

# Anaerobic Respiration (Fermentation)

by

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## 1. Introduction

Have you noticed that sometimes when you are running a race, you tend to run out of breath and cramp up after a while? How will this affect your performance? When you have trouble breathing it means that not enough oxygen is being delivered to your cells. Aerobic respiration can only take place when there is oxygen present; therefore your body is not producing enough usable energy, adenosine triphosphate (ATP), for your muscles to use as you compete in the race.

How do you feel the day after the race? Most likely, your legs will be very sore. This happens because in the absence of oxygen, the muscles switch to fermentation in order to produce energy, and the final product of fermentation in the body is lactic acid. The build up of lactic acid in the muscles cause the pH level of the body to become more acidic, the muscles to get tired, and cramps to develop.

In this lab we will be studying anaerobic respiration, or fermentation, which is the process of breaking down sugar to produce energy in the absence of oxygen. You will be using yeast as the living organism (enzyme) that breaks down the different sugars to produce energy, with alcohol as the byproduct of fermentation instead of lactic acid.

## 2. Background

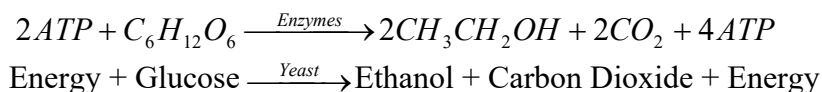
### Cellular Respiration:

Living organisms require energy to function. There is potential energy in the chemical bonds of all organic molecules such as sugars, and some of the common sugars are: glucose, sucrose, fructose, and lactose. However, this energy can not be used directly by the cells and so these bonds need to be broken to release the energy in a form that is useful to the cells. Cellular respiration is the process where energy that is stored in organic compounds is eventually converted to a usable form called ATP (adenosine triphosphate). ATP is like money of the body, cells in different muscles use ATP in order to do their work.

The two phases of cellular respiration is anaerobic and aerobic respiration. Anaerobic respiration is phase 1 and it takes place in the cytoplasm. At the end of anaerobic respiration 2 molecules of ATP are produced. If there is oxygen,  $O_2$  present then aerobic respiration or phase 2 can take place. If there is no oxygen present then phase 2 of cellular respiration cannot take place.

Fermentation begins by splitting a glucose molecule into 2 molecules with 3 carbon atoms each. This glucose splitting process is called glycolysis, during which ten individual

chemical reactions occur, with each reaction controlled by a different enzyme. The end result of anaerobic respiration, or fermentation, is 2 ATP and an alcohol, or an acid. Anaerobic respiration can be summarized with the following chemical equation:



Yeast is used as the enzyme because it contains the enzymes that are needed for fermentation.

Structural Formula of Sugar Molecules:

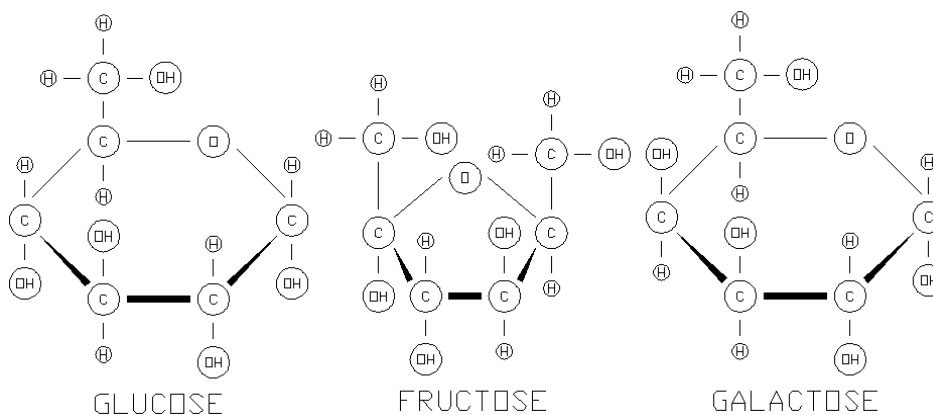
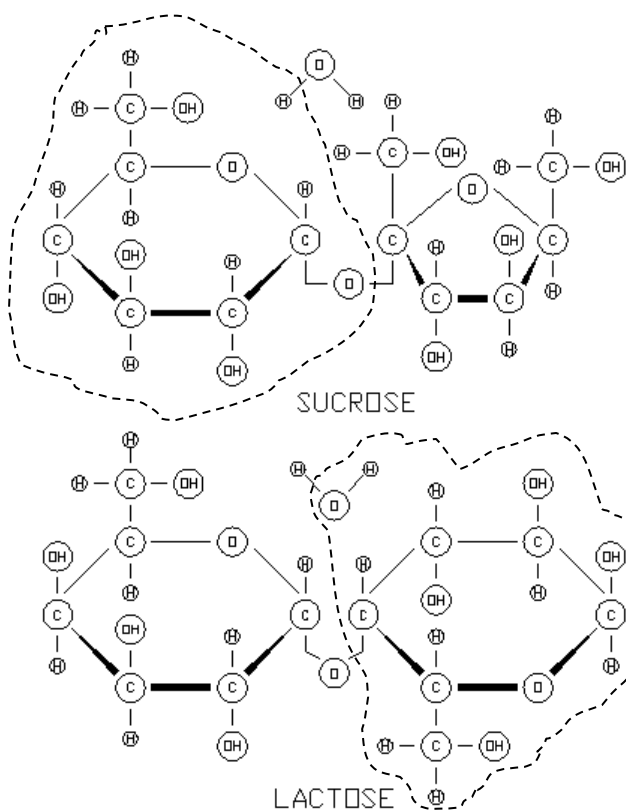


Figure 1



Glucose is the most fundamental molecular composition of all the sugars; its structural formula is shown in Figure 1. That means that the chemical bonds between its elements are the easiest to break compared to the bonds in a fructose molecule. Galactose is another monosaccharide, or a single sugar, that has very weak chemical bonds. Galactose is an isomer of glucose, thus it is obtained by reorienting the location of one of the hydroxyl groups, *OH*, of the glucose as shown above in Figure 1.

The “glucosidic” links in the disaccharides, or double sugars, are more difficult to break down than the interior bonds of the monosaccharides. The “glucosidic” link is the oxygen bond between the two sugar molecules, as shown to the left in Figure 2.

Figure 2

### 3. Objective

- Determine the rate of fermentation while using yeast as an enzyme to breakdown different sugars.
- Learn how the type of sugar involved affects the rate of fermentation.

### 4. Equipment

Computer	Milk
Vernier computer interface	600 ml beaker (for water bath)
Logger Pro Software	Hot and cold water
Vernier Gas Pressure Sensor	Thermometer
Single-holed rubber stopper	Two 18 x 150 mm test tube
Plastic tubing with Luer-lock fitting	10 mL graduated cylinder
Gatorade	Utility clamp
Coke with $CO_2$ completely released	Ring-stand
100% fruit juice	Yeast suspension

### 5. Experimental Procedure

1. Incubate the yeast solution in a  $37 - 40\text{ }^{\circ}\text{C}$  water bath to ensure that the yeast will remain in a constant and controlled temperature. To prepare the water bath, pour warm and cold water into the 600 mL beaker until the water temperature reaches  $37 - 40\text{ }^{\circ}\text{C}$ . The beaker should be filled with about 300 – 400 mL water, and leave the thermometer in the water bath for the duration of the experiment to monitor the temperature of the water bath.
2. While the yeast is incubating, connect the Gas Pressure Sensor to Channel 1 of the Vernier computer interface. Prepare the computer for data collection by opening the file “Anaerobic Respiration” from the Living Environment folder.
3. Connect the plastic tubing to the valve on the Gas Pressure Sensor
4. Label the two test tubes 1 and 2
5. Your group will test two of the four drinks, as directed by your instructor. Using the graduated cylinder:
  - Pour 2.5 mL of the first solution into the test tube labeled 1
  - Pour 2.5 mL of the second solution into the test tube labeled 2Record which solutions you tested in Table 1.
6. Lightly stir the yeast suspension to mix the yeast that settled to the bottom. Using the graduated cylinder pour 2.5 mL of the yeast suspension into the test tube labeled 1. Gently mix the yeast and sugar solution.

7. Insert the single-holed rubber stopper into the test tube. Firmly twist the stopper for an airtight fit and then secure the test tube with a utility clamp to the ring-stand. Then connect the free end of the plastic tubing to the connector in the rubber stopper, shown in Figure 3.

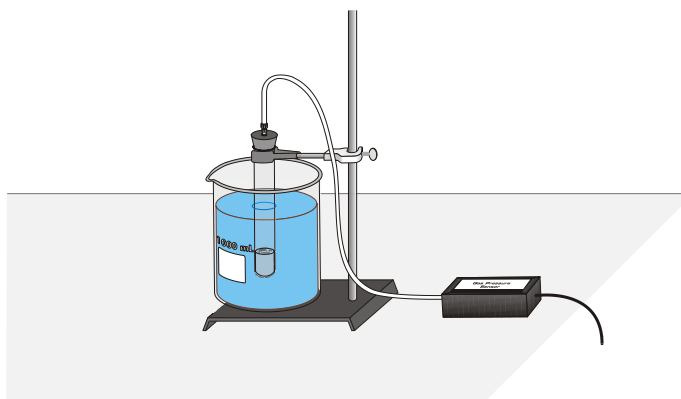


Figure 3 (Masterman, 2003)

8. Then incubate the test tube for 10 minutes in the water bath. Make sure to keep the water bath at a constant temperature (37-40<sup>0</sup> C) and the test tube completely covered by the water. Periodically check to ensure that the temperature remains constant. If the temperature falls below or above acceptable levels, add cold or hot water accordingly. (If water is being added to the bath, first remove the same amount of water that will be added to the beaker to prevent overflow.)
9. After 10 minutes have elapsed you can begin measuring the gas pressure by clicking on the Collect Data button, . Disconnect the plastic tubing from the Gas Pressure Sensor if the pressure exceeds 130 kPa, because the pressure inside the test tube is too great. The file is set up to collect data for 8 minutes.
10. After data collection has finished, remove the rubber stopper from the test tube and discard the contents in a waste basket.
11. Determine the rate of fermentation:
  - First change the name of the Header in the Data Collection Window on the left to the drink that is being tested (Gatorade, Coke, Juice, or Milk). This can be done by double clicking the cell and then typing in the title into the little window that opens.
  - To highlight the data move the mouse pointer to the point where the data values begin to increase. The data should start increasing around 60 seconds. Drag the pointer to the end of the data and release the mouse button.
  - To obtain the rate of fermentation a best fit line needs to be drawn for the data. After the data has been highlighted, click on the Linear Fit button, . A floating box will appear with the formula for a best fit line.
  - Record the slope of the line,  $m$ , as the rate of respiration in Table 1.

- Close the linear regression floating box and move your data to a stored run. To do this, choose Store Latest Run from the Experiment menu.

12. Use a notebook or notepad to fan air across the opening of the Gas Pressure Sensor.
13. Repeat Steps 6– 12 for the second sample.
14. Save all your data by choosing “save as” from the file menu, and save the file as Anaerobic Respiration (Group #).
15. Share your data with the class by recording the fermentation rates you obtained for the two drinks in the appropriate spot on the board.
16. After all the groups have recorded their data on the board, copy down all the values into Table 2.

## 6. Results

<b>Table 1: Group Data</b>		
Drink Tested	Actual Temperature (°C)	Rate of Fermentation (kPa/s)
Gatorade		
Coke		
Fruit Juice		
Milk		

<b>Table 2: Class Data</b>				
Drink Tested	Group 1 – Rate of Fermentation (kPa/s)	Group 2 – Rate of Fermentation (kPa/s)	Group 3 – Rate of Fermentation (kPa/s)	Group 4– Rate of Fermentation (kPa/s)
Gatorade				
Coke				
Fruit Juice				
Milk				

## 7. Analysis

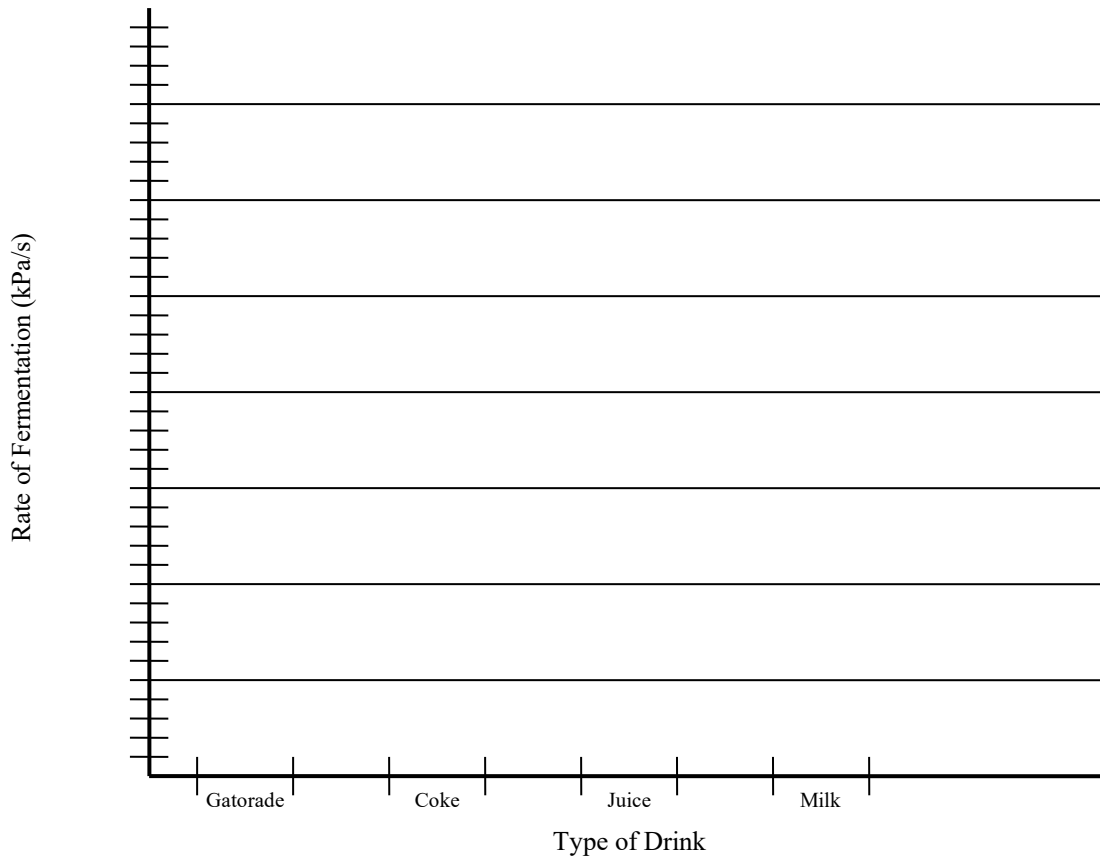
1. Calculate the average rate of fermentation for each of the five drinks that were tested. Record the class averages for the fermentation rates in Table 3.

<b>Table 3: Class Average</b>	
Drink Tested	Rate of Fermentation

	(kPa/min)
Gatorade	
Coke	
Fruit Juice	
Milk	

2. Make a bar graph, using the graph space provided below, of the rate of fermentation vs. type of drink. Use the fermentation rates calculated in Table 3. The rate values should be plotted on the y-axis, and the type of drink on the x-axis.

**Bar Graph of Rate of Fermentation**



3. Looking at the bar graph, can yeast utilize all the sugars equally? Explain.

4. Explain why some of the sugars in the drinks were metabolized and the other sugars were not metabolized as much.

5. Why do you need to incubate the yeast at 37-40°C before you start collecting the data?

6. Why does all the  $CO_2$  from the coke need to be released before the yeast is added?

7. Explain some possible sources of error?

## Reference

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## Anaerobic Respiration (Fermentation)

### Teacher Information

1. To Prepare Yeast Suspension: Dissolve 7 g of dried yeast for every 100 mL of water. Incubate the suspension in 37 - 40 °C water for at least 10 minutes; then transfer the yeast to dispensing tubes. Each group will need at least 5 mL of yeast.
2. Make sure that all the  $CO_2$  from the coke has been released.