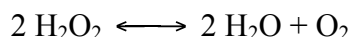


# Enzyme Action: Testing Catalase Activity

## 50 Points

Many organisms can decompose hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) 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.

$\text{H}_2\text{O}_2$  is toxic to most living organisms. Many organisms are capable of enzymatically destroying the  $\text{H}_2\text{O}_2$  before it can do much damage.  $\text{H}_2\text{O}_2$  can be converted to oxygen and water, as follows:



Although this reaction occurs spontaneously, enzymes increase the rate considerably. At least two different enzymes are known to catalyze this reaction: *catalase*, found in animals and protists, and *peroxidase*, found in plants. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions. The rate of a chemical reaction may be studied in a number of ways including:

- measuring the rate of appearance of a product (in this case,  $\text{O}_2$ , which is given off as a gas)
- measuring the rate of disappearance of substrate (in this case,  $\text{H}_2\text{O}_2$ )
- measuring the pressure of the product as it appears (in this case,  $\text{O}_2$ ).

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 concentration of oxygen gas formed as  $\text{H}_2\text{O}_2$  is destroyed using an  $\text{O}_2$  Gas Sensor. 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 concentration is the same as the atmosphere. 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  $\text{O}_2$  is produced at lower rates. When no more peroxide is left,  $\text{O}_2$  is no longer produced.

## OBJECTIVES

In this experiment, you will

- Use 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  $\text{H}_2\text{O}_2$ .
- 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.

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- 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.

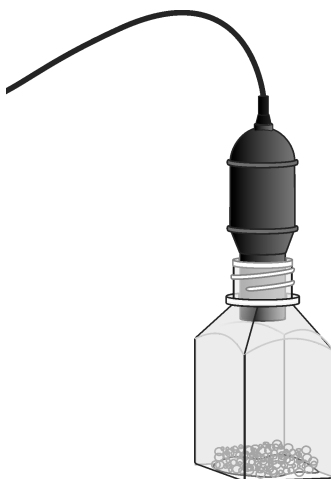


Figure 1

## MATERIALS

LabQuest  
LabQuest app  
Vernier O<sub>2</sub> Gas Sensor  
400 mL beaker  
10 mL graduated cylinder  
three 18 × 150 mm test tubes  
250 mL Nalgene bottle  
3.0% H<sub>2</sub>O<sub>2</sub>

enzyme suspension  
ice  
pH buffers  
test tube rack  
thermometer  
three dropper pipettes  
Logger *Pro* (optional)

## PROCEDURE

1. Obtain and wear goggles.
2. Connect the O<sub>2</sub> Gas Sensor to LabQuest and choose New from the File menu. If you have an older sensor that does not auto-ID, manually set up the sensor.
3. On the Meter screen, tap Rate. Change the data-collection rate to 0.2 samples/second and the data-collection length to 180 seconds.

**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 5 mL of 3.0% H<sub>2</sub>O<sub>2</sub> and 5 mL of water.
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, start data collection.
6. When data collection is complete, a graph of O<sub>2</sub> gas vs. time will be displayed. Remove the O<sub>2</sub> Gas Sensor from the Nalgene bottle. Rinse the bottle with water and dry with a paper towel.
7. Perform a linear regression to calculate the rate of reaction.
  - a. Choose Curve Fit from the Analyze menu.
  - b. Select Linear for the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form
$$y = mx + b$$
  - c. Enter the absolute value of the slope,  $m$ , as the reaction rate in Table 2.
  - d. Select OK.
8. Store the data from the first run by tapping the File Cabinet icon.
9. 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 5–8.
  - b. Add 20 drops of the enzyme solution to test tube 3. Repeat Steps 5–7.
10. Graph all three runs of data on a single graph.
  - a. Tap Run 3, and select All Runs. All three 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 I.

**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.
11. Rinse the three numbered test tubes used for Part I. Fill each test tube with 5 mL of 3.0% H<sub>2</sub>O<sub>2</sub> and 5 mL of water then place the test tubes in the water bath. The test tubes should be

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in the water bath for 5 minutes before proceeding to Step 15. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 3.

12. Tap Table. Choose Clear All Data from the Table menu.
13. Tap Graph to display the graph.
14. 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 5–7. Record the reaction rate in Table 3.
  - b. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 5–7. Record the reaction rate in Table 3.
  - c. Add 10 drops of the enzyme solution to test tube 3. Repeat Steps 5–7. Record the reaction rate in Table 3.
15. Calculate the average rate for the three trials you tested. Record the average in Table 3.
16. 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

17. Place three clean test tubes in a rack and label them pH 4, pH 7, and pH 10.
18. 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.

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

19. Tap Table. Choose Clear All Data from the Table menu.
20. Tap Graph to display the graph.
21. Using the test tube labeled pH 4, add 10 drops of enzyme solution and repeat Steps 5–8.
22. Using the test tube labeled pH 7, add 10 drops of enzyme solution and repeat Steps 5–8.
23. Using the test tube labeled pH 10, add 10 drops of enzyme solution and repeat Steps 5–7.
24. Graph all three runs of data on a single graph.
  - a. Tap Run 3 and select All Runs. All three runs will now be displayed on the same graph axes.
  - b. Use the displayed graph and the data in Table 5 to answer the questions for Part III.



**CONCLUSION** (SUMMARY OF EXPERIMENT, ANALYSIS OF DATA, DISCUSSION OF ERROR)

**QUESTIONS**

**Part I Effect of Enzyme Concentration**

1. How does changing the concentration of enzyme affect the rate of decomposition of  $\text{H}_2\text{O}_2$ ?
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?