Enzyme Action: Testing Catalase Activity

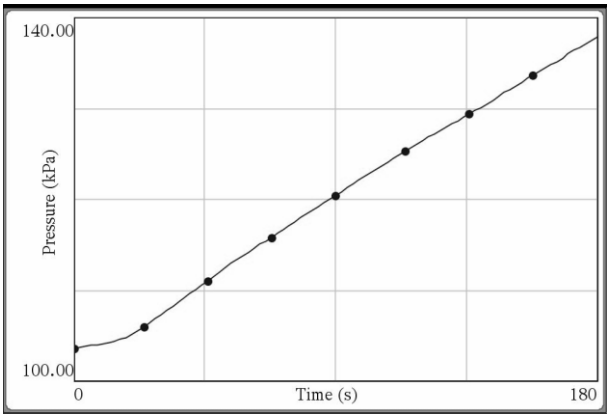
Many organisms can decompose hydrogen peroxide (H_2O_2) enzymatically. Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. They act as *catalysts*, substances that speed up chemical reactions without being destroyed or altered during the process. Enzymes are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second. Both the temperature and the pH at which enzymes function are extremely important. Most organisms have a preferred temperature range in which they survive, and their enzymes most likely function best within that temperature range. If the environment of the enzyme is too acidic or too basic, the enzyme may irreversibly *denature*, or unravel, until it no longer has the shape necessary for proper functioning.

H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2 can be converted to oxygen and water, as follows:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Although this reaction occurs spontaneously, the enzyme catalase increases the rate considerably. Catalase is found in most living organisms. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions.

In this experiment, you will measure the rate of enzyme activity under various conditions, such as different enzyme concentrations, pH values, and temperatures. It is possible to measure the pressure of oxygen gas formed as H_2O_2 is destroyed. If a plot is made, it may appear similar to the graph shown.



At the start of the reaction, there is no product, and the pressure is the same as the atmospheric pressure. After a short time, oxygen accumulates at a rather constant rate. The slope of the curve at this initial time is constant and is called the *initial rate*. As the peroxide is destroyed, less of it is available to react and the O_2 is produced at lower rates. When no more peroxide is left, O_2 is no longer produced.

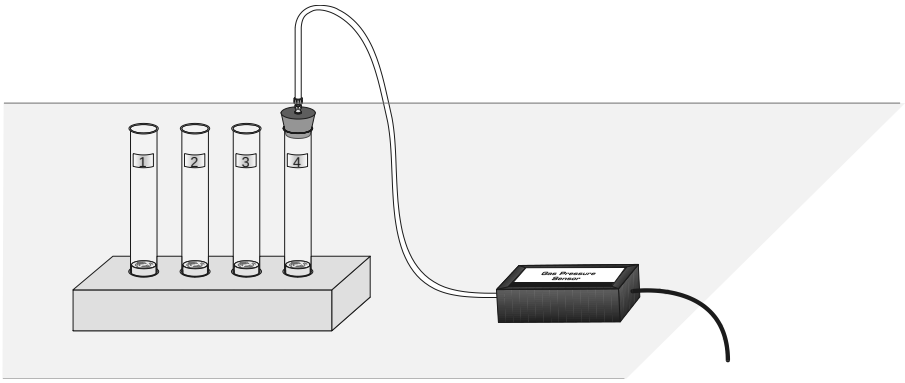


Figure 1

OBJECTIVES

In this experiment you will

- Use a Gas Pressure 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.

MATERIALS

TI-Nspire handheld **or**
computer and TI-Nspire software
data-collection interface
Vernier Gas Pressure Sensor
rubber-stopper assembly
10 mL graduated cylinder
250 mL beaker of water
3% H_2O_2
600 mL beaker
enzyme suspension
four 18 × 150 mm test tubes
ice
pH buffers
test tube rack
thermometer
four dropper pipettes

PROCEDURE

1. Obtain and wear goggles.

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

3. Connect the Gas Pressure Sensor to the data-collection interface. Connect the interface to the TI-Nspire handheld or computer.

4. Choose New Experiment from the Experiment menu. Choose Collection Setup from the Experiment menu. Enter **0.5** as the rate (samples/second) and **180** as the experiment duration in seconds. The number of points collected should be 91. Select OK.

Part I Testing the Effect of Enzyme Concentration

5. Place four test tubes in a rack and label them 1, 2, 3, and 4.

6. Add 3 mL of 3.0% H_2O_2 and 3 mL of water to each test tube.

7. Use a clean dropper pipette to add 1 drop of enzyme suspension to test tube 1. **Note:** Be sure not to let the enzyme fall against the side of the test tube.

8. Stopper the test tube and gently swirl to thoroughly mix the contents. The reaction should begin. The next step should be completed as rapidly as possible.

9. Connect the free-end of the plastic tubing to the connector in the rubber stopper as shown in Figure 2. Start data collection ( ).

10. Monitor the pressure readings displayed on the screen. If the pressure exceeds 130 kPa, 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 kPa.

11. When data collection has finished, an auto-scaled graph of pressure *vs*. time will be displayed. 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.

12. Click any data point and use ► and ◄ to examine the data pairs on the displayed graph.

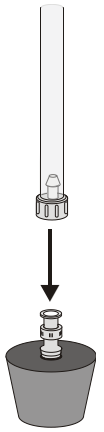


Figure 2

13. Determine the rate of enzyme activity for the curve of pressure *vs*. time. To help make comparisons between experimental runs, choose your data points at the same time values.
    a. Examine the graph and identify the most linear region.
    b. Select the linear region of the data.
    c. Choose Curve Fit ►Linear from the Analyze menu. The linear-regression statistics for these two data columns are displayed for the equation in the form
    $$y = mx + b$$
    d. Enter the slope, *m*, as the reaction rate in Table 1.

14. Find the rate of enzyme activity for test tubes 2, 3, and 4.
    a. Click the Store Latest Data Set button ( ) to save the first run. Add 2 drops of the enzyme solution to test tube 2. Repeat Steps 8–13.
    b. Click the Store Latest Data Set button ( ) to save the second run. Add 3 drops of the enzyme solution to test tube 3. Repeat Steps 8–13.
    c. Click the Store Latest Data Set button ( ) to save the third run. Add 4 drops of the enzyme solution to test tube 4. Repeat Steps 8–13.

15. Graph all four runs of data on a single graph.
    a. Click **run4**, and select All. All four runs will now be displayed on the same graph axes.
    b. Use the displayed graph and the data in Table 1 to answer the questions for Part I.

Part II Testing the Effect of Temperature

16. Insert a new **problem** in the document. Insert a new DataQuest App into problem 2. Choose Collection Setup from the Experiment menu. Enter **0.5** as the rate (samples/second) and **180** as the experiment duration in seconds. The number of points collected should be 91. Select OK.

17. Place four clean test tubes in a rack and label them T 0–5, T 20–25, T 30–35, and T 50–55.

18. Add 3 mL of 3.0% H_2O_2 and 3 mL of water to each test tube.

19. Measure the enzyme activity at 0–5°C.

    a. Prepare a water bath at a temperature in the range of 0–5°C by placing ice and water in a 600 mL beaker. Using a thermometer check that the temperature remains in this range throughout this test. See Figure 3.
    b. Place test tube T 0–5 in the cold water bath for 5 minutes so that it reaches a temperature in the 0–5°C range. Record the actual temperature of the test-tube contents in Table 2.
    c. Add 2 drops of the enzyme solution to test tube T 0–5. Repeat Steps 8–13, except this time record the reaction rate in Table 2.

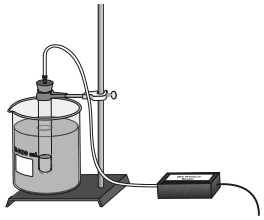


Figure 3

20. Measure the enzyme activity at 30–35°C.

    a. Prepare a water bath at a temperature in the range of 30–35°C by placing warm water in a 600 mL beaker. Using a thermometer, check that the temperature remains in this range throughout this test.
    b. Place test tube T 30–35 in the warm water bath for 5 minutes so that it reaches a temperature in the 30–35°C range. Record the actual temperature of the test-tube contents in the blank in Table 2.
    c. Add 2 drops of the enzyme solution to test tube T 30–35.
    d. Click the Store Latest Data Set button () to save the first run. Repeat Steps 8–13, again recording the reaction rate in Table 2.

21. Measure the enzyme activity at 50–55°C.

    a. Prepare a water bath at a temperature in the range of 50–55°C by placing hot water in a 600 mL beaker (hot tap water will probably work fine). Check that the temperature remains in this range throughout this test.
    b. Place test tube T 50–55 in the warm water bath until the temperature of the mixture reaches a temperature in the 50–55°C range. Record the actual temperature of the test-tube contents in the blank in Table 2.
    c. Add 2 drops of the enzyme solution to test tube T 50–55.
    d. Click the Store Latest Data Set button () to save the second run. Repeat Steps 8–13, again recording the reaction rate in Table 2.

22. Measure the enzyme activity at 20–25°C (room temperature).

    a. Record the temperature of test tube T 20–25 in Table 2.
    b. In the tube labeled T 20–25, add 2 drops of the enzyme solution.
    c. Click the Store Latest Data Set button () to save the third run. Repeat Steps 8–13, again recording the reaction rate in Table 2.

23. Graph all four runs of data on a single graph.
    a. Click **run4**, and select All. All four runs will now be displayed on the same graph axes.
    b. Use the displayed graph and the data in Table 2 to answer the questions for Part II.

Part III Testing the Effect of pH

24. Insert a new **problem** in the document. Insert a new DataQuest App into problem 3. Choose New Experiment from the Experiment menu. Choose Collection Setup from the Experiment menu. Enter **0.5** as the rate (samples/second) and **180** as the experiment duration in seconds. The number of points collected should be 91. Select OK.

25. Place three clean test tubes in a rack and label them pH 4, pH 7, and pH 10.

26. Add 3 mL of 3% H_2O_2 and 3 mL of the appropriate pH buffer to each labeled test tube label.

27. In the tube labeled pH 4, add 2 drops of the enzyme solution. Repeat Steps 8–13, except this time record the reaction rate in Table 3.

28. Click the Store Latest Data Set button () to save the first run. In the tube labeled pH 7, add 2 drops of the enzyme solution. Repeat Steps 8–13, again recording the reaction rate in Table 3.

29. Click the Store Latest Data Set button () to save the second run. In the tube labeled pH 10, add 2 drops of the enzyme solution. Repeat Steps 8–13, again recording the reaction rate in Table 3.

30. Graph all three runs of data on a single graph.
    a. Click **run3** and select All. All three runs will now be displayed on the same graph axes.
    b. Use the displayed graph and the data in Table 3 to answer the questions for Part III.

DATA

Table 1

Label	Rate (kPa/s)	Reaction Rate (kPa/min)
1 drop		
2 drops		
3 drops		
4 drops		

DATA (CONT.)

Table 2			
Label	Actual Temperature (°C)	Rate (kPa/s)	Reaction Rate (kPa/min)
0–5°C			
20–25°C			
30–35°C			
50–55°C			

Table 3		
Label	Rate (kPa/s)	Reaction Rate (kPa/min)
pH 4		
pH 7		
pH 10		

PROCESSING THE DATA

1. Convert your reaction rates from kPa/s to kPa/min. Record the rates in the appropriate tables.

2. Create summary graphs for the data in each part.
   a. Insert a new problem in the document, then Insert a new DataQuest App into problem 4. Click on the Table View tab ( ) to view the Table.
   b. Double click on the *X* column to access the column options. Enter **Test Tube** for the column name. Change the Display Precision to 0 decimal places. Select OK.
   c. Double click on the *Y* column to access the column options. Enter **Rate** for the column name. Enter **kPa/min** as the units. Select OK.
   d. Using the data from Table 1, enter the values in the DataQuest Table. Use the number of drops in the Test Tube column.
   e. Choose New Data Set from the Data menu. Using the data from Table 2, enter the values in the DataQuest Table. Use the actual temperature in the Test Tube column.
   f. Choose New Data Set from the Data menu. Using the data from Table 3, enter the values in the DataQuest Table. Use the pH value in the Test Tube column.
   g. For each data set, double click the data set name and change the name to something more meaningful (for example, change run1 to Concentration).
   h. Click on the Graph View tab ( ) to view the summary graphs. To view the different summary graphs, click on the run indicator and select the desired run.

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 the concentration of enzyme is increased to five drops? Predict what the rate would be for 5 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? Does this follow a pattern you anticipated?

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 catalase on the reaction rate.

4. Explain how environmental factors affect the rate of enzyme-catalyzed reactions.