Experiment
6A

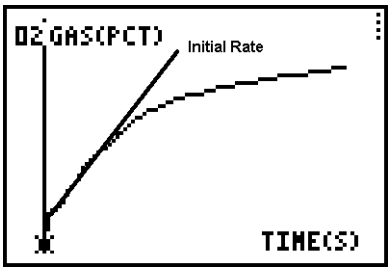
Enzyme Action: Testing Catalase Activity

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:

$$2\ H_2O_2 \longleftrightarrow 2\ H_2O + O_2$$

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, 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).

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

OBJECTIVES

In this experiment, you will

- use 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 H_2O_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.

Figure 1

MATERIALS

LabPro or CBL 2 interface
TI Graphing Calculator
DataMate program
Vernier O_2 Gas Sensor
400-mL beaker
10-mL graduated cylinder
250-mL Nalgene bottle
1.5% H_2O_2
3.0% H_2O_2
enzyme suspension
three 18 x 150 mm test tubes
ice
pH buffers
test tube rack
thermometer
three dropper pipettes
Graphical Analysis (optional)

PROCEDURE

1. Obtain and wear goggles.

2. Plug the O_2 Gas Sensor into Channel 1 of the LabPro or CBL 2 interface. Use the link cable to connect the TI Graphing Calculator to the interface. 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 calculator and interface for an O_2 Gas Sensor.
   a. Select SETUP from the main screen.
   b. If the calculator displays OXYGEN GAS (PCT) in CH 1, proceed directly to Step 5. If it does not, continue with this step to set up your sensor manually.
   c. Press ENTER to select CH 1.
   d. Select OXYGEN GAS from the SELECT SENSOR menu.
   e. Select percent (PCT) as the unit.

5. 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 "5" as the time between samples in seconds.
   e. Enter "36" as the number of samples (data will be collected for 3 minutes).
   f. Select OK to return to the setup screen.
   g. Select OK to return to the main screen.

Part I Testing the Effect of Enzyme Concentration

6. Place three test tubes in a rack and label them 1, 2, and 3. Fill each test tube with 10 mL of 1.5% H_2O_2.

7. 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. The sensor is designed to seal the bottle with minimal force.
   f. When 30 seconds has passed, select START on the calculator to begin data collection.

8. When data collection has finished, a graph of O2 GAS VS. TIME will be displayed. Press ENTER to return to the main screen.

9. Remove the O_2 Gas Sensor from the Nalgene bottle. Rinse the bottle with water and dry with a paper towel.

10. Perform a linear regression to calculate the rate of reaction.
    a. Select ANALYZE from the main screen.
    b. Select CURVE FIT from the ANALYZE OPTIONS menu.
    c. Select LINEAR (CH 1 VS TIME) from the CURVE FIT menu.
    d. The linear-regression statistics for these two lists are displayed for the equation in the form:

$$Y=A*X+B$$

    e. Enter the absolute value of the slope, *A*, as the reaction rate in Table 2.

f. Press ENTER to view a graph of the data and the regression line.
g. Press ENTER to return to the ANALYZE menu.
h. Select RETURN TO MAIN SCREEN from the ANALYZE menu.

11. Store the data from the first run so that it can be used later.
    a. Select TOOLS from the main screen.
    b. Select STORE LATEST RUN from the TOOLS MENU.
12. Find the rate of enzyme activity for test tubes 2, and 3:
    a. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 7 – 11.
    b. Add 20 drops of the enzyme solution to test tube 3. Repeat Steps 7 – 10.
13. Graph all three runs of data on a single graph. To do this:
    a. Select GRAPH from the main screen, then press ENTER.
    b. Select MORE, then select L2, L3 AND L4 VS L1 from the MORE GRAPHS menu.
    c. All three runs should now be displayed on the same graph. Each point of the 5-drop run is plotted with a cross, each point of the 10-drop run is plotted with a box, and each point of the 20-drop run is plotted with a dot. Use the displayed graph and the data in Table 2 to answer the questions for Part I.
    d. When finished with the graph, press ENTER to exit.
    e. Select RETURN TO GRAPHS SCREEN from the MORE GRAPHS menu.
    f. Select MAIN SCREEN from the graph screen.

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

14. Rinse the three numbered test tubes used for Part I. Fill each test tube with 10 mL of 1.5% H_2O_2 and 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. 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 7 – 10. Record the reaction rate in Table 3.
    b. Add 10 drops of the enzyme solution to test tube 2. Repeat Steps 7 – 10. Record the reaction rate in Table 3.
    c. Add 10 drops of the enzyme solution to test tube 3. Repeat Steps 7 – 10. Record the reaction rate in Table 3.
16. Calculate the average rate for the three trials you tested. Record the average in Table 3.

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

Part III Testing the Effect of pH

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

19. 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

20. Using the test tube labeled pH 4, add 10 drops of enzyme solution and repeat Steps 7 – 11.

21. Using the test tube labeled pH 7, add 10 drops of enzyme solution and repeat Steps 7 – 11.

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

23. Graph all three runs of data on a single graph. To do this:
    a. Select GRAPH from the main screen, then press ENTER.
    b. Select MORE, then select L2, L3 AND L4 VS L1 from the MORE GRAPHS menu.
    c. All three runs should now be displayed on the same graph. Use the displayed graph and the data in Table 5 to answer the questions for Part III.
    d. When finished with the graph, press ENTER to exit.
    e. Select RETURN TO GRAPHS SCREEN from the MORE GRAPHS menu.
    f. Select MAIN SCREEN from the graph screen.

DATA

Part I Effect of Enzyme Concentration

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

Part II Effect of Temperature

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

Table 4 (Class data)	
Temperature tested	Average rate

Part III Effect of pH

Table 5	
Test tube label	Slope, or rate (%/s)
pH 4	
pH 7	
pH 10	

PROCESSING THE DATA

1. For Part II of this experiment, make a graph of the rate of enzyme activity *vs*. temperature by hand or by using Graphical Analysis. 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. If one increases the concentration of enzyme to thirty drops, what do you think will happen to the rate of reaction? 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?

EXTENSIONS

1. Repeat Step 12a to collect data with 10 drops of enzyme suspension. Using the Graphical Analysis computer software, import your collected data into a computer. In Graphical Analysis, use the mouse to select each of the time intervals from Table 6—calculate the rate using the Regression function found in the Analyze menu.

Table 6 Time intervals (Minutes)					
Rates	0-30 s	30-60 s	60-90 s	90-120 s	120-180 s
10 Drops					

Questions

When is the reaction rate highest? Explain why.

When is the reaction rate lowest? Why?

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.

TEACHER INFORMATION

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 13, 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.

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 grams (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.

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 if the reaction occurs to quickly.

6. 3% H_2O_2, to be used in Part III, may be purchased from any supermarket. If refrigerated, bring it to room temperature before starting the experiment. To prepare 100 mL of 1.5% H_2O_2 (for Parts I and II), add 50 mL of distilled water to 50 mL of 3% H_2O_2.

7. When not being used, the O_2 Gas Sensor should be stored upright in the box in which is was shipped. Storing the sensor in this position will extend the sensor's life.

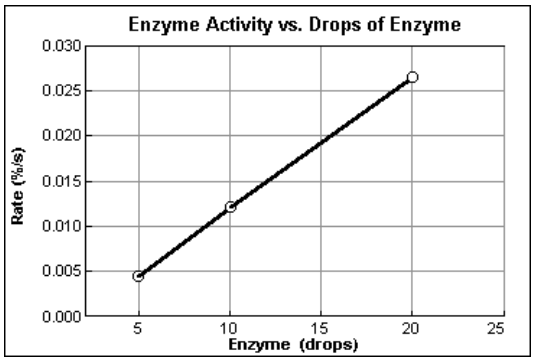
8. Vernier Software sells a pH buffer package for preparing buffer solutions with pH values of 4, 7, and 10 (order code PHB, $10.00). Simply add the capsule contents to 100 mL of distilled water.

9. You can also prepare pH buffers using the following recipes:
   - pH 4.00: Add 2.0 mL of 0.1 M HCl to 1000 mL of 0.1 M potassium hydrogen phthalate.
   - pH 7.00: Add 582 mL of 0.1 M NaOH to 1000 mL of 0.1 M potassium dihydrogen phosphate.
   - pH 10.00: Add 214 mL of 0.1 M NaOH to 1000 mL of 0.05 M sodium bicarbonate.

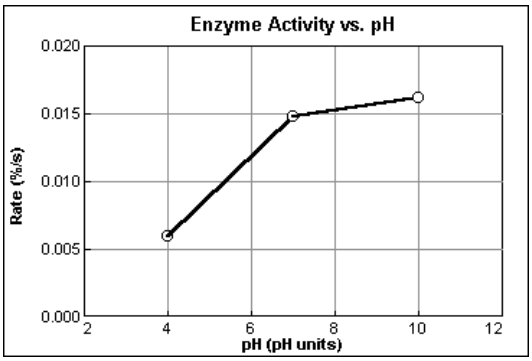
10. You may need to let students know that at pH values above 10 enzymes will become denatured and the rate of activity will drop. If you have pH buffers higher than 10, have students perform an experimental run using them.

SAMPLE RESULTS

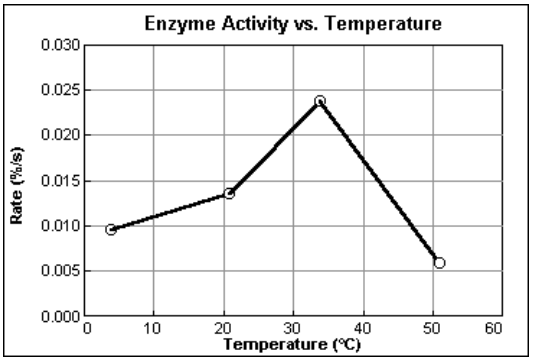
Sample class data	
Test tube label	Slope, or rate (%/s)
5 Drops	0.0045
10 Drops	0.0122
20 Drops	0.0265
0 – 5 °C range: 4°C	0.0097
20 – 25 °C range: 21 °C	0.0137
30 – 35 °C range: 34°C	0.0238
50 – 55 °C range: 51°C	0.0060
pH 4	0.0060
pH 7	0.0148
pH 10	0.0162



The effect of H_2O_2 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

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 30 drops, the rate in the above experiment should be about 0.041 %/s.

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.

ANSWERS TO EXTENSION 1

1. 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

2. 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.