



## Calculator 6A

---

- Measure the production of oxygen gas as hydrogen peroxide is destroyed by the enzyme catalase or peroxidase at various temperatures.
- Measure and compare the initial rates of reaction for the enzyme at each temperature.
- Measure the production of oxygen gas as hydrogen peroxide is destroyed by the enzyme catalase or peroxidase at various pH values.
- Measure and compare the initial rates of reaction for the enzyme at each pH value.

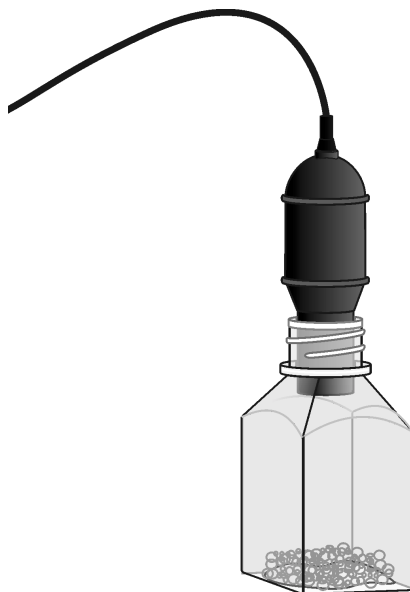


Figure 1

## MATERIALS

TI-83 Plus or TI-84 Plus graphing calculator  
EasyData application  
data-collection interface  
Vernier O<sub>2</sub> Gas Sensor  
400 mL beaker  
10 mL graduated cylinder  
250 mL Nalgene bottle  
1.5% H<sub>2</sub>O<sub>2</sub>  
3.0% H<sub>2</sub>O<sub>2</sub>

enzyme suspension  
three 18 × 150 mm test tubes  
ice  
pH buffers  
test tube rack  
thermometer  
three dropper pipettes  
Logger *Pro* (optional)

## PROCEDURE

1. Obtain and wear goggles.
2. Turn on the calculator. Connect the O<sub>2</sub> Gas Sensor, data-collection interface, and calculator.
3. Set up the data-collection mode.
  - a. Start the EasyData application if it is not already running.
  - b. Select **[File]** from the Main screen, and then select **New** to reset the application.
  - c. Select **[Setup]** from the Main screen, then select **Time Graph...**

- d. Select **(Edit)** on the Time Graph Settings screen.
- e. Enter **5** as the time between samples in seconds and select **(Next)**.
- f. Enter **36** as the number of samples and select **(Next)** (data will be collected for 3 minutes).
- g. Select **(OK)** to return to the Main screen.

### **Part I Testing the Effect of Enzyme Concentration**

4. Place three test tubes in a rack and label them 1, 2, and 3. Fill each test tube with 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub>.
5. Initiate the enzyme catalyzed reaction.
  - a. Using a clean dropper pipette, add 5 drops of enzyme suspension to test tube 1.
  - b. Begin timing with a stopwatch or clock.
  - c. Cover the opening of the test tube with a finger and gently invert the test tube two times.
  - d. Pour the contents of the test tube into a clean 250 mL Nalgene bottle.
  - e. Place the O<sub>2</sub> Gas Sensor into the bottle as shown in Figure 1. Gently push the sensor down into the bottle until it stops. The sensor is designed to seal the bottle with minimal force.
  - f. When 30 seconds has passed, select **(Start)** to begin data collection.
6. When data collection has finished, a graph of O<sub>2</sub> gas vs. time will be displayed.
7. Remove the O<sub>2</sub> Gas Sensor from the Nalgene bottle. Rinse the bottle with water and dry with a paper towel.
8. Perform a linear regression to calculate the rate of reaction.
  - a. Select **(Anlyz)**, and then select **Linear Fit**.
  - b. The linear-regression statistics for these two lists are displayed for the equation in the form:
$$y=ax+b$$
  - c. Enter the absolute value of the slope, *a*, as the reaction rate in Table 2.
  - d. Select **(OK)** to view a graph of the data and the regression line.
  - e. Select **(Main)** to return to the Main screen.
9. Store the data from the first run so that it can be used later.
  - a. Select **(File)**, and then select **Store Run**.
  - b. Select **(OK)** to store your latest data and overwrite the data in Lists 3 and 4 (L3 and L4).
10. Find the rate of enzyme activity for test tubes 2, and 3:
  - a. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 5–9. **Note:** After selecting **(Start)** to begin data collection, select **(OK)** to start collecting data. Your stored data will not be overwritten.
  - b. Add 20 drops of the enzyme solution to test tube 3. Repeat Steps 5–8.

## Calculator 6A

---

11. Graph all three runs of data on a single graph. To do this:
  - a. From the Main screen, select **(Graph)**.
  - b. Select **(Adv)** and then select **L2, L3 and L4 vs L1**.
  - c. All three runs should now be displayed on the same graph. Each point of the 5-drop run is plotted with a plus sign, each point of the 10-drop run is plotted with a square, and each point of the 20-drop run is plotted without a marker. Use the displayed graph and the data in Table 2 to answer the questions for Part I.
  - d. When finished with the graph, select **(Main)** to return to the Main screen.

### Part II Testing the Effect of Temperature

Your teacher will assign a temperature range for your lab group to test. Depending on your assigned temperature range, set up your water bath as described below. Place a thermometer in your water bath to assist in maintaining the proper temperature.

- 0–5°C: 400-mL beaker filled with ice and water.
  - 20–25°C: No water bath needed to maintain room temperature.
  - 30–35°C: 400-mL beaker filled very warm water.
  - 50–55°C: 400-mL beaker filled hot water.
12. Rinse the three numbered test tubes used for Part I. Fill each test tube with 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub> and then place the test tubes in the water bath. The test tubes should be in the water bath for 5 minutes before proceeding to Step 13. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 3.
  13. Find the rate of enzyme activity for test tubes 1, 2, and 3:
    - a. Add 10 drops of the enzyme solution to test tube 1. Repeat Steps 5–8. Record the reaction rate in Table 3. **Note:** After selecting **(Start)** to begin data collection, select **(OK)** to start collecting data.
    - b. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 5–8. Record the reaction rate in Table 3.
    - c. Add 10 drops of the enzyme solution to test tube 3. Repeat Steps 5–8. Record the reaction rate in Table 3.
  14. Calculate the average rate for the three trials you tested. Record the average in Table 3.
  15. Record the average rate and the temperature of your water bath from Table 3 on the class chalkboard. When the entire class has reported their data on the chalkboard, record the class data in Table 4.

### Part III Testing the Effect of pH

16. Place three clean test tubes in a rack and label them pH 4, pH 7, and pH 10.
17. Add 5 mL of 3% H<sub>2</sub>O<sub>2</sub> and 5 mL of a pH buffer to each test tube, as in Table 1.

| Table 1      |   |                       |
|--------------|---|-----------------------|
| pH of buffer | Volume of 3% H <sub>2</sub> O <sub>2</sub> (mL) | Volume of buffer (mL) |
| pH 4         | 5   | 5                     |
| pH 7         | 5   | 5                     |
| pH 10        | 5   | 5                     |



**Part III Effect of pH**

| Table 5         |                      |
|-----------------|----------------------|
| Test tube label | Slope, or rate (%/s) |
| pH 4            |                      |
| pH 7            |                      |
| pH 10           |                      |

**PROCESSING THE DATA**

1. For Part II of this experiment, make a graph of the rate of enzyme activity vs. temperature by hand or by using *Logger Pro*. Plot the rate values for the class data in Table 4 on the y-axis and the temperature on the x-axis. Use this graph to answer the questions for Part II.

**QUESTIONS**

**Part I Effect of Enzyme Concentration**

1. How does changing the concentration of enzyme affect the rate of decomposition of  $H_2O_2$ ?
2. If one increases the concentration of enzyme to thirty drops, what do you think will happen to the rate of reaction? Predict what the rate would be for 30 drops.

**Part II Effect of Temperature**

3. At what temperature is the rate of enzyme activity the highest? Lowest? Explain.
4. How does changing the temperature affect the rate of enzyme activity? Does this follow a pattern you anticipated?
5. Why might the enzyme activity decrease at very high temperatures?

**Part III Effect of pH**

6. At what pH is the rate of enzyme activity the highest? Lowest?
7. How does changing the pH affect the rate of enzyme activity?

## EXTENSIONS

1. Repeat Step 13a to collect data with 10 drops of enzyme suspension. Using the *Logger Pro* computer software, import your collected data into a computer. In *Logger Pro*, use the mouse to select each of the time intervals from Table 6. Calculate the rate using the Linear Fit function found in the Analyze menu.

| Table 6<br>Time intervals (Minutes) |        |         |         |          |           |
|-------------------------------------|--------|---------|---------|----------|-----------|
| Rates                               | 0–30 s | 30–60 s | 60–90 s | 90–120 s | 120–180 s |
| 10 Drops                            |        |         |         |          |           |

### Questions

- When is the reaction rate highest? Explain why.
- When is the reaction rate lowest? Why?
2. Different organisms often live in very different habitats. Design a series of experiments to investigate how different types of organisms might affect the rate of enzyme activity. Consider testing a plant, an animal, and a protist.
  3. Presumably, at higher concentrations of  $\text{H}_2\text{O}_2$ , there is a greater chance that an enzyme molecule might collide with  $\text{H}_2\text{O}_2$ . If so, the concentration of  $\text{H}_2\text{O}_2$  might alter the rate of oxygen production. Design a series of experiments to investigate how differing concentrations of the substrate hydrogen peroxide might affect the rate of enzyme activity.
  4. Design an experiment to determine the effect of boiling the catalase on the rate of reaction.
  5. Explain how environmental factors affect the rate of enzyme-catalyzed reactions.

## TEACHER INFORMATION

## Enzyme Action: Testing Catalase Activity

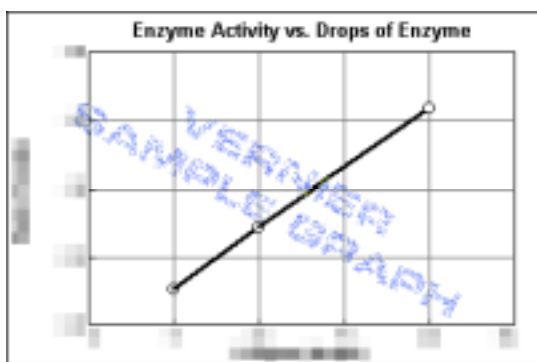
1. The student pages with complete instructions for data-collection using LabQuest App, Logger *Pro* (computers), EasyData or DataMate (calculators), and DataPro (Palm handhelds) can be found on the CD that accompanies this book. See *Appendix A* for more information.
2. This experiment may take a single group several lab periods to complete. A good breaking point is after the completion of Step 10, when students have tested the effect of different enzyme concentrations. Alternatively, if time is limited, different groups can be assigned one of the three tests and the data can be shared.
3. Your hot tap water may be in the range of 50-55°C for the hot-water bath. If not, you may want to supply pre-warmed temperature baths for Part II, where students need to maintain very warm water. Warn students not to touch the hot water.
4. Many different organisms may be used as a source of catalase in this experiment. If enzymes from an animal, a protist, and a plant are used by different teams in the same class, it will be possible to compare the similarities and differences among those organisms. Often, either beef liver, beef blood, or living yeast are used.
5. To prepare the yeast solution, dissolve 7 g (1 package) of dried yeast per 100 mL of 2% glucose solution. A 2% glucose is made by adding 20 g of glucose to enough distilled water to make 1 L of solution. Incubate the suspension in 37 – 40°C water for at least 10 minutes to activate the yeast. Test the experiment before the students begin. The yeast may need to be diluted if the reaction occurs too rapidly. The reaction in Step 4, with 6 mL of 1.5% hydrogen peroxide, and 5 drops of suspension produces enough oxygen to exceed a measured concentration of 22% in 40 to 60 seconds.
6. To prepare a liver suspension, homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water. You will need to test the suspension before use, as its activity varies greatly depending on its freshness. Dilute the suspension until the reaction in Step 4, with 6 mL of 1.5% hydrogen peroxide, and 5 drops of suspension produces enough oxygen to exceed a measured concentration of 22 % in 40 to 60 seconds. The color of the suspension will be a faint pink. Keep the suspension on ice until used in an experiment.
7. 3% H<sub>2</sub>O<sub>2</sub> may be purchased from any supermarket. If refrigerated, bring it to room temperature before starting the experiment.
8. To extend the life of the O<sub>2</sub> Gas Sensor, always store the sensor upright in the box in which it was shipped.
9. Vernier Software sells a pH buffer package for preparing buffer solutions with pH values of 4, 7, and 10 (order code PHB). Simply add the capsule contents to 100 mL of distilled water.
10. You can also prepare pH buffers using the following recipes:
  - pH 4: Add 2.0 mL of 0.1 M HCl to 1000 mL of 0.1 M potassium hydrogen phthalate.
  - pH 7: Add 582 mL of 0.1 M NaOH to 1000 mL of 0.1 M potassium dihydrogen phosphate.
  - pH 10: Add 214 mL of 0.1 M NaOH to 1000 mL of 0.05 M sodium bicarbonate.

## Experiment 6A

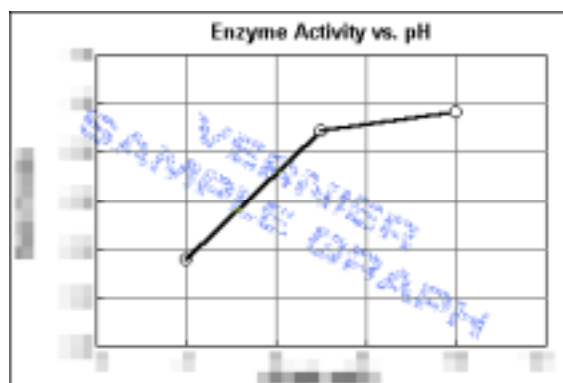
11. You may need to let students know that at pH values above 10, enzymes will become denatured and the rate of activity will drop. If you have pH buffers higher than 10, have students perform an experimental run using them.

### SAMPLE RESULTS

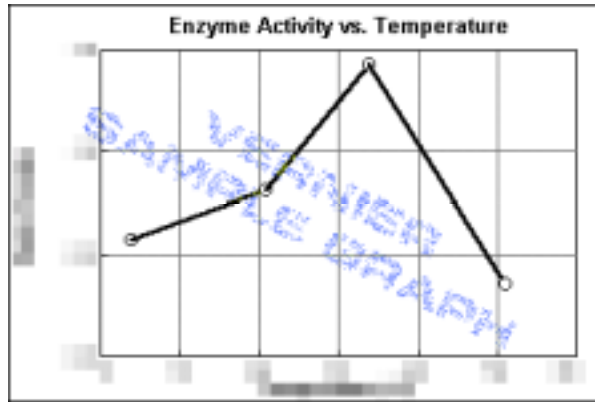
| Sample class data       |                        |
|-------------------------|------------------------|
| Test tube label         | Slope, or rate (%/min) |
| 5 Drops                 | xxxx                   |
| 10 Drops                | xxxx                   |
| 20 Drops                | xxxx                   |
| 0 – 5 °C range: 4°C     | xxxx                   |
| 20 – 25 °C range: 21 °C | xxxx                   |
| 30 – 35 °C range: 34°C  | xxxx                   |
| 50 – 55 °C range: 51°C  | xxxx                   |
| pH 4                    | xxxx                   |
| pH 7                    | xxxx                   |
| pH 10                   | xxxx                   |



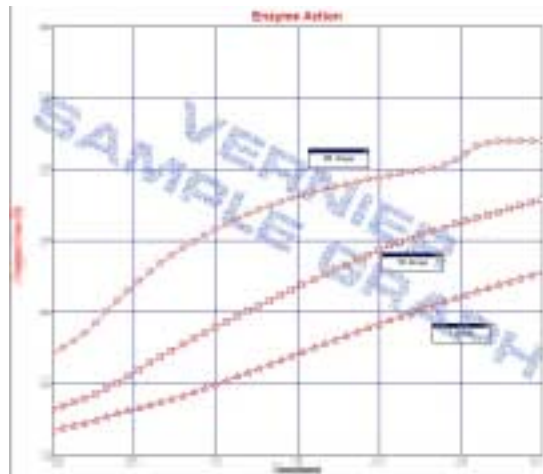
*The effect of H<sub>2</sub>O<sub>2</sub> concentration on the rate of enzyme activity*



*The effect of pH on the rate of enzyme activity*



*The effect of temperature on the rate of enzyme activity*



*Sample Data: Effect of H<sub>2</sub>O<sub>2</sub> concentration on the rate of enzyme activity.*

## **ANSWERS TO QUESTIONS**

Answers have been removed from the online versions of Vernier curriculum material in order to prevent inappropriate student use. Graphs and data tables have also been obscured. Full answers and sample data are available in the print versions of these labs.