cellular respiration in yeasts
- Determine the effect of different variables on cellular respiration in yeast
- Illustrate how different organisms can affect the level of carbon dioxide in the atmosphere
- Deduce that oxygen is used by germinating seeds
- Measure heat released as a by- product of cellular respiration

INTRODUCTION

Energy is required by all living organisms for metabolism. Where does that energy come from? The process of cellular respiration involves the breakdown of complex organic molecules. By breaking bonds in these molecules, energy is released in the form of adenosine triphosphate (ATP). The ATP can be used to drive a number of cellular metabolic reactions in an organism. The following chemical reaction illustrates the overall reaction that occurs in respiration.



The equation above summarizes the very complex process of cellular respiration which involves a series of many biochemical reactions in a metabolic pathway. These reactions have been organized into three stages: **glycolysis**, the **Krebs Cycles (citric acid cycle)**, and the **electron transport chain**. Some organisms can also generate ATP by the process of fermentation. The processes of cellular respiration and fermentation are illustrated in the diagram below;



Answer the following questions before you come to lab:

1. What is the initial substrate for cellular respiration? -----
2. How many ATP molecules are produced during glycolysis? Krebs' cycle? Electron transport chain?

Glycolysis	
Krebs' cycle	
Electron transport chain	
Total	

3. What is the final electron acceptor in aerobic respiration? _____
4. Why is heat released during cellular respiration when it cannot be used by cells?

5. If oxygen is not available this type of respiration is called _____
6. Name 2 molecules that are used as the final electron acceptor if oxygen is not available. State the products formed in each case.

Final electron acceptors in fermentation	Products

- How many ATP molecules are produced from fermentation? _____
- Name the three different molecules that make up a molecule of ATP.

In the following exercises, you will measure both examine and fermentation.

FERMENTATION

IMPORTANT NOTE: Wear gloves.

Dispose of sodium pyruvate, sodium fluoride and phenol red in the appropriate waste jar.

Exercise 1: Fermentation in Yeast

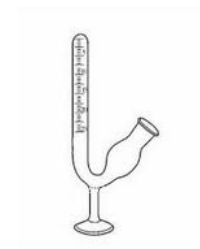
One type of anaerobic respiration most common to us involves the use of yeast in the production of bread and alcoholic beverages e.g. beer and wine. The yeasts use sugar present in the extract (e.g. grape juice) as a substrate and produce CO₂ and alcohol as byproducts. This process is known as fermentation. It is anaerobic since no oxygen is involved. We can measure the amount of CO₂ produced by the yeasts as an indication of how efficiently they are able to carry out fermentation.

- Obtain 4 fermentation tubes and fill them according to the table below. Your instructor will demonstrate the proper way to fill the tubes so that no air bubbles are present in the neck of the tubes.

Table 1 – Solution amounts of fermentation experiment.

Tube #	3M sodium pyruvate	0.1 M NaF (poison)	5% glucose	water
1			2.5 ml	5.0 ml
2		5.0 ml	2.5 ml	
3	5.0 ml		2.5 ml	
4				7.5 ml

- Finish filling the tubes with 25 ml of the yeast solution provided.
- Incubate the tubes at 40°C for 45 minutes.
- After 45 minutes, use a ruler to measure the height of the bubble (CO₂) in each tube, and write your results in Table 2.



Which tube would you expect to produce the most CO₂? The least? Explain your answers.

Table 2 – Size of CO₂ bubble.

Tube #	Height of CO ₂ bubble (mm)
1	
2	
3	
4	

Exercise 2: Effect of Temperature on Fermentation

- Obtain 2 more fermentation tubes (tubes 5 and 6).

- Prepare them exactly as Tube 1. Place 2.5 ml glucose and 5.0 ml water into each tube, and fill with yeast solution.
Put tube 5 at room temperature (20°C) and tube 6 in the refrigerator (4°C)
Tube 1 (40°C) from the previous exercise is included in this experiment
- Measure the size of the bubble after 40 minutes and record your results in Table 3.

Table 3 – Size of CO₂ bubble.

Tube #	Temperature	Height of CO ₂ bubble (mm)
1		
5		
6		

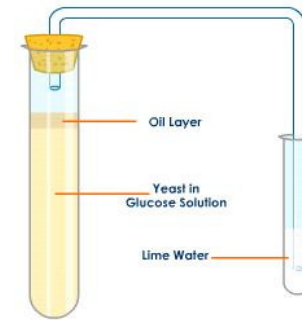
Which tube would you expect to produce the most CO₂? The least? Explain your answers.

Exercise 3: Release of carbon dioxide during anaerobic respiration

In this experiment you are going to use a yeast mixture. The yeast mixture was prepared by first boiling a sugar solution (to remove all dissolved air) and then adding the yeast after it was cooled.

Procedure

- Use the apparatus provided to setup the experiment as shown in the diagram below.
- Half fill the large test tube with the yeast mixture.
- Pour in oil just to cover the surface of the mixture.



What is the purpose of the oil layer?

- Half fill the other tube with phenol red.

What is the reason for using phenol red?

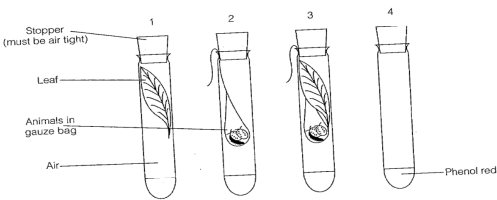
- Place the stopper with the delivery tube tightly (BE CRAEFUL!) on the tube with the yeast.
- Arrange both tubes on the test tube rack so that the end of the delivery tube is submerged in the phenol red.
- Set up an identical experiment but use killed yeast instead.

What is the purpose of this duplicate experiment? _____

Why was oil added to the surface of the yeast?

What is the color of phenol red in a neutral solution?

Explain why the color changed.



Exercise 4: How organisms affect the amount of carbon dioxide in the atmosphere.

Procedure

1. Label four large test tubes 1-4.
2. Place 5 ml phenol red into each tube.
3. Place the following into the tubes and stopper tightly (see diagram).

Tube	Organism(s)
1	a green leaf
2	some small animals in a piece of gauze
3	a green leaf and animals in a piece of gauze
4	no organisms

4. Place the tubes in a rack on the bench and observe periodically for about an hour.
5. Gently shake the tubes every 10 minutes and observe the time taken for any color change. Record your results in the table below.

Time (mins)	Color			
Start	Tube 1	Tube 2	Tube 3	Tube 4
10				
20				
30				
40				
50				
60				

Which tube changed first? Explain why this tube changed first.

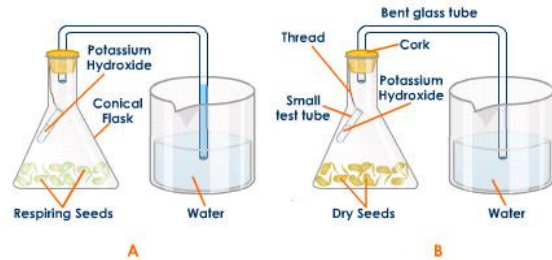
What color change took place in tube 1? Explain.

Describe what was happening in tube 3.

Exercise 5: Use of oxygen during respiration by germinating seeds.

Soda lime is a chemical substance that absorbs carbon dioxide. Any carbon dioxide produced by the organisms is removed by the soda lime.

Observe the experiment, which has been set up.



When the organisms use up oxygen, what will happen to the pressure in the flask?

Describe what you observe taking place in each of the glass tubing.

What is the function of the potassium hydroxide (soda lime) pellets?

Explain why the level of the colored water rose in one glass tubing and not in the other.

Exercise 6: Release of heat by germinating seeds.

The experiment was set up as follows:

1. Moist cotton wool was placed at the bottom of a thermos flask and the flask was filled with germinating seeds.
2. A thermometer was inserted and the flask and cotton wool used as a stopper.
3. A second flask was set up in the same way with non-germinating seeds.
4. Note the temperature in each flask:

Flask with live seeds _____

Flask with dead seeds _____

Why were thermos flasks used?

Why was the flask with non-germinating seeds included in the experiment?

Since respiration releases heat energy, what type of biochemical process is it?

Review Questions

Exercise 1: Anaerobic Respiration

Tube #	Contents of Tube	Height of CO ₂ bubble (mm)
1		
2		
3		
4		

1. What was the function of Tube 1 and Tube 4?

2. Although Tube 4 contained no glucose a bubble was still produced by the yeast. Where did they get the sugar for respiration?

3. NaF is a poison. Which substances, essential for metabolic processes could be inhibited by the NaF? Explain.

4. (a) Yeast carry out alcohol fermentation. How is alcohol fermentation different from lactic acid fermentation?

- (b) What effect did the addition of pyruvate have on the reaction in tube 3? Explain.

Exercise 2: Effect of temperature on respiration

Tube #	Temperature	Height of CO ₂ bubble (mm)
1		
5		
6		

5. Which of the fermentation tubes (1, 5, or 6) produced the greatest amount of CO₂? Explain your results *fully*.
