

OBJECTIVES

In this experiment, you will

- Use a computer and an Oxygen Gas Sensor to measure the production of oxygen gas as hydrogen peroxide is destroyed by the enzyme catalase or peroxidase at various enzyme concentrations.
- Measure and compare the initial rates of reaction for this enzyme when different concentrations of enzyme react with H_2O_2 .
- 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.

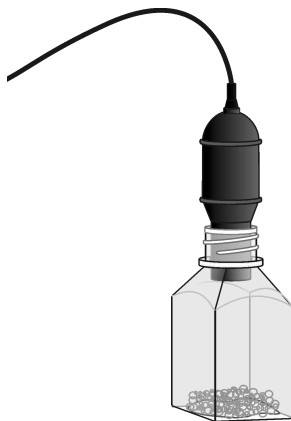


Figure 1

MATERIALS


computer
Vernier computer interface
LoggerPro
Vernier O_2 Gas Sensor
400 mL beaker
10 mL graduated cylinder
250 mL Nalgene bottle
three dropper pipettes

3.0% H_2O_2
enzyme suspension
three 18 × 150 mm test tubes
ice
pH buffers
test tube rack
thermometer

PROCEDURE

1. Obtain and wear goggles.
2. Connect the Oxygen Gas Sensor to the computer interface. Prepare the computer for data collection by opening the file “06A Enzyme (O_2)” from the *Biology with Vernier* folder of *LoggerPro*.

Part I Testing the Effect of Enzyme Concentration

3. Place three test tubes in a rack and label them 1, 2, and 3. Fill each test tube with 3 mL of 3.0% H₂O₂ and 3 mL of water.
4. 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 O₂ 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 without the need for unnecessary force.
 - f. When 30 seconds has passed, Click to begin data collection.
5. When data collection has finished, remove the O₂ gas sensor from the Nalgene bottle. Rinse the bottle with water and dry with a paper towel.
6. Move your data to a stored run. To do this, choose Store Latest Run from the Experiment menu.
7. Collect data for test tubes 2 and 3:
 - Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 4–6.
 - Add 20 drops of the enzyme solution to test tube 3. Repeat Steps 4–5.
8. Using the mouse, select the initial linear region of your data on the graph. Click on the Linear Fit button, . Click and a best-fit linear regression line will be shown for each run selected. In your data table, record the value of the slope, *m*, for each of the three solutions. (The linear regression statistics are displayed in a floating box for each of the data sets.)
9. To print a graph of concentration vs. volume showing all three data runs:
 - a. Label all three curves by choosing Text Annotation from the Insert menu, and typing “5 Drops” (or “10 Drops”, or “20 Drops”) in the edit box. Then drag each box to a position near its respective curve. Adjust the position of the arrow head.
 - b. Print a copy of the graph, with all three data sets and the regression lines displayed. Enter your name(s) and the number of copies of the graph you want.
10. Determine the rate of reaction for each of the time intervals listed in Table 3 using the procedure outlined in Step 8. Record the rates for all three data runs in the Table 3.

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 with very warm water.
 - 50–55°C: 400 mL beaker filled with hot water.
11. Rinse the three numbered test tubes used for Part I. Fill each test tube with 3 mL of 3.0% H₂O₂ and 3 mL of water. Place the test tubes in the water bath. The test tubes should be in the water bath for 5 minutes before proceeding to Step 12. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 4.
 12. Find the rate of enzyme activity for test tubes 1, 2, and 3:
 - Add 10 Drops of the enzyme solution to test tube 1. Repeat Steps 4–6.
 - Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 4–6.
 - Add 10 drops of the enzyme solution to test tube 3. Repeat Steps 4–5.
 13. Repeat Step 8 and record the reaction rate for each data set in Table 4. Calculate and record the average rate in Table 4.
 14. Record the average rate and the temperature of your water bath from Table 4 on the class data table. When the entire class has reported their data, record the class data in Table 5.

Part III Testing the Effect of pH

15. Place three clean test tubes in a rack and label them pH 4, pH 7, and pH 10.
16. Add 3 mL of 3% H₂O₂ and 3 mL of a pH buffer to each test tube, as in Table 1.

Table 1		
pH of buffer	Volume of 3% H ₂ O ₂ (mL)	Volume of buffer (mL)
pH 4	5	5
pH 7	5	5
pH 10	5	5

17. Using the test tube labeled pH 4, add 10 drops of enzyme solution and repeat Steps 4–6.
18. Using the test tube labeled pH 7, add 10 drops of enzyme solution and repeat Steps 4–6.
19. Using the test tube labeled pH 10, add 10 drops of enzyme solution and repeat Steps 4–5.
20. Repeat Steps 8 and 9 to calculate the rate of reaction and print your graph. Record the reaction rate for each pH value in Table 6.

DATA

Part I Effect of Enzyme Concentration

Table 2	
Test tube label	Slope, or rate (%/min)
5 Drops	
10 Drops	
20 Drops	

Table 3 Time intervals (Minutes)					
Rates	0–0.5 min	0.5–1.0 min	1.0–1.5 min	1.5–2.0 min	2.0–3.0 min
5 Drops					
10 Drops					
20 Drops					

Part II Effect of Temperature

Table 4	
Test tube label	Slope, or rate (%/min)
Trial 1	
Trial 2	
Trial 3	
Average	
Temperature range: ____ °C	

Table 5 (Class Data)	
Temperature tested	Average rate

Part III Effect of pH

Table 6	
Test tube label	Slope, or rate (%/min)
pH 4	
pH 7	
pH 10	

PROCESSING THE DATA

1. On Page 2 of this experiment file, create a graph of the rate of enzyme activity vs. temperature. Plot the rate values for the class data in Table 5 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. What do you think will happen to the rate of reaction if one increases the concentration of enzyme to twenty-five drops? 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. 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.
2. Presumably, at higher concentrations of H_2O_2 , there is a greater chance that an enzyme molecule might collide with H_2O_2 . If so, the concentration of H_2O_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.
3. Design an experiment to determine the effect of boiling the catalase on the rate of reaction.
4. Explain how environmental factors affect the rate of enzyme-catalyzed reactions.

TEACHER INFORMATION

Enzyme Action: Testing Catalase Activity

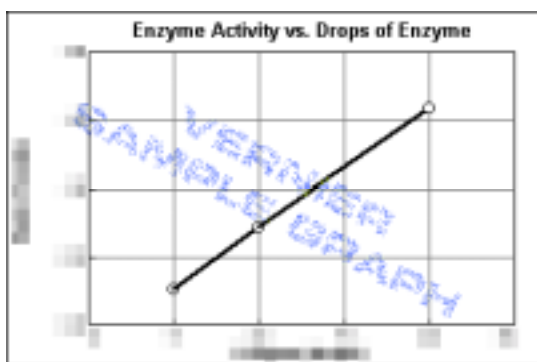
1. The student pages with complete instructions for data-collection using LabQuest App, Logger *Pro* (computers), EasyData or DataMate (calculators), and DataPro (Palm handhelds) can be found on the CD that accompanies this book. See *Appendix A* for more information.
2. This experiment may take a single 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% H₂O₂ may be purchased from any supermarket. If refrigerated, bring it to room temperature before starting the experiment.
8. To extend the life of the O₂ 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

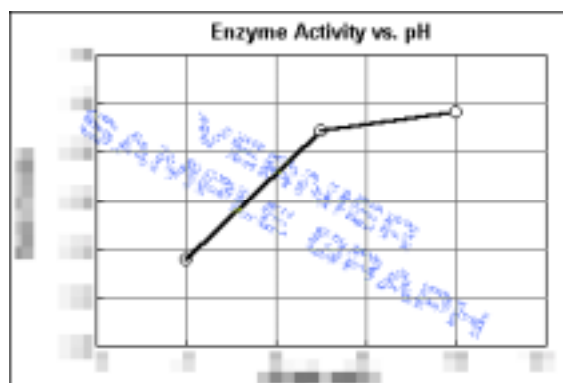
11. You may need to let students know that at pH values above 10, enzymes will become denatured and the rate of activity will drop. If you have pH buffers higher than 10, have students perform an experimental run using them.

SAMPLE RESULTS

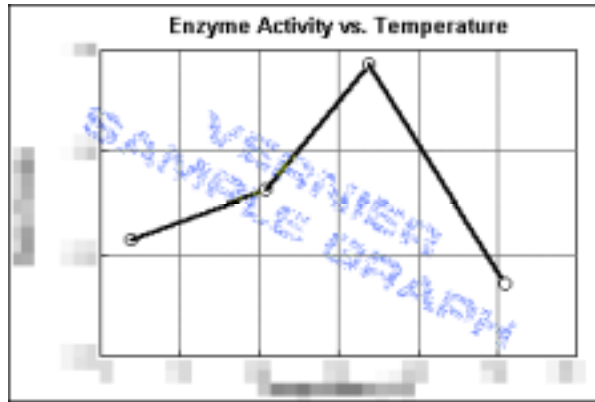
Sample class data	
Test tube label	Slope, or rate (%/min)
5 Drops	xxxx
10 Drops	xxxx
20 Drops	xxxx
0 – 5 °C range: 4°C	xxxx
20 – 25 °C range: 21 °C	xxxx
30 – 35 °C range: 34°C	xxxx
50 – 55 °C range: 51°C	xxxx
pH 4	xxxx
pH 7	xxxx
pH 10	xxxx



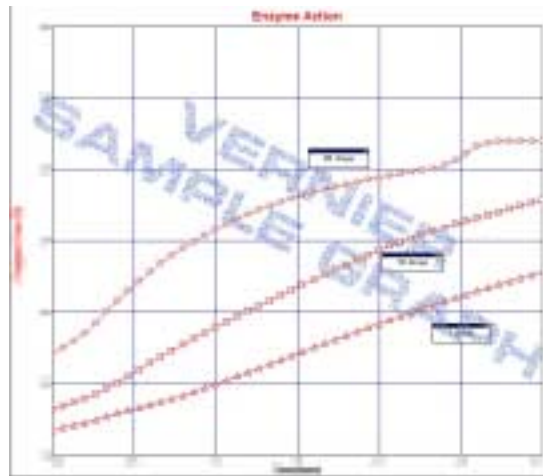
The effect of H₂O₂ concentration on the rate of enzyme activity



The effect of pH on the rate of enzyme activity



The effect of temperature on the rate of enzyme activity



Sample Data: Effect of H₂O₂ 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.