ENZYME ACTION: TESTING CATALASE ACTIVITY

LAB ENZ 1.CALC
From Biology with Calculators, Vernier Software & Technology, 2000

INTRODUCTION

Many organisms can decompose hydrogen peroxide (H₂O₂) 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.

H₂O₂ is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H₂O₂ before it can do much damage. H₂O₂ can be converted to oxygen and water, as follows:

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

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.

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 H₂O₂ 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](image-url)

**PURPOSE**

The purpose of this experiment is to study the effect of varying conditions such as enzyme concentration, pH, and temperature on the rate of enzyme activity.

**EQUIPMENT/MATERIALS**

- LabPro interface
- TI Graphing calculator
- DataMate program
- Vernier Gas Pressure Sensor
- rubber-stopper assembly
- 10-mL graduated cylinder
- 250-mL beaker of water
- 1.5% $H_2O_2$
- 3% $H_2O_2$
- enzyme suspension
- 4, 18 x 150 mm test tubes
- ice
- pH buffers (4, 7, 10)
- test tube rack
- thermometer
- 3, dropper pipets
- Graphical Analysis (optional)
- 600-mL beaker

**SAFETY**

- Always wear goggles in the lab.
PROCEDURE
1. Connect the plastic tubing to the valve on the Gas Pressure Sensor

2. Plug the Gas Pressure Sensor into Channel 1 of the LabPro. Use the link cable to connect the TI Graphing Calculator to the LabPro. Firmly press in the cable ends.

3. Turn on the calculator and start the DATAMATE program. Press CLEAR to reset the program.

4. Set up the data-collection mode.
   a. To select MODE, press (the up arrow key) once and press ENTER.
   b. Select TIME GRAPH from the SELECT MODE menu.
   c. Select CHANGE TIME SETTINGS from the TIME GRAPH SETTINGS menu.
   d. Enter “3” as the time between samples in seconds.
   e. Enter “60” as the number of samples (data will be collected for 3 minutes).
   f. Select 1:OK to return to the setup screen.
   g. Select 1:OK to return to the main screen.

Part I: Testing the Effect of Enzyme Concentration

5. Place four test tubes in a rack and label them 1, 2, 3, and 4. These stand for 1 drop, 2 drops, 3 drops, and 4 drops.

6. Add 6 mL of 1.5% H₂O₂ to each test tube.
7. Using a clean dropper pipette, 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. Select 2:START to begin data collection. Data collection will end after 3 minutes.

10. Monitor the pressure readings displayed on the calculator 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 on the calculator screen. As you move the cursor right or left, the time (X) and pressure (Y) values of each data point are displayed below the graph.

12. 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.

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 determine the most linear region.
   b. Move the flashing cursor to the first point of the region. Enter the initial pressure, \( P_i \), and initial time, \( t_i \), in Data Table 1.
   c. Move the flashing cursor to the last point of the region. Enter the final pressure, \( P_f \), and final time, \( t_f \), in Data Table 1.

14. Press \( \text{ENTER} \) to return to the main screen.

15. Find the rate of enzyme activity for test tubes 2, 3 and 4:
   a. Add 2 drops of the enzyme solution to test tube 2. Repeat Steps 8 – 14.
   b. Add 3 drops of the enzyme solution to test tube 3. Repeat Steps 8 – 14.
   c. Add 4 drops of the enzyme solution to test tube 4. Repeat Steps 8 – 14.
Part II: Testing the Effect of Temperature

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

17. Add 6 mL of 1.5% H₂O₂ to each test tube.

18. 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 the blank in Data Table 2.
   c. Add 2 drops of the enzyme solution to test tube T 0-5. Repeat Steps 8 – 14.

19. 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 Data Table 2.
   c. Add 2 drops of the enzyme solution to test tube T 30-35. Repeat Steps 8 – 14.

20. 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 Data Table 2.
   c. Add 2 drops of the enzyme solution to test tube T 50-55. Repeat Steps 8 – 14.

21. Measure the enzyme activity at 20-25°C (room temperature):
   a. Record the temperature of test tube T 20-25 in Data Table 2.
   b. In the tube labeled T 20-25, add 2 drops of the enzyme solution. Repeat Steps 8 – 14.
Part III: Testing the Effect of pH

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

23. Add 3 mL of 3% H$_2$O$_2$ and 3 mL of each pH buffer to each test tube, as in Table 1.

<table>
<thead>
<tr>
<th>pH of buffer</th>
<th>Volume of 3% H$_2$O$_2$ (mL)</th>
<th>Volume of buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>pH 7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>pH 10</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

24. In the tube labeled pH 4, add 2 drops of the enzyme solution. Repeat Steps 8 – 14.

25. In the tube labeled pH 7, add 2 drops of the enzyme solution. Repeat Steps 8 – 14.

## DATA SHEET

Name ________________________
Name ________________________
Period _______ Class ___________
Date ___________

### ENZYME ACTION: TESTING CATALASE ACTIVITY

#### DATA TABLES

<table>
<thead>
<tr>
<th>Data Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="" /></td>
</tr>
</tbody>
</table>

### Data Table 1

<table>
<thead>
<tr>
<th>Label</th>
<th>Point 1</th>
<th>Point 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_i$ (kPa)</td>
<td>$t_i$ (s)</td>
</tr>
<tr>
<td>1 Drop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 5°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 25°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 – 35°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 – 55°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test tube label</td>
<td>Slope, or Rate (kPa/s)</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------</td>
<td></td>
</tr>
<tr>
<td>1 Drop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 5°C range:</td>
<td>______ °C</td>
<td></td>
</tr>
<tr>
<td>20 – 25°C range:</td>
<td>______ °C</td>
<td></td>
</tr>
<tr>
<td>30 – 35°C range:</td>
<td>______ °C</td>
<td></td>
</tr>
<tr>
<td>50 – 55°C range:</td>
<td>______ °C</td>
<td></td>
</tr>
<tr>
<td>pH 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PROCESSING THE DATA**

1. Calculate the rate of enzyme activity for each part of this experiment recorded in Data Table 1. The rate of enzyme activity is equal to the slope. Record the rates in Data Table 2.

2. For Part I of this experiment, make a graph of the rate of enzyme activity vs. enzyme concentration by hand or by using Graphical Analysis. Plot the rate values from Data Table 2 on the y-axis, and the number of drops of enzyme on the x-axis.

3. For Part II of this experiment, make a graph of the rate of enzyme activity vs. temperature. Plot the rate values from Data Table 2 on the y-axis, and the temperature on the x-axis.

4. For Part III of this experiment, make a graph of the rate of enzyme activity vs. pH. Plot the rate values from Data Table 2 on the y-axis, and the pH on the x-axis.
QUESTIONS
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 five drops, what do you think will happen to the rate of reaction? Predict what the rate would be for five drops.

3. At what pH is the rate of enzyme activity the highest? Lowest?

4. How does changing the pH affect the rate of enzyme activity?

5. At what temperature is the rate of enzyme activity the highest? Lowest? Explain.
6. How does changing the temperature affect the rate of enzyme activity? Does this follow a pattern you anticipated?

7. Why might the enzyme activity decrease at very high temperatures?

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 \( \text{H}_2\text{O}_2 \), there is a greater chance that an enzyme molecule might collide with \( \text{H}_2\text{O}_2 \). If so, the concentration of \( \text{H}_2\text{O}_2 \) 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.