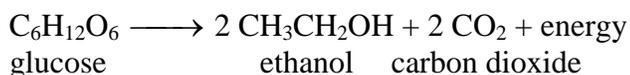# SUGAR FERMENTATION IN YEAST

LAB ENZ 2.CALC
From *Biology with Calculators,* Vernier Software & Technology, 2000.

INTRODUCTION

Yeast are able to metabolize some foods, but not others. In order for an organism to make use of a potential source of food, it must be capable of transporting the food into its cells. It must also have the proper enzymes capable of breaking the food's chemical bonds in a useful way. Sugars are vital to all living organisms. Yeast are capable of using some, but not all sugars as a food source. Yeast can metabolize sugar in two ways, *aerobically,* with the aid of oxygen, or *anaerobically*, without oxygen.

In this lab, you will try to determine whether yeast are capable of metabolizing a variety of sugars. Although aerobic fermentation of sugar is much more efficient, in this experiment we will have yeast ferment sugars anaerobically. When the yeast respire aerobically, oxygen gas is consumed at the same rate that CO_2 is produced—there would be no change in the gas pressure in the test tube. When yeast ferment the sugars anaerobically, however, CO_2 production will cause a change in the pressure of a closed test tube, since no oxygen is being consumed. We can use this pressure change to monitor the respiration rate and metabolic activity of the organism. A Gas Pressure Sensor will be used to monitor the fermentation of sugar.

The fermentation of glucose can be described by the following equation:

$$C_6H_{12}O_6 \longrightarrow 2\ CH_3CH_2OH + 2\ CO_2 + energy$$

glucose   ethanol   carbon dioxide

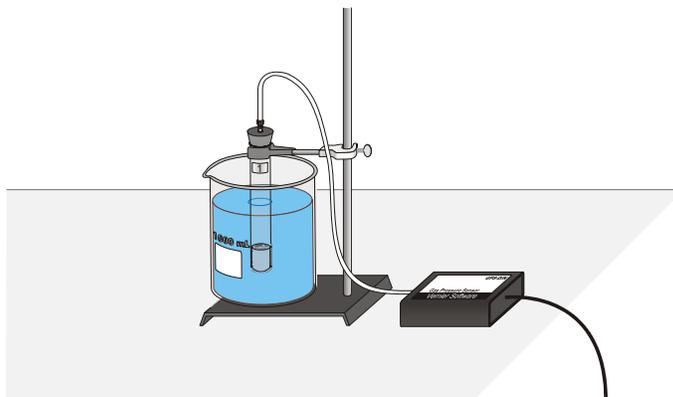Note that alcohol is a byproduct of this fermentation.

Figure 1

PURPOSE

The purpose of this lab is to study the ability of yeast to respire anaerobically using various sugars. A Gas Pressure Sensor will be used to measure the production of CO_2, which will be used to determine the respiration rate and metabolic activity of yeast.

EQUIPMENT/MATERIALS

LabPro	18 x 150 mm test tube
TI Graphing Calculator	1-L beaker (for water bath)
DataMate program	disposable plastic pipets
Vernier Gas Pressure Sensor	hot and cold water
Graphical Analysis (optional)	test tube rack
ring stand	utility clamp
hot plate	stirring rods
rubber-stopper assembly	thermometer
5% glucose, sucrose, lactose, and one other sugar solution	yeast suspension
	vegetable oil in dropper bottle

SAFETY

- Always wear an apron and goggles in the lab.

PROCEDURE

1. Obtain and wear goggles.

2. Connect the plastic tubing to the valve on the Gas Pressure Sensor.

3. Plug the Gas Pressure Sensor into Channel 1 of the LabPro. Use the link cable to connect the TI Graphing Calculator to the interface. Firmly press in the cable ends.

4. Turn on the calculator and start the DATAMATE program. Press CLEAR to reset the program.

5. Set up the calculator and interface for a Gas Pressure Sensor.

   a. If the calculator displays PRESS(KPA) in CH 1, proceed directly to Step 6. If it does not, continue with this step to set up your sensor manually.

   b. Select SETUP from the main screen.

   c. Press ENTER to select CH 1.

   d. Select PRESSURE from the SELECT SENSOR menu.

   e. Select the correct pressure sensor from the PRESSURE menu.

   f. Select the calibration listing for (KPA).

   g. Select OK to return to the main screen.

6. Prepare a water bath for the yeast. A water bath is simply a large beaker of water at a certain temperature. This ensures that the yeast will remain at a constant and controlled temperature. To prepare the water bath, obtain some warm and cool water from your teacher. Combine the warm and cool water in the 1-liter beaker until it reaches 38 – 40°C. The beaker should be filled with about 600 – 700 mL of water. Place the thermometer in the water bath to monitor the temperature during the experiment.

7. Obtain two test tubes and label them 1 and 2.

8. Your team will test two of the four sugar solutions. Obtain two of the four sugar solutions: glucose, sucrose, lactose, and one other sugar solution, as directed by your instructor. Place 2.5 mL of the first sugar solution into test tube 1 and 2.5 mL of the second sugar solution into test tube 2. Record which solutions you tested in Table 1.

9. Set the test tubes into the water bath.

10. Obtain the yeast suspension. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Using a 10-mL pipette or graduated cylinder, transfer 2.5 mL of yeast into test tube 1. Gently mix the yeast into the sugar solution. Be gentle with the yeast—they are living organisms!

Vegetable oil

Yeast/glucose

Figure 2

11. In the test tube, place enough vegetable oil to completely cover the surface of the yeast/glucose mixture as shown in Figure 2. Be careful to not get oil on the inside wall of the test tube. Set the test tube in the water bath.

12. Insert the single-holed rubber-stopper into the test tube. **Note**: *Firmly* twist the stopper for an *airtight* fit. Secure the test tube with a utility clamp and ring-stand as shown in Figure. 1.

13. Incubate the test tube for 10 minutes in the water bath. Be sure to keep the temperature of the water bath constant. If you need to add more hot or cold water, first remove about as much water as you will be adding, or the beaker may overflow. Use a basting bulb to remove excess water.
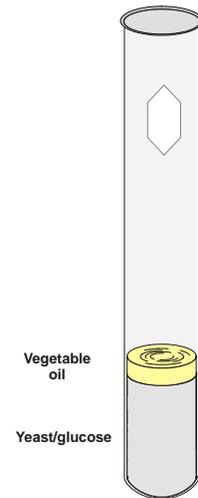
   Note: Be sure that most of the test tube is completely covered by the water in the water bath. The temperature of the air in the tube must be constant for this experiment to work well.

14. When incubation has finished, connect the free-end of the plastic tubing to the connector in the rubber stopper as shown in Figure 3.

15. Select START to begin data collection. Maintain the temperature of the water bath during the course of the experiment.

16. Data collection will end after 15 minutes. Monitor the pressure readings displayed on the calculator screen. If the pressure exceeds 130 kilopascals, the pressure inside the tube will be too great and the rubber stopper is likely to pop off. Disconnect the plastic tubing from the Gas Pressure Sensor if the pressure exceeds 130 kilopascals.
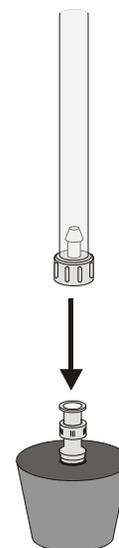
17. When data collection has finished, an auto-scaled graph of pressure *vs.* time will be displayed on the calculator screen. As you move the cursor right or left, the time (X) and pressure (Y) values of each data point are displayed below the graph.

Figure 3

18. Disconnect the plastic tubing connector from the rubber stopper. Remove the rubber stopper from the test tube and discard the contents in a waste beaker.

19. Determine the rate of respiration for the curve of pressure *vs.* time. The rate of respiration can be measured by examining the slope of the pressure *vs.* time curve:

    a. Examine the graph and determine the most linear region.
    b. Move the flashing cursor to the first point of the region. Enter the initial pressure, P_i, and initial time, t_i, in Table 1.
    c. Move the flashing cursor to the last point of the region. Enter the final pressure, P_f, and final time, t_f, in Table 1.
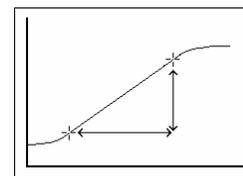
20. Press ⌊ENTER⌋ to return to the main screen.

21. Repeat Steps 9 – 20 using test tube 2.

22. Select QUIT from the main screen to exit the DATAMATE program.

DATA SHEET

Name _____

Name _____

Period _____ Class _____

Date _____

SUGAR FERMENTATION IN YEAST

DATA TABLES

Table 1					
	Point 1		Point 2		
Test Tube/Sugar	P_i (kPa)	t_i (s)	P_f (kPa)	t_f (s)	Slope $= \dfrac{P_f - P_i}{t_f - t_i}$
1 _____					
2 _____					

Table 2			
Test	Type of Sugar	Rate of Respiration (kPa/s)	Class Average (kPa/s)
1			
2			
3			
4			

PROCESSING THE DATA

1. Calculate the slope of the graph for both of the experimental runs recorded in Table 1. The slope of each graph is equal to the rate of respiration for that experiment. Record the calculated slopes in the rate column of Table 2.

2. Share your data with the rest of the class by recording the sugar type you tested and the rate of respiration on the board.

3. Using the class data, calculate the average rates of respiration for each of the sugar types tested. Record the average rates in Table 2, along with the names of the 4 sugars tested.

4. Using the class data in Table 2, make a bar graph of sugar type *vs.* rate of respiration. The rate values should be plotted on the y-axis, and the sugar type on the x-axis.

QUESTIONS

1. Considering the results of this experiment, do yeast equally utilize all sugars? Explain.

2. Hypothesize why some sugars were not metabolized while other sugars were.

3. Why do you need to incubate the yeast before you start monitoring air pressure?

4. Why do you need a layer of oil over your yeast/sugar mix during the experiment?

5. Yeast live in many different environments. Make a list of some locations where yeast might naturally grow. Estimate the possible food sources at each of these locations.