

ENZYME ACTION: TESTING CATALASE ACTIVITY



Westminster College

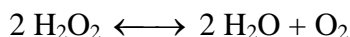
STANDARDS

- **3.3.10B** - Explain cell functions and processes in terms of chemical reactions and energy changes.
- **3.3.12B** - Identify and describe factors affecting metabolic function (e.g., temperature, acidity, hormones). Evaluate metabolic activities using experimental knowledge of enzymes.

INTRODUCTION

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:



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, O_2 , which is given off as a gas)
- measuring the rate of disappearance of substrate (in this case, H_2O_2)
- measuring the pressure of the product as it appears (in this case, O_2).

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 O_2 is produced at lower rates. When no more peroxide substrate is left, O_2 is no longer produced.

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.

GUIDING QUESTIONS

- What is being measured as the yeast enzyme catalase breaks down the hydrogen peroxide?
- What do you observe about the rate of the reaction when the concentration of the catalase enzyme is varied?
- How is the catalase activity affected when the temperature and pH of the enzyme reaction is altered?

SAFETY

The students may want to wear goggles during the lab. Hydrogen peroxide will irritate the eyes if it is accidentally splashed in them.

MATERIALS

LabQuest
 LabQuest App
 Vernier O₂ Gas Sensor
 400 mL beaker
 10 mL graduated cylinder
 three 18 × 150 mm test tubes
 250 mL Nalgene bottle
 3.0% H₂O₂

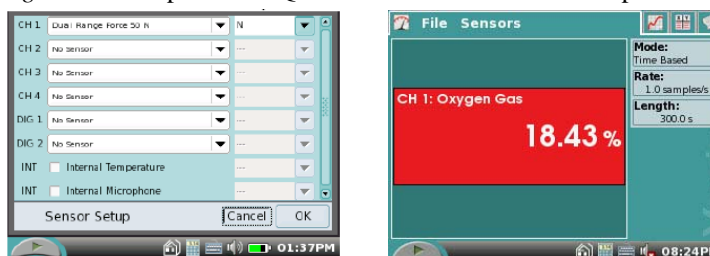
enzyme suspension
 ice
 pH buffers
 test tube rack
 thermometer
 three dropper pipettes
 Logger *Pro* (optional)
 Water baths (35°C, 55°C)

PROCEDURE

1. Connect the O₂ Gas Sensor to the LabQuest via a channel port and choose New from the File menu. If you have an older sensor that does not auto-ID, manually set up the sensor. To do this manually:

- Stay in the Meter mode and select Sensors → Sensor Setup....→. A sensor set-up screen will appear showing all the available probe ports (*Fig. 1a*).
- Select the channel that the O₂ Gas sensor is plugged into (ex. CH1 for channel 1), and tap the arrow to the side of the channel box.
- A list of compatible probes will appear in alphabetical order. Scroll down the list and select “O₂ Gas Old”. Select **OK** to return to the Meter mode screen.
- A red box will now be in this window

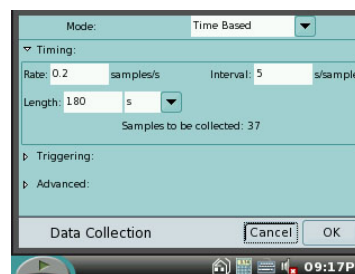
Figure 1. Example of LabQuest Screens and Sensor Set-Up



a. Sensor set-up screen

b. Meter mode screen


Figure 2. Data Collection Screen




displaying the channel the O₂ Gas sensor is plugged into (*Fig. 1b*). The LabQuest is reading in % O₂.

- To the right of the Meter screen, tap the gray Rate box. This will bring up the Data Collection screen (*Fig. 2*). Change the data-collection rate to **0.2 samples/second** and the data-collection length to **180 seconds**.

Part I. The Uncatalyzed Rate of H₂O₂ Decomposition

- Obtain approximately 5 mL “day old” 3.0% mL H₂O₂ from your teacher. This H₂O₂ was allowed to decompose spontaneously in an open beaker for at least 24 hours at room temperature. This is what will be used to determine the rate of spontaneous conversion of H₂O₂ to H₂O and O₂.
- Place a clean test tube in a rack and fill the tube with 5 mL of 3.0% H₂O₂ and 5 mL of water.
- Initiate the enzyme catalyzed reaction.
 - Using a clean dropper pipette, add 10 drops of enzyme suspension to the test tube.
 - Begin timing with a stopwatch or clock.
 - Cover the opening of the test tube with Parafilm and gently invert the test tube two times.
 - Pour the contents of the test tube into a clean 250 mL Nalgene bottle.
 - Place the O₂ Gas Sensor into the bottle as shown in Figure 1. Gently push the sensor down into the bottle until it stops (*Fig. 3*). The sensor is designed to seal the bottle with minimal force.
 - After 30 seconds have passed, tap the Start icon  to begin data collection.
- When data collection is complete, a graph of O₂ gas vs. Time(s) will be displayed. Remove the O₂ 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. If the graph is not completely linear, tap and drag the stylus across a linear portion of the data. Gray shading on the graph will indicate the section of data that will be analyzed using the following steps.

- While still in the Graph mode , choose Curve Fit from the Analyze menu.
- Select Linear for the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form

$$y = mx + b$$






- Enter the absolute value of the slope, m , as the reaction rate in Table 2.
- Select .



Figure 3. Set-up of O₂ sensor and Nalgene bottle for catalase reactions.



Part II Testing the Effect of Enzyme Concentration

8. Place three test tubes in a rack and label them 1, 2, and 3. Fill each test tube with 5 mL of 3.0% H₂O₂ and 5 mL of water.
9. Tap the Table icon  at the top of the screen. Choose Clear All Data from the Table menu. Select when prompted.
10. Tap the Graph icon  to display the graph again.
11. Find the rate of enzyme activity for test tubes 1, 2, and 3:
 - a. Add 5 drops of the enzyme solution to test tube 1. Repeat Steps 5b-7 from Part I.
 - b. Store the data from the first run by tapping the File Cabinet icon  to the right of the screen.
 - c. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 5b-7.
 - d. Store the data from the second run by tapping the File Cabinet icon  to the right of the screen.
 - e. Add 20 drops of the enzyme solution to test tube 3. Repeat Steps 5b-7.
12. Graph all three runs of data on a single graph.
 - a. Tap the gray Run 3 box at the right of the screen, and select All Runs from the drop down menu. 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 II.

Part III Testing the Effect of Temperature





13. 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.
14. Rinse the three numbered test tubes used for Part I. Fill each test tube with 5 mL of 3.0% H₂O₂ 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 15. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 3.
15. Tap the Table icon  at the top of the screen. Choose Clear All Data from the Table menu. Select when prompted.
16. Tap the Graph icon  to display the graph again.
17. 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 5b–7 from Part I. Record the reaction rate in Table 4.
 - b. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 5b–7. Record the reaction rate in Table 4.
 - c. Add 10 drops of the enzyme solution to test tube 3. Repeat Steps 5b–7. Record the reaction rate in Table 4.
18. Calculate the average rate for the three trials you tested. Record the average in Table 4.
19. Record the average rate and the temperature of your water bath from Table 4 on the class chalkboard. When the entire class has reported their data on the chalkboard, record the class data in Table 5.

Part IV Testing the Effect of pH

20. Place three clean test tubes in a rack and label them pH 4, pH 7, and pH 10.
21. Add 5 mL of 3% H₂O₂ and 5 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

22. Tap the Table icon  at the top of the screen. Choose Clear All Data from the Table menu. Select when prompted.
23. Tap the Graph icon  to display the graph again.
24. Using the test tube labeled pH 4, add 10 drops of enzyme solution and repeat Steps 5b–7. Store the data from the first run by tapping the File Cabinet icon  to the right of the screen.
25. Using the test tube labeled pH 7, add 10 drops of enzyme solution and repeat Steps 5b–7. Store the data from the first run by tapping the File Cabinet icon  to the right of the screen.
26. Using the test tube labeled pH 10, add 10 drops of enzyme solution and repeat Steps 5b–7.
27. Graph all three runs of data on a single graph. Tap Run 3 and select All Runs. All three runs will now be displayed on the same graph axes. Use the displayed graph and the data in Table 6 to answer the questions for Part III.

REFERENCES

The College Board Advanced Placement Program. Biology Lab Manual for Students. Lab Two: Enzyme Catalysis. © 2001 by the College Examination Board. Pp. 19-28.

The College Board Advanced Placement Program. Biology Lab Manual for Teachers. Lab Two: Enzyme Catalysis. © 2001 by the College Examination Board. Pp. 11-18.

Kelly Redding and David Masterman. Advanced Biology with Vernier. (2008) Enzyme Action: Testing Catalase Activity (Method 1-O₂). Vernier Software & Technology; 13979 S.W. Millikan Way, Beaverton, OR pp. 2-1(O₂) to 2-6(O₂), 2T-1(O₂) to 2T-3(O₂).

CREDITS

Special thanks to Chris Cassano of Wilmington High School, New Wilmington, PA and John Thompson of Neshannock High School, Neshannock Township, PA for testing, editing and reviewing this protocol. The lab was revised and adapted from the above references by Dr. Stephanie Corrette-Bennett.

DATA SHEET

Name: _____

Group: _____

Date: _____

Part I Spontaneous Decomposition of H₂O₂

Table 2	
# drops enzyme	Slope, or rate (% O ₂ /s)
10	

Part II Effect of Enzyme Concentration

Table 3	
Test tube label	Slope, or rate (% O ₂ /s)
5 Drops	
10 Drops	
20 Drops	

Part III Effect of Temperature

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

Table 5 (Class data)	
Temperature tested	Average rate
0-5°C	
20-25°C	
30-35°C	
50-55°C	

Part IV Effect of pH

Table 6	
Test tube label	Slope, or rate (% O ₂ /s)
pH 4	
pH 7	
pH 10	

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 IV 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?