# 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.

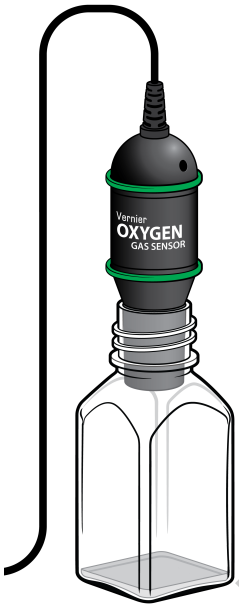


*Figure 1*

## MATERIALS

computer
Vernier computer interface
Logger *Pro*
$O_2$ Gas Sensor
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. Connect the Oxygen Gas Sensor to the computer interface. Prepare the computer for data collection by opening the file "06A Enzyme (O2)" from the *Biology with Vernier* folder of Logger *Pro*.

### Part I Effect of enzyme concentration

3. 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.

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 $O_2$ 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 has passed, click Collect to begin data collection.

5. 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.

6. Choose Store Latest Run from the Experiment menu to move your data to a stored run.

7. Collect data for test tubes 2 and 3:
   - Add 10 drops of enzyme suspension to test tube 2. Repeat Steps 4–6.
   - Add 20 drops of enzyme suspension to test tube 3. Repeat Steps 4–5.

8. Determine the slope for each of the solutions in Table 2.
   a. Select the initial linear region of your data on the graph.
   b. Click Linear Fit, . Click OK and a best-fit linear regression line will be shown for each run selected.
   c. In your data table, record the value of the slope, *m*, for each of the three solutions in Table 2.

### 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

9. 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. Place the test tubes in the water bath. The test tubes should be in the water bath for 5 minutes before proceeding to Step 10. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 3.

10. Find the rate of enzyme activity for test tubes 1, 2, and 3:
    - Add 10 drops of enzyme suspension to test tube 1. Repeat Steps 4–6.
    - Add 10 drops of enzyme suspension to test tube 2. Repeat Steps 4–6.
    - Add 10 drops of enzyme suspension to test tube 3. Repeat Steps 4–5.

11. Repeat Step 8 and record the reaction rate for each data set in Table 3. Calculate and record the average rate in Table 3.

12. 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**

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

14. 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 |

15. 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 4–6.
    - Add 10 drops of enzyme suspension to test tube pH 7. Repeat Steps 4–6.
    - Add 10 drops of enzyme suspension to test tube pH 10. Repeat Steps 4–5.

16. Repeat Step 8 and record the reaction rate for each pH value in Table 5.

# 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

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 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.

# Enzyme Action: Testing Catalase Activity

1. In the Electronic Resources you will find multiple versions of each student experiment—one for each supported data-collection software or app (Logger *Pro*, Graphical Analysis 4, Spectral Analysis, LabQuest App, and EasyData). Deliver to your students the version that supports the software and hardware they will use. Sign in to your account at **vernier.com/account** to access the Electronic Resources. See Appendix A for more information. **Note**: The printed version of the book and the PDF of the entire book (found in the Electronic Resources) include only the Logger *Pro* versions of the experiments.

2. Different sensors can be used for this experiment. The sensors that can be used are: $O_2$ (only) or Gas Pressure (only). All versions of the experiment can be found in the Electronic Resources. **Note**: The printed version of the book and the PDF of the entire book contain the $O_2$ (only) version of the experiment.

3. Test the experiment before the students begin. Depending on the type of enzyme you use, the activity will vary greatly, you may need to dilute the enzyme solution or make a new solution to get the ideal reaction rate.

4. This experiment may take a single group several lab periods to complete. A good breaking point is after the completion of Part I, 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.

5. 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.

6. Many different organisms may be used as a source of catalase in this experiment. Beef liver, potato, or living yeast can be used for 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.

7. We recommend purchasing purified catalase enzyme from Flinn Scientific, Ward's Natural Science, or Sigma-Aldrich. The concentration of enzyme varies from 2000–5000 units/mg and depends on the bottle. Store the catalase powder as instructed. Enzyme activity may decrease from year to year, but will remain viable for up to three years.

8. Follow the instructions below to prepare an enzyme solution
   a. Purified catalase
      i. Make a stock solution of 1000 units/mL.
      ii. Dilute the stock solution to 200 units/mL for use by the students.

b. Yeast suspension
   i. Dissolve 1 package (7 g) of dried yeast per 100 mL of 2% sugar solution. To prepare a 2% sugar solution, add 20 grams of sugar to make one liter of solution.
   ii. Incubate the suspension in 37–40°C water for at least 10 minutes to activate the yeast.
   iii. To ensure a uniform yeast concentration, make the suspension available on a magnetic stirrer and instruct your students to withdraw their samples from the center as the suspension is being stirred.
   iv. The yeast may need to be diluted if the reaction occurs too rapidly.

c. Liver suspension
   i. Homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water.
   ii. Keep the suspension on ice until it is to be used.
   iii. Dilute the suspension as needed based on reaction rate.

9. You can purchase 3% $H_2O_2$ at any supermarket. If refrigerated, bring it to room temperature before starting the experiment.

10. Vernier Software sells a pH buffer package for preparing buffer solutions with pH values of 4, 7, and 10 (order code: PH-BUFCAP). Simply add the capsule contents to 100 mL of distilled water.

11. 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.

12. 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.

13. All Vernier Oxygen Gas Sensors should always be used and stored upright. Do not get the sensor wet.

14. If using a Gas Pressure Sensor or a Go Direct Pressure Sensor, the accessory items used in this experiment are the #1 single hole stopper fitted with a tapered valve connector and the section of plastic tubing fitted with Luer-lock connectors. Remind your students that a 1/2 to 3/4 turn of the Luer lock is sufficient to tighten the connection. Tightening down the Luer lock too much can damage the fitting.

15. If you are using Go Direct sensors, see **www.vernier.com/start/go-direct** for information about how to connect your sensor.

16. For additional information about the Vernier probeware used in this experiment, including tips and product specifications, visit **www.vernier.com/manuals** and download the appropriate user manual.

## ESTIMATED TIME

We estimate that setup and data collection can be completed in two 45-minute class periods.

## NEXT GENERATION SCIENCE STANDARDS (NGSS)

| Science and Engineering Practices | Disciplinary Core Ideas | Crosscutting Concepts |
|---|---|---|
| Analyzing and Interpreting Data<br>Developing and Using Models | LS1.A: Structure and Function | Cause and Effect<br>Structure and Function |

## SAMPLE RESULTS

Sample data for all versions of the student experiment are provided.

**Using an $O_2$ Gas Sensor**

| Test tube | Slope (or rate) (%/min) |
|---|---|
| 5 drops | 0.27 |
| 10 drops | 0.73 |
| 20 drops | 1.59 |
| 0–5°C range: 4°C | 0.58 |
| 20–25°C range: 21°C | 0.82 |
| 30–35°C range: 34°C | 1.43 |
| 50–55°C range: 51°C | 0.36 |
| pH 4 | 0.36 |
| pH 7 | 0.89 |
| pH 10 | 0.97 |

**Using a Gas Pressure Sensor**

| Test tube | Slope (or rate) (kPa/min) |
|---|---|
| 1 drop | 10.23 |
| 2 drops | 44.98 |
| 3 drops | 59.36 |
| 4 drops | 98.26 |
| 0–5°C range: 4°C | 41.43 |
| 20–25°C range: 21°C | 48.02 |
| 30–35°C range: 34°C | 73.85 |
| 50–55°C range: 51°C | 27.55 |
| pH 4 | 36.57 |
| pH 7 | 66.86 |
| pH 10 | 75.27 |

## ANSWERS TO QUESTIONS

1. The rate should be highest when the concentration of enzyme is highest. With higher concentration of enzyme, there is a greater chance of an effective collision between the enzyme and $H_2O_2$ molecule.

2. Roughly, the rate doubles when the concentration of enzyme doubles. Since the data are somewhat linear, the rate is proportional to the concentration. At a concentration of 5 drops, the rate in the above experiment should be about 111 kPa/min.

3. The temperature at which the rate of enzyme activity is the highest should be close to 30°C. The lowest rate of enzyme activity should be at 60°C.

4. The rate increases as the temperature increases, until the temperature reaches about 50°C. Above this temperature, the rate decreases.

5. At high temperatures, enzymes lose activity as they are denatured.

6. Student answers may vary. Activity is usually highest at pH 10 and lowest at pH 4.

7. Student answers may vary. Usually, the enzyme activity increases from pH 4 to 10. At low pH values, the protein may denature or change its structure. This may affect the enzyme's ability to recognize a substrate or it may alter its polarity within a cell.