Enzyme Action: Testing Catalase Activity

(O_2 Gas Sensor)

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 typically 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 breaking down the H_2O_2 before it can do much damage. H_2O_2 can be converted to oxygen and water, as follows:

$$2\ H_2O_2 \leftrightarrow 2\ H_2O + O_2$$

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. The rate of a chemical reaction may be studied in a number of ways including:

- Measuring the rate of appearance of a product
- Measuring the rate of disappearance of substrate
- Measuring the pressure of the product as it appears

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 H_2O_2 is destroyed using an O_2 Gas Sensor.

OBJECTIVES

- Measure the production of oxygen gas as hydrogen peroxide is broken down 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 broken down 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 broken down 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.

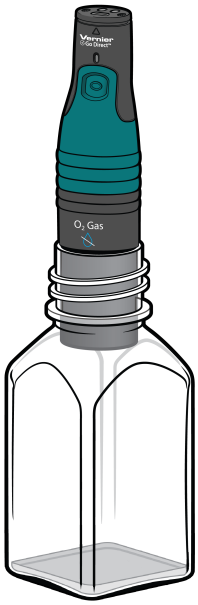

Figure 1

MATERIALS

Chromebook, computer, **or** mobile device
Graphical Analysis 4 app
Go Direct O_2 Gas
400 mL beaker
10 mL graduated cylinder
250 mL Nalgene bottle
three Beral pipettes
3.0% H_2O_2
enzyme suspension
three 18 × 150 mm test tubes
ice
pH buffers
test tube rack
thermometer
goggles
(optional) Stir Station with magnetic stir bar

PROCEDURE

1. Obtain and wear goggles.

2. Launch Graphical Analysis. Connect the O_2 Gas Sensor to your Chromebook, computer, or mobile device.

3. Set up the data-collection mode.
   a. Click or tap Mode to open Data Collection Settings.
   b. Change Rate to 0.25 samples/s.
   c. Set End Collection to 180 s. Click or tap Done.

Part I 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_2O_2 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_2 Gas Sensor into the bottle as shown in Figure 1. Gently push the sensor down into the bottle until it stops. **Note**: The sensor is designed to seal the bottle with minimal force.
   f. When 30 seconds have passed, click or tap Collect to start data collection.

6. When data collection has finished, remove the O_2 gas sensor from the Nalgene bottle. Rinse the bottle with water and dry with a paper towel.

7. Determine the rate of enzyme activity.
   a. Select the data in the most linear region of the graph.
   b. Click or tap Graph Tools, , and choose Apply Curve Fit.
   c. Select Linear as the curve fit. Click or tap Apply.
   d. Record the slope, *m*, as the reaction rate in Table 2.
   e. Dismiss the Curve Fit box.

8. Find the rate of enzyme activity for test tubes 2 and 3:
   a. Add 10 drops of enzyme solution to test tube 2. Repeat Steps 5–7. **Note**: The previous data set is automatically saved.
   b. Add 20 drops of enzyme solution to test tube 3. Repeat Steps 5–7.

9. Display all three runs of data on a single graph.
   a. To display multiple data sets on a single graph, click or tap the y-axis label and select the data sets you want to display. Dismiss the box to view the graph.
   b. Use the graph and the data in Table 2 to answer the questions for Part I.

Part II 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 warm water
- 50–55°C: 400 mL beaker filled with hot water

10. Rinse the three numbered test tubes used for Part I. Fill each test tube with 5 mL of 3.0% H_2O_2 and 5 mL of water 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 11. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 3.

11. Find the rate of enzyme activity for test tubes 1, 2, and 3:
    a. Add 10 drops of enzyme solution to test tube 1. Repeat Steps 5–7. Record the reaction rate in Table 3.
    b. Add 10 drops of enzyme solution to test tube 2. Repeat Steps 5–7. Record the reaction rate in Table 3.
    c. Add 10 drops of enzyme solution to test tube 3. Repeat Steps 5–7. Record the reaction rate in Table 3.

12. Calculate the average rate for the three trials you tested. Record the average in Table 3.

13. Record the average rate and the temperature of your water bath from Table 3 on the class data table. When the entire class has reported their data, record the class data in Table 4.

Part III Effect of pH

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

15. Add 5 mL of 3% H_2O_2 and 5 mL of a pH buffer to each test tube, as in Table 1.

Table 1

pH of buffer	Volume of 3% H_2O_2 (mL)	Volume of buffer (mL)
pH 4	5	5
pH 7	5	5
pH 10	5	5

16. Find the rate of enzyme activity for test tubes labeled pH 4, pH 7, and pH 10:
    - Add 10 drops of enzyme suspension to test tube pH 4. Repeat Steps 5–7. Record the reaction rate in Table 5.

- Add 10 drops of enzyme suspension to test tube pH 7. Repeat Steps 5–7. Record the reaction rate in Table 5.
- Add 10 drops of enzyme suspension to test tube pH 10. Repeat Steps 5–7. Record the reaction rate in Table 5.

17. Displayed all three runs of data on a single graph. Use the graph and the data in Table 5 to answer the questions for Part III.

DATA

Part I Effect of enzyme concentration

Table 2	
Sample	Reaction rate (%/min)
5 drops	
10 drops	
20 drops	

Part II Effect of temperature

Table 3	
Sample	Reaction rate (%/min)
Trial 1	
Trial 2	
Trial 3	
Average	
Temperature range: _____° C	

Table 4: Class Data	
Temperature tested (°C)	Average rate (%/min)

Part III Effect of pH

Table 5	
Sample	Reaction rate (%/min)
pH 4	
pH 7	
pH 10	

PROCESSING THE DATA

For Part II of this experiment, make a graph of the rate of enzyme activity *vs*. temperature in Graphical Analysis or by hand. 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. What do you think will happen to the rate of reaction if one increases the concentration of enzyme to 25 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? Does this follow a pattern you anticipated?

EXTENSIONS

1. Determine the reaction rates of trials in Part I for each 30 second interval. What patterns do you see? What could explain the different rates you determined?

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 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.

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.