Enzyme Action:
Testing Catalase Activity

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. 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, O₂, which is given off as a gas)
- measuring the rate of disappearance of substrate (in this case, H₂O₂)
- measuring the pressure of the product as it appears (in this case, O₂).

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 H₂O₂ is destroyed using an O₂ 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 O₂ is produced at lower rates. When no more peroxide is left, O₂ is no longer produced.
OBJECTIVES

In this experiment, you will

- Use a computer and 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.
- 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.

MATERIALS

- computer
- Vernier computer interface
- LoggerPro
- Vernier O$_2$ Gas Sensor
- 400 mL beaker
- 10 mL graduated cylinder
- 250 mL Nalgene bottle
- three dropper pipettes
- 3.0% H$_2$O$_2$
- enzyme suspension
- three 18 × 150 mm test tubes
- ice
- pH buffers
- test tube rack
- thermometer

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 Computers folder of LoggerPro.
Part I Testing the Effect of Enzyme Concentration

3. Place three test tubes in a rack and label them 1, 2, and 3. Fill each test tube with 3 mL of 3.0% H₂O₂ and 3 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 O₂ 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 without the need for unnecessary force.
   f. When 30 seconds has passed, Click [Collect] to begin data collection.

5. When data collection has finished, remove the O₂ gas sensor from the Nalgene bottle. Rinse the bottle with water and dry with a paper towel.

6. Move your data to a stored run. To do this, choose Store Latest Run from the Experiment menu.

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

8. Using the mouse, select the initial linear region of your data on the graph. Click on the Linear Fit button, [ ] Click [OK] and a best-fit linear regression line will be shown for each run selected. In your data table, record the value of the slope, \( m \), for each of the three solutions. (The linear regression statistics are displayed in a floating box for each of the data sets.)

9. To print a graph of concentration vs. volume showing all three data runs:
   a. Label all three curves by choosing Text Annotation from the Insert menu, and typing “5 Drops” (or “10 Drops”, or “20 Drops”) in the edit box. Then drag each box to a position near its respective curve. Adjust the position of the arrow head.
   b. Print a copy of the graph, with all three data sets and the regression lines displayed. Enter your name(s) and the number of copies of the graph you want.

10. Determine the rate of reaction for each of the time intervals listed in Table 3 using the procedure outlined in Step 8. Record the rates for all three data runs in the Table 3.
**Experiment 6A**

**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 with very warm water.
- 50 – 55°C: 400 mL beaker filled with hot water.

11. Rinse the three numbered test tubes used for Part I. Fill each test tube with 3 mL of 3.0% H₂O₂ and 3 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 12. Record the temperature of the water bath, as indicated on the thermometer, in the space provided in Table 4.

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

13. Repeat Step 8 and record the reaction rate for each data set in Table 4. Calculate and record the average rate in Table 4.

14. Record the average rate and the temperature of your water bath from Table 4 on the class data table. When the entire class has reported their data, record the class data in Table 5.

**Part III  Testing the Effect of pH**

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

16. Add 3 mL of 3% H₂O₂ and 3 mL of a pH buffer to each test tube, as in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of buffer</td>
</tr>
<tr>
<td>pH 4</td>
</tr>
<tr>
<td>pH 7</td>
</tr>
<tr>
<td>pH 10</td>
</tr>
</tbody>
</table>

17. Using the test tube labeled pH 4, add 10 drops of enzyme solution and repeat Steps 4 – 6.

18. Using the test tube labeled pH 7, add 10 drops of enzyme solution and repeat Steps 4 – 6.


20. Repeat Steps 8 and 9 to calculate the rate of reaction and print your graph. Record the reaction rate for each pH value in Table 6.
**Enzyme Action: Testing Catalase Activity**

**DATA**

**Part I Effect of Enzyme Concentration**

<table>
<thead>
<tr>
<th>Test tube label</th>
<th>Slope, or rate (%/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Drops</td>
<td></td>
</tr>
<tr>
<td>10 Drops</td>
<td></td>
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<tr>
<td>20 Drops</td>
<td></td>
</tr>
</tbody>
</table>

**Part II Effect of Temperature**

<table>
<thead>
<tr>
<th>Test tube label</th>
<th>Slope, or rate (%/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td></td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
</tr>
<tr>
<td>Trial 3</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
</tr>
</tbody>
</table>

Temperature range: _____°C

<table>
<thead>
<tr>
<th>Temperature tested</th>
<th>Average rate</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<td></td>
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</tbody>
</table>

**Part III Effect of pH**

<table>
<thead>
<tr>
<th>Test tube label</th>
<th>Slope, or rate (%/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4</td>
<td></td>
</tr>
<tr>
<td>pH 7</td>
<td></td>
</tr>
<tr>
<td>pH 10</td>
<td></td>
</tr>
</tbody>
</table>
PROCESSING THE DATA

1. 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 5 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 H₂O₂?

2. What do you think will happen to the rate of reaction if one increases the concentration of enzyme to twenty-five drops? Predict what the rate would be for 25 drops.

3. When is the reaction rate highest? Explain why.

4. When is the reaction rate lowest? Why?

Part II Effect of Temperature

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?

Part III Effect of pH

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

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

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 H₂O₂, there is a greater chance that an enzyme molecule might collide with H₂O₂. If so, the concentration of H₂O₂ 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.

3. Design an experiment to determine the effect of boiling the catalase on the rate of reaction.

4. Explain how environmental factors affect the rate of enzyme-catalyzed reactions.
Enzyme Action:
Testing Catalase Activity

1. This experiment may take a single group several lab periods to complete. A good breaking point is after the completion of Step 10, 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.

2. 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.

3. Many different organisms may be used as a source of catalase in 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. Often, either beef liver, beef blood, or living yeast are used.

4. To prepare the yeast solution, dissolve 7 g (1 package) of dried yeast per 100 mL of 2% glucose solution. A 2% glucose is made by adding 20 g of glucose to enough distilled water to make 1 L of solution. Incubate the suspension in 37 – 40°C water for at least 10 minutes to activate the yeast. Test the experiment before the students begin. The yeast may need to be diluted if the reaction occurs too rapidly. The reaction in Step 4, with 10 mL of 1.5% hydrogen peroxide, and 5 drops of suspension produces enough oxygen to exceed a measured concentration of 22% in 40 to 60 seconds.

5. To prepare a liver suspension, homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water. You will need to test the suspension before use, as its activity varies greatly depending on its freshness. Dilute the suspension until the reaction in Step 4, with 10 mL of 1.5% hydrogen peroxide, and 5 drops of suspension produces enough oxygen to exceed a measured concentration of 22% in 40 to 60 seconds. The color of the suspension will be a faint pink. Keep the suspension on ice until used in an experiment.

6. 3% H₂O₂ may be purchased from any supermarket. If refrigerated, bring it to room temperature before starting the experiment.

7. To extend the life of the O₂ Gas Sensor, always store the sensor upright in the box in which it was shipped.

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

9. 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.

10. 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.
SAMPLE RESULTS

<table>
<thead>
<tr>
<th>Test tube label</th>
<th>Slope, or rate (%/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Drops</td>
<td>0.27</td>
</tr>
<tr>
<td>10 Drops</td>
<td>0.73</td>
</tr>
<tr>
<td>20 Drops</td>
<td>1.59</td>
</tr>
<tr>
<td>0 – 5 °C range: 4°C</td>
<td>0.58</td>
</tr>
<tr>
<td>20 – 25 °C range: 21 °C</td>
<td>0.82</td>
</tr>
<tr>
<td>30 – 35 °C range: 34°C</td>
<td>1.43</td>
</tr>
<tr>
<td>50 – 55 °C range: 51°C</td>
<td>0.36</td>
</tr>
<tr>
<td>pH 4</td>
<td>0.36</td>
</tr>
<tr>
<td>pH 7</td>
<td>0.89</td>
</tr>
<tr>
<td>pH 10</td>
<td>0.97</td>
</tr>
</tbody>
</table>

**The effect of H₂O₂ concentration on the rate of enzyme activity**

**The effect of pH on the rate of enzyme activity**

**The effect of temperature on the rate of enzyme activity**
Sample Data: Effect of $H_2O_2$ concentration on the rate of enzyme activity.

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 25 drops, the rate would be about 2.42 %/min.

3. Student answers vary. Ideal data would have the rate being the highest during the first (and maybe second) interval. This is because there are a large number of substrate molecules in comparison to the number of enzyme molecules and there will be a maximum number of collisions between the enzyme and the substrate.

4. Student answers vary. Ideal data would have the rate being lowest (the rate would be zero or would approach zero) during the last intervals. As the number of substrate molecules decreases and the number of product molecules increases, the number of collisions between the enzyme and the substrate decreases.

5. 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.

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

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

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

9. 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.