

NAME _____

DATE _____

Aerobic Cellular Respiration and Fermentation

Overview

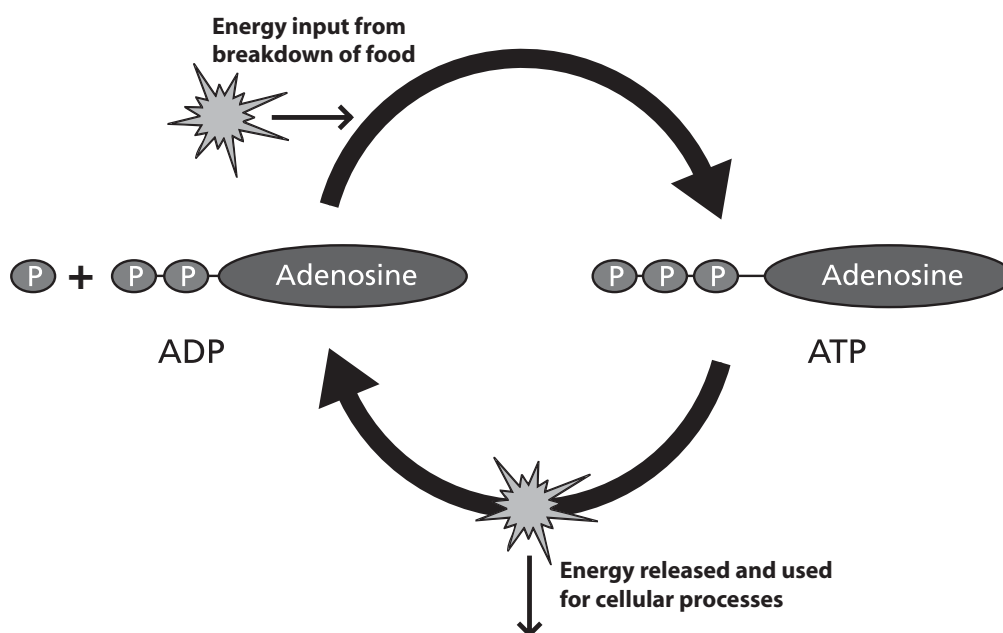
The following activities demonstrate physiological differences between aerobic cellular respiration and fermentation. Through the Background and Pre-Laboratory Questions, you will compare the products of aerobic cellular respiration and alcohol fermentation in yeast. You will observe carbon dioxide production as a pH change in liquids containing respiring wheat seeds and fermenting yeasts. Then, you will investigate fermentation of different types of sugars by yeast. You and your classmates will use your combined data from this investigation to address a hypothesis.

Background

All cells need energy. Many cells obtain energy either from aerobic cellular respiration, an oxygen-requiring process, or fermentation, a process that does not require the presence of oxygen.

Organic compounds such as carbohydrates, proteins, and fats store energy in their molecular structure. This laboratory activity addresses how energy is derived from carbohydrates. When the bonds of a carbohydrate molecule are broken in a series of small steps with the help of specific enzymes, energy is released from the bonds. The energy can be stored in a molecule called **adenosine triphosphate (ATP)**.

ATP is the chief energy source of cells. Energy is stored in the structure of its three-phosphate tail. The removal of a phosphate from the ATP molecule releases energy that powers almost all metabolic processes. When a phosphate is removed, adenosine triphosphate becomes adenosine diphosphate (ADP). There is a constant cycling between ATP and ADP through the various metabolic processes of the cell.



We will discuss two methods of obtaining energy for ATP production: cellular aerobic respiration and fermentation. Both methods begin with a series of chemical reactions called glycolysis.

Glycolysis

Glycolysis is a 10-step series of reactions that splits a six-carbon glucose molecule into 2 three-carbon molecules called **pyruvate**. The process occurs in the cytoplasm of a cell. For every molecule of glucose broken down, there is a net yield of two ATP and two NADH molecules (the function of NADH will be discussed further below). Oxygen is not required for glycolysis. What happens next depends on the abilities of the organism and on the presence or absence of oxygen.

Aerobic Cellular Respiration

All higher organisms and many microorganisms have the necessary enzymes to perform **aerobic cellular respiration** to generate additional ATP from glucose, but only if oxygen is present. This is possible through the pathway called the Krebs cycle and the electron transport chain.

Steps of Aerobic Cellular Respiration

Process	Oxygen Required	Location	Starting material (per glucose)	Energy containing outputs (per glucose)	
				Electron carriers	ATPs
Glycolysis	No	Cytoplasm	Glucose	2 NADH	2 ATP
Acetyl-CoA synthesis & Krebs cycle	No*	Mitochondria matrix	2 Pyruvate	8 NADH 2 FADH ₂	2 ATP
Electron transport chain	Yes	Mitochondria inner membrane	10 NADH 2 FADH ₂		32 ATP
Total					36 ATP

*Although O₂ is not used directly in these reactions, they occur only in the presence of O₂ because the NADH and FADH₂ they produce must be processed by the electron transport chain.

Krebs Cycle

If oxygen is present, the pyruvate enters the mitochondria of the cell, where it is initially converted to a molecule called acetyl-CoA; as this happens, one molecule of carbon dioxide (CO₂) and one molecule of NADH are produced. The acetyl-CoA is then broken down through a series of six-enzyme reactions referred to as the **Krebs cycle** or citric acid cycle. As each acetyl-CoA is broken down, two CO₂ molecules are produced and energy is released and stored in ATP. Some of the energy also passes as electrons (plus some hydrogen atoms) to the electron carrier molecules NAD⁺ and FAD, yielding NADH and FADH₂. The electrons held in these electron carriers can be used to make additional ATP through the electron transport chain. Thus, within a mitochondrion, each pyruvate is completely broken down to three CO₂ molecules. Release of this CO₂ into water, as in the demonstration, produces carbonic acid, which acidifies the solution.

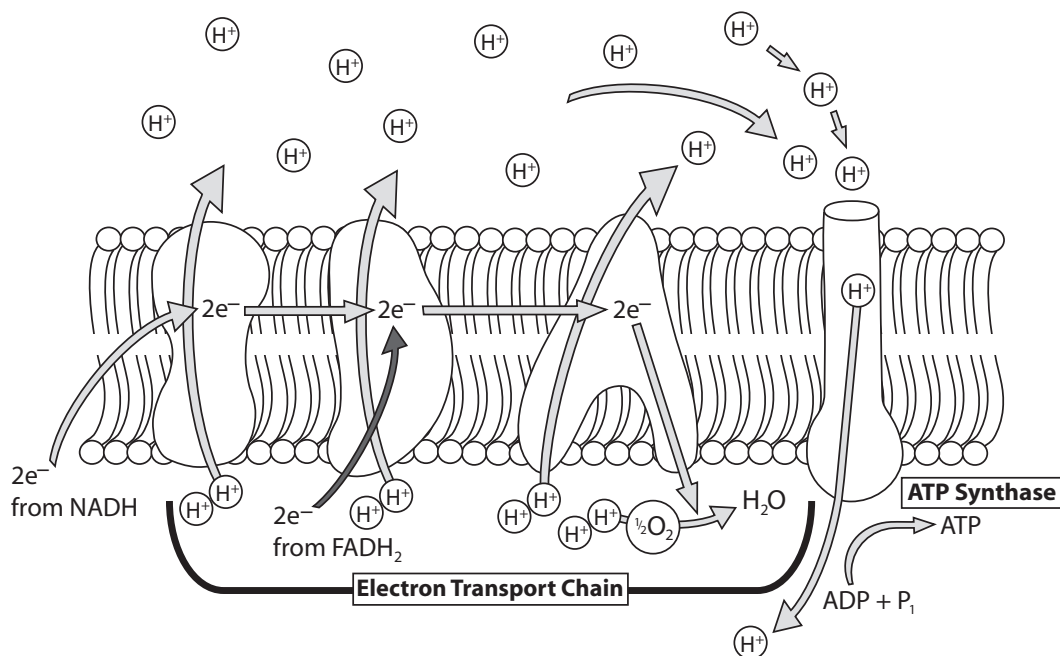
Electron Transport Chain

The NADH and FADH₂ produced during glycolysis and the Krebs cycle are used to generate more ATP in the **electron transport chain**. NADH and FADH₂ molecules are called electron carriers because they transport electrons and associated hydrogen atoms to a series of membrane-embedded proteins called the electron transport chain. Here, the hydrogens and electrons are stripped from the electron carriers, leaving NAD⁺ from NADH and FADH from FADH₂. The forms of these molecules cycle continually in the cell.

As the electrons from the NADH and FADH₂ are passed between the membrane-embedded proteins of the electron transport chain, the energy released is used to form a hydrogen ion gradient across a membrane.

This gradient provides the energy to power an enzyme, called ATP synthase, to add another phosphate group to ADP. In eukaryotic cells, the electrons from each NADH yield 3 ATP molecules and those from each FADH_2 yield 2 ATP molecules. The electron transport chain produces 16 ATP for each pyruvate molecule. Remember that there are two pyruvate molecules for every glucose molecule that enters the system. For each glucose molecule, 36 ATP molecules are made.

Free oxygen (O_2) comes into play only during the final step of the electron transport chain. When the electrons get to the end of the transport chain, they do not simply “fall off”—the electrons and hydrogens are passed to oxygen, resulting in the formation of water (H_2O). For this reason oxygen is called the **terminal electron acceptor**. As oxygen picks up electrons at the end of the electron transport chain, more electrons are passed through. If oxygen is not available to pick up electrons, no more electrons can enter the system from NADH and FADH_2 . Thus, in the absence of oxygen, the electron transport and Krebs cycle processes cease to function.



Anaerobic Cellular Respiration

Some microorganisms can generate ATP through steps similar to those in aerobic cellular respiration but using other molecules such as sulfate (SO_4^{2-}) or nitrate (NO_3^-) in place of oxygen as the terminal electron acceptor. When a molecule other than oxygen is used as the terminal electron acceptor, the method of respiration is referred to as **anaerobic cellular respiration**. As you might expect, microorganisms found growing in anaerobic environments such as water-logged soils often use this process. The sulfurous odor characteristic of swamps is due to the hydrogen sulfide gas produced when bacteria convert SO_4^{2-} to H_2S through anaerobic cellular respiration.

Fermentation

Cells can produce ATP in the absence of oxygen, but only through glycolysis. In order to break down another molecule of glucose in the absence of oxygen, the cell must cycle NADH back to NAD^+ (the NADH produced during glycolysis is normally converted back into NAD^+ by the electron transport chain). This need is met through a process called **fermentation**. During fermentation, 2 ATP molecules are produced per glucose molecule. Cells convert the pyruvate to either lactic acid, or to ethanol and CO_2 . The two common types of fermentation are called lactic acid fermentation and alcohol fermentation.

Lactic Acid Fermentation

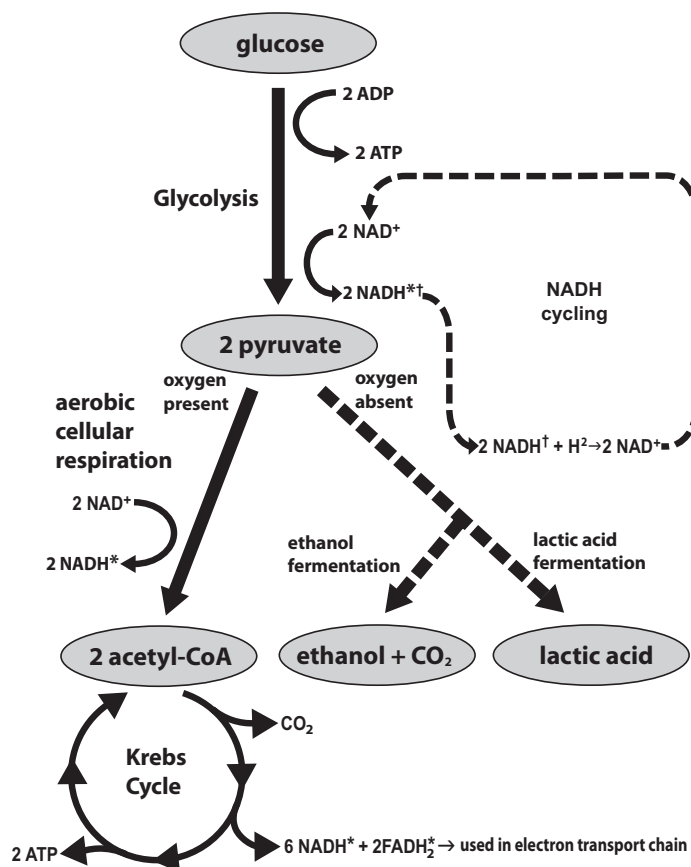
Animals and some microorganisms such as fungi and bacteria carry out **lactic acid fermentation** in the absence of oxygen. During lactic acid fermentation, the pyruvate produced during glycolysis is converted to lactic acid, and the NADH is converted back into NAD⁺.

Animal cells require a great deal of ATP. Aerobic cellular respiration provides most of the ATP to meet the cells' energy requirements. If oxygen is in short supply, another mechanism, lactic acid fermentation, can provide a small amount of ATP to sustain cell function for a short time until the oxygen supply is restored. For example, during prolonged strenuous physical activity, muscle cells require vast amounts of energy. If the circulatory system is unable to transport oxygen fast enough from the lungs to the muscle cells, the cells may resort to lactic acid fermentation. During and right after a run, you might breathe rapidly. This helps restore oxygen to the muscles, so that aerobic respiration can supply your energy needs. Fermentation alone cannot produce enough ATP to sustain energy needs for very long.

Alcohol Fermentation

Yeasts use alcohol fermentation in the absence of oxygen to remove pyruvate and cycle NADH. In alcohol fermentation, the pyruvate is converted to ethanol and CO₂. As this occurs, NADH is converted back to NAD⁺. The waste products of fermentation, ethanol and CO₂, are essential to the baking and brewing industries—carbon dioxide to leaven bread, and ethanol for beer, wine, and distilled liquors. Also, yeast fermentation of plant materials is used in the production of ethanol for fuel.

Cellular Respiration and Fermentation Pathways



*NADH and FADH₂ are utilized in the electron transport chain to generate large amounts of ATP. O₂ serves as the terminal electron acceptor.

† During fermentation, NADH does not enter the electron transport chain but instead is converted to NAD⁺ for reuse in glycolysis.

Comparison of Aerobic Cellular Respiration and Fermentation

Process	Starting material	Final waste products (per glucose)	Total ATP production (per glucose)
Aerobic Cellular Respiration	Glucose	6 CO ₂ and 6 H ₂ O	36 ATP
Lactic Acid Fermentation	Glucose	2 Lactic Acid	2 ATP
Alcohol fermentation	Glucose	2 CO ₂ and 2 Ethanol	2 ATP

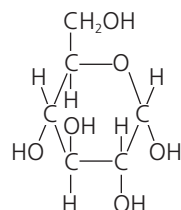
Yeasts

Yeasts are unicellular fungi. The type of yeast used in these activities is *Saccharomyces cerevisiae*. Yeasts reproduce by "budding" off small cells that then enlarge to full size. Scientists study *S. cerevisiae* as a model research organism to learn about fundamental life processes that are shared with more complex multicellular plants and animals. Yeast is easy to grow and can be stored for long periods of time in a dormant state (e.g., the packets available in grocery stores).

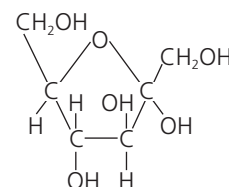
Yeasts can generate ATP by either aerobic cellular respiration or alcohol fermentation. They are equipped with the ability to produce energy in the absence of oxygen. The energy needs of a single-celled organism are much lower than those of a complex, multicellular organism, and yeasts can survive by fermentation alone. Also, yeast cells can import and use carbohydrates very rapidly, in effect, compensating for the inefficiency of fermentation by processing large quantities of carbohydrate.

Sugars

Simple sugars or **monosaccharides** are the basic units of a carbohydrate. Three examples of monosaccharides are glucose, fructose, and galactose. Glucose, the most common sugar in nature, is the one transported in your blood. Fructose is found in many fruits, and galactose is found in many vegetables.

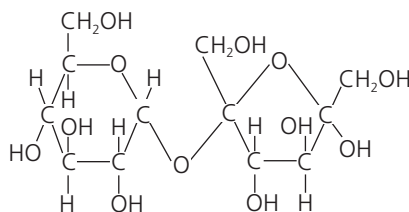


Glucose

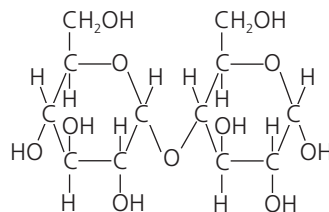


Fructose

A **disaccharide** molecule, or double sugar, consists of two bound monosaccharides. Sucrose, common table sugar, contains a glucose and fructose molecule linked together. Sucrose is produced by many plants; most of the sucrose we eat comes from sugar cane and sugar beets. Lactose is the sugar found in milk. Each lactose molecule contains a glucose and galactose molecule linked together. Some people cannot digest lactose because they lack a protein needed to split the glucose and galactose apart.

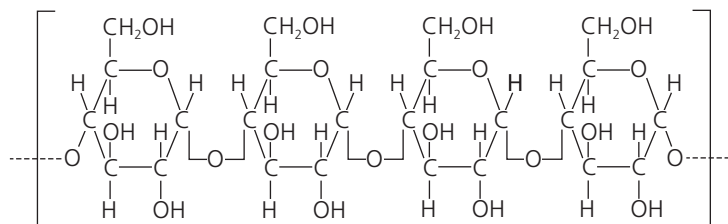


Sucrose



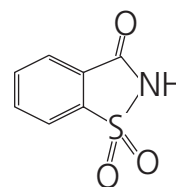
Lactose

Polysaccharides are long chains of many monosaccharides bound together. Starch is a polysaccharide made of many glucose molecules.



Starch

Saccharin and some other artificial sweeteners are not sugars but are different types of organic compounds that have been found to taste sweet and deemed safe to eat.



Saccharin

What types of sugars can yeast ferment?

To answer this question, you will examine fermentation by observing the production of carbon dioxide, one of the waste products of fermentation. The carbon dioxide released by yeast in a syringe pushes a water droplet through a calibrated measuring pipet, and the volume of carbon dioxide produced can be measured over time by the position of a water droplet.

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Pre-laboratory Questions

1. Why is the ability to perform lactic acid fermentation important to an animal cell when oxygen is scarce?
2. When athletes undergo extreme exertion, their circulatory system may be unable to supply enough oxygen, and their muscle cells may experience anaerobic conditions. Do you think this situation is more likely to occur in a bodybuilder or a long-distance runner. Why?
3. Why do you think anaerobic conditions in the muscle cells may lead to reduced performance?
4. In the fermentation activity, how will you determine if the yeast is performing fermentation?

5. Your class will test for yeast fermentation of glucose, lactose, sucrose, and saccharin. Which do you predict that yeast cells will ferment? Your answer to this question will be your experimental hypothesis. The class data will support or refute your hypothesis.

Use what you know about cellular respiration and fermentation of yeast to fill out the following chart.

Cellular respiration in yeast under aerobic conditions

Molecules of glucose processed	Molecules of CO ₂ produced	Molecules of ethanol produced	Molecules of ATP produced	Total ATP produced
1				
2				
3				
4				
5				
6				

Cellular respiration in yeast under anaerobic conditions

Molecules of glucose processed	Molecules of CO ₂ produced	Molecules of ethanol produced	Molecules of ATP produced	Total ATP produced
1				
2				
3				
4				
5				
6				

6. Compare the breakdown of six glucose molecules by aerobic cellular respiration and alcohol fermentation as described in the chart.
- How many more ATP molecules are produced by aerobic cellular respiration?
 - How many times more ATP is produced by aerobic cellular respiration than by fermentation?

Materials

For each student pair:

- 2 copies of the Student Guide
- 2 syringes
- 2 pipets
- 2 pieces of tubing

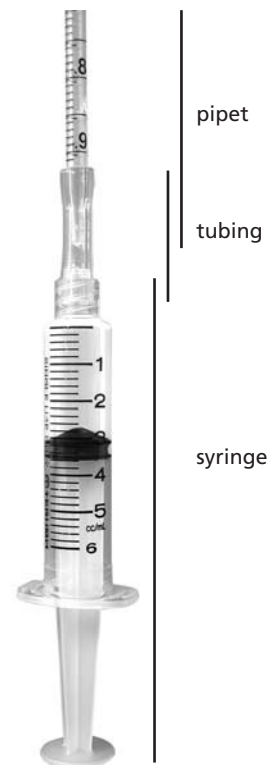
At each station:

- water cup
- sugar solution cup(s)
- yeast cup
- 2 test tube racks to support the fermentation apparatus

Procedure

1. Place a water drop into the plastic pipet.
 - a. Draw a small amount of water into the syringe.
 - b. While holding the pipet and syringe parallel to the floor, insert the syringe tip into the pipet and expel about 0.05 mL of water.
 - c. Remove the syringe and expel the rest of the water from the syringe.
 - d. Draw a small amount of air into the syringe, hold it to the pipet tip, and press the plunger, expelling the air into the pipet behind the water drop to place the drop between 0.85 and 0.9 mL.
 - e. Set the pipet down until you are ready to use it.

Note: Remember that 1 mL is equal to 1 cc.
2. Aspirate the yeast and sugar solutions.
 - a. Make sure the plunger is fully depressed.
 - b. Aspirate 1 mL of water into the syringe. Invert the syringe and flick the side of the syringe to bring any air bubbles into the tip. Press the plunger up until all the bubbles are expelled and 1 mL water fills the syringe.
 - c. Add 2 mL of yeast solution to the syringe by placing the syringe tip into the suspension and slowly pulling the plunger past the 3-cc graduation line (1 mL of water is already present). Invert the syringe and flick it to bring any bubble into the tip. Press the plunger up until all the bubbles are expelled and 3 mL yeast suspension fills the syringe.
 - d. Next, aspirate 2 mL of sugar solution by placing the tip of the syringe in the sugar solution and pulling the plunger. The total volume will now be 5 mL.
 - e. Invert the syringe and add 1 mL of air above the yeast suspension by pulling the plunger down to 6 cc.
3. Set up the fermentation apparatus.
 - a. Attach the tubing piece to the tapered end of the pipet.
 - b. Attach the other end of the tube securely to the tip of the syringe with the yeast and sugar suspension. Be careful not to jostle the yeast or tip the pipet.
 - c. Carefully adjust the syringe plunger until the bottom of the water droplet rests at the 0.9-mL mark.
 - d. Carefully, place the apparatus in the test tube rack so that it sits vertically.



Taking measurements

1. The top of the drop should still be at 0.85 mL. The pipet is inverted so the labeled increments are upside-down. You will have to calculate the difference between the initial reading of the pipet and the final reading to figure out the change in volume of gas.

Examples: If the top of the water drop moved from 0.85 to 0.7 mL, subtract 0.7 from 0.85 to get 0.15. The gas volume is 0.15 mL. If it moved to -0.2, subtract -0.2 from 0.85 to get 1.05. The gas volume is 1.05 mL.

2. Once the apparatus is set up, start the time. Do not touch the apparatus once the time has started.
3. In the data tables provided, you will record the graduation closest to the level of the bottom of the liquid drop in the pipet every minute for 15 minutes.

Glucose

Minutes	Reading on Pipet (mL)	-	Initial Reading	=	CO ₂ Volume
0		-		=	
1		-		=	
2		-		=	
3		-		=	
4		-		=	
5		-		=	
6		-		=	
7		-		=	
8		-		=	
9		-		=	
10		-		=	
11		-		=	
12		-		=	
13		-		=	
14		-		=	
15		-		=	

Solution 2: _____

Minutes	Reading on Pipet (mL)	-	Initial Reading	=	CO ₂ Volume
0		-		=	
1		-		=	
2		-		=	
3		-		=	
4		-		=	
5		-		=	
6		-		=	
7		-		=	
8		-		=	
9		-		=	
10		-		=	
11		-		=	
12		-		=	
13		-		=	
14		-		=	
15		-		=	

4. After you have finished collecting data, share your results with the class.
5. Graph the fermentation rate of both of your solutions. In order to graph your data, you need to determine which values to plot on the x-axis (horizontal) and y-axis (vertical). Time is the independent variable, and the measured carbon dioxide volume is the dependent variable (because the carbon dioxide volume is dependent upon the time you choose to measure it). Label the axis and then graph the data.

Post-Laboratory Questions

1. How did you know whether each type of sugar was fermented or not?
2. Summarize the class findings in this table. Place an X in the appropriate space for each sample.

	Was fermented	Was not fermented
Control	_____	_____
Glucose	_____	_____
Lactose	_____	_____
Sucrose	_____	_____
Saccharin	_____	_____

3. Did the results support your hypothesis? Explain.

4. Reread the Background information about the different types of sugars. Drawing upon this information, offer an explanation for the different results obtained for each sample.

Glucose:

No sugar control:

Lactose:

Sucrose:

Saccharin:

5. You were asked to graph the relationship between carbon dioxide volume and elapsed time in the experiment. The slope of the line created by your data points is the fermentation rate (mL CO₂/minute; i.e., the change of carbon dioxide volume over the change in time). Use the following formula to calculate the fermentation rate of yeast with your glucose solution between 5 and 15 minutes.

$$\text{fermentation rate} = \frac{\text{CO}_2 \text{ volume at 15 minutes} - \text{CO}_2 \text{ volume at 5 minutes}}{15 \text{ minutes} - 5 \text{ minutes}}$$

6. Does the color change of the bromthymol blue in the demonstration show conclusively that the wheat seeds are carrying out aerobic cellular respiration rather than fermentation? What other information would you like to know (or what additional analysis would you like to perform) that would help answer this question?

7. Look at the table summarizing all of the class data. Did all groups obtain the same values? What are some possible reasons for the different results? Discuss potential experimental error and uncontrolled variables.

8. Why is the average value of several replica experiments more reliable than results for a single test?

9. Someone might suggest that carbon dioxide released in this lab does not require the presence of carbohydrate; how does the design of the experiment address that suggestion?

10. Is it possible that carbon dioxide can be released from sugar without yeasts under the conditions in our laboratory experiment? What additional control experiment could you perform to test this alternative explanation?

11. What causes the water droplet to move up the measuring tube?

14. How do the products of yeast fermentation aid in bread and beverage production?