# EFFECT OF TEMPERATURE ON

**FERMENTATION with LabQuest**

## LAB 16

From *Biology with Vernier*

# INTRODUCTION



**Westminster College**

Temperature changes have profound effects upon living things. Enzyme-catalyzed reactions are especially sensitive to small changes in temperature. Because of this, the metabolism of *poikilotherms,* organisms whose internal body temperature is determined by their surroundings are often determined by the surrounding temperature. Bakers who use yeast in their bread making are very aware of this. Yeast is used to leaven bread (make it rise). Yeast leavens bread by fermenting sugar, producing carbon dioxide, CO2, as a waste product. Some of the carbon dioxide is trapped by the dough and forms small “air” pockets that make the bread light. If the yeast is not warmed properly, it will not be of much use as a leavening agent; the yeast cells will burn sugar much too slowly. In this experiment, you will watch yeast cells ferment (burn sugar in the absence of oxygen) at different temperatures and measure their rates of fermentation. Each team will be assigned one temperature and will share their results with other class members.

You will observe the yeast under anaerobic conditions and monitor the change in air pressure due to CO2 released by the yeast. When yeast burn sugar under anaerobic conditions, ethanol (ethyl alcohol) and CO2 are released as shown by the following equation.

C6 H12 O6 → 2 CH3 CH2 OH + 2 CO2 + energy

sugar ethanol carbon dioxide

Thus, the metabolic activity of yeast may be measured by monitoring the pressure of gas in the test tube. If the yeast were to respire aerobically, there would be no change in the pressure of gas in the test tube, because oxygen gas would be consumed at the same rate as CO2 is produced.

# OBJECTIVES

* Use a Gas Pressure Sensor to measure the change in pressure due to carbon dioxide released during respiration.
* Determine the rate of fermentation of yeast at different temperatures.

# MATERIALS

LabQuest Gas Pressure Sensor

5% Sugar Solution 1 L beaker (for water bath)

Beral pipet, graduated 10 mL graduated cylinder

18 x 150 mm test tube test tube rack

Yeast suspension vegetable oil in a dropper bottle

1-hole rubber stopper assembly utility clamp Plastic tubing with Luer-lock fitting ring stand Thermometer goggles

 *Figure 1*

# PROCEDURE

1. Connect the plastic tubing to the valve on the Gas Pressure Sensor.
2. Connect the Gas Pressure Sensor to LabQuest and chose New from the File menu.
3. Prepare a water bath for the experiment.
	1. To prepare the water bath, obtain some warm water and some cool water.
	2. Combine the warm and cool water into the 1 L beaker until it reaches the temperature you were assigned. The beaker should be filled with about 600-700 mL water.
	3. Place the thermometer in the water bath to monitor the temperature during the experiment.
4. Pipet 2.5 mL of the sugar solution into a clean 18 x 155 mm test tube.
5. Using a different pipet, pipet 2.5 mL of yeast suspension into the test tube. Gently swirl the test tube to thoroughly mix the yeast into the solution. **Important:** The yeast suspension must be removed from the middle of a yeast source that is being stirred by a magnetic stirrer at a constant stirring speed.
6. In the test tube, place 1 mL of vegetable oil to completely cover the surface of the yeast/sugar mixture as shown in Figure 2. **Note:** Be careful to not get oil on the inside wall of the test tube. Set the test tube in the water bath.

1. 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.
2. Connect the free end of the plastic tubing to the connector in the rubber stopper as shown in Figure 3.

*Figure 2*

*Figure 3*

1. Set the test tube in the water bath and secure it with a utility clamp and ring-stand as shown in Figure 1. **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 test tube must be constant for this experiment to work well. Be sure to keep the temperature of the water bath constant.
2. Start data collection. **Note:** Maintain the temperature of the water bath during the course of the experiment.
3. Record the temperature of the water bath in Table 1.
4. Monitor the pressure readings. 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.
5. When data collection has finished, 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.
6. Determine the rate of fermentation.
	1. Tap and drag your stylus across the most linear region to select these data points.
	2. Choose Curve Fit from the Analyze menu.
	3. Select Linear as the Fit Equation.
	4. Record the slope of the line, *m,* as the rate of fermentation in Table 1.
	5. Select OK.
7. Record your temperature and respiration values form table 1 on the classroom board. When all groups have reported their data, calculate an average respiration for each temperature tested. Fill in Table 2 with the calculated class averages.

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| --- |
| Table 1: Lab Group Data |
| Temperature (°C) | Rate of fermentation (ppm/s) |
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| --- |
| Table 2: Class Data |
| Temperature (°C) | Rate of fermentation (ppm/s) |
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|  |  |
|  |  |
|  |  |
|  |  |

# PROCESSING THE DATA

1. Record the data from the other teams in Table 2.
2. Create a graph of rate of fermentation *vs.* temperature. The rate values should be plotted on the y-axis, and the temperature on the x-axis.

# QUESTIONS

1. On the basis of the results of this experiment, does temperature affect the rate of fermentation of yeast? Explain
2. Is there an optimal temperature for yeast to ferment sugar? How does this compare to the temperature yeast are at when bread rises? You may have to consult a cookbook to answer this.
3. Why does the fermentation rate decrease at very high temperatures?
4. It is sometimes said that a metabolism of poikilotherms double with every 10°C increase in temperature. Does your data support this statement? Explain.
5. What is the purpose of the oil on top of the mixture?
6. Do yeast always live in conditions where their consumption of energy is optimal? Explain
7. Why do you need to incubate the yeast before you start monitoring air pressure?
8. Yeast live in many different environments. Make a list of some locations where yeast might naturally grow. Estimate the temperatures of each of these locations and compare them to your results.