Name _____ Date _____ Period _____

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,* as 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.

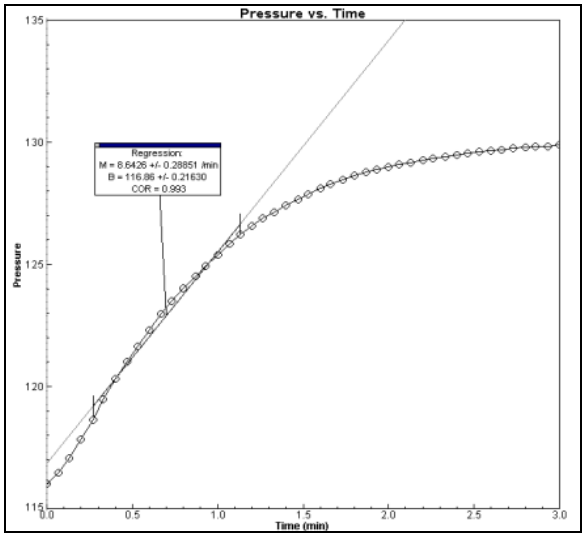
H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2 can be converted to oxygen and water, as follows:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

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

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 H_2O_2 is destroyed. If a plot is made, it may appear similar to the graph shown.

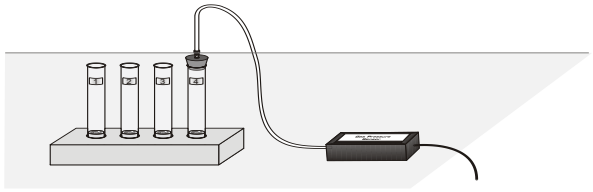


At the start of the reaction, there is no product, and the pressure is the same as the atmospheric pressure. After a short time, oxygen accumulates at a rather constant rate. The slope of the curve at this initial time is constant and is called the *initial rate*. As the peroxide is destroyed, less of 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.

OBJECTIVES

In this experiment, you will

- Use a computer and Gas Pressure 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.



HYPOTHESES

1. If more drops of enzyme are added to a substrate, then the reaction rate will _____ [increase / decrease].

2. If an enzyme-catalyzed reaction occurs at different temperatures, then the reaction rate will be highest at _____ °C.

3. If an enzyme-catalyzed reaction occurs at different pH values, then the reaction rate will be highest at pH _____.

GROUP MATERIALS

MacBook Air with Logger Pro software
LabQuest
Vernier Gas Pressure Sensor
rubber-stopper assembly
test tube rack
13 test tubes
13 test tube labels
13 pieces of parafilm
2 10 mL graduated cylinders (H_2O, H_2O_2)
250 mL beaker of water
100 mL beaker of 3% H_2O_2
enzyme suspension (liver purée)
3 dropper pipettes (liver, H_2O, H_2O_2)

SHARED MATERIALS

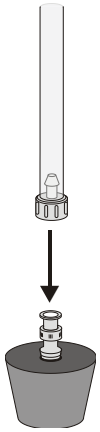
1000 mL beaker of ice water
room temperature beaker of water
37°C water bath
99°C water bath or beaker of boiling water
4 dropper pipettes for water samples
4 thermometers
pH buffers (1, 4, 7, 10, 12)
5 graduated cylinders for pH buffers
5 dropper pipettes for pH buffers

PROCEDURE

1. Obtain and wear goggles.
2. Connect the Gas Pressure Sensor to the computer interface. Prepare the computer for data collection by opening the file "06B Enzyme (Pressure)" from the *Biology with Vernier* folder of Logger*Pro*.
3. Connect the plastic tubing to the valve on the Gas Pressure Sensor. Connect the free-end of the plastic tubing to the connector in the rubber stopper.

Part I – Testing the Effect of Enzyme Concentration

4. Place four test tubes in a test tube rack and label them **1**, **2**, **3**, and **4**.
5. Add 3 mL of water to each test tube. Add 1 drop of liver purée to test tube **1**. Add 2 drops of liver purée to test tube **2**. Add 3 drops of liver purée to test tube **3**. Add 4 drops of liver purée to test tube **4**.
6. Cover the test tube with parafilm and invert to mix. Throw away the used pieces of parafilm.



7. Measure 3 mL of hydrogen peroxide (H_2O_2) in a graduated cylinder. Click Collect to begin data collection. Pour the H_2O_2 into test tube **1**. Immediately stopper the test tube. The reaction should begin. Stop data collection after the reaction begins to slow down.
8. When data collection has finished, disconnect the plastic tubing connector from the rubber stopper. Rinse off the rubber stopper and reconnect it to the plastic tubing.
9. If the reaction was so strong that the data cannot all be seen at once, change the range of the Y axis so that you can see all of it.
10. Find the rate of enzyme activity:
    a. Move the mouse pointer to the point where the data values begin to increase. Hold down the mouse button. Drag the mouse pointer to the point where the pressure values no longer increase and release the mouse button.
    b. Click the Linear Fit button, , to perform a linear regression. A floating box will appear with the formula for a best-fit line.
    c. Record the slope of the line, *m*, as the rate of enzyme activity.
    d. Close the linear regression floating box.
11. Repeat steps 7-10 for test tubes **2**, **3**, and **4**.

Part II – Testing the Effect of Temperature

12. Place four clean test tubes in a rack and label them **T 0–5**, **T 20–25**, **T 35–40**, and **T 95–100**.
13. Add 3 mL of water to each test tube. **Make sure to use the water that has been placed in the appropriate temperature water bath. Record the actual temperature of the water in Data Table #2.**

14. Add 2 drops of liver purée to each test tube. Cover the test tube with parafilm and invert to mix. Throw away the used pieces of parafilm.
15. Measure 3 mL of hydrogen peroxide (H_2O_2) in a graduated cylinder. Click Collect to begin data collection. Pour the H_2O_2 into test tube **T 0–5**. Immediately stopper the test tube. The reaction should begin. Stop data collection after the reaction begins to slow down.
16. When data collection has finished, disconnect the plastic tubing connector from the rubber stopper. Rinse off the rubber stopper and reconnect it to the plastic tubing.
17. If the reaction was so strong that the data cannot all be seen at once, change the range of the Y axis so that you can see all of it.
18. Find the rate of enzyme activity:
    a. Move the mouse pointer to the point where the data values begin to increase. Hold down the mouse button. Drag the mouse pointer to the point where the pressure values no longer increase and release the mouse button.
    b. Click the Linear Fit button, , to perform a linear regression. A floating box will appear with the formula for a best-fit line.
    c. Record the slope of the line, *m*, as the rate of enzyme activity.
    d. Close the linear regression floating box.
19. Repeat steps 15-18 for test tubes **T 20–25**, **T 35–40**, and **T 95–100**.

Part III – Testing the Effect of pH

20. Place five clean test tubes in a rack and label them **pH 1**, **pH 4**, **pH 7**, **pH 10**, and **pH 12**.
21. Add 3 mL of buffer to each test tube. **Make sure to use the appropriate pH buffer.**
22. Add 2 drops of liver purée to each test tube. Cover the test tube with parafilm and invert to mix. Throw away the used pieces of parafilm.
23. Measure 3 mL of hydrogen peroxide (H_2O_2) in a graduated cylinder. Click Collect to begin data collection. Pour the H_2O_2 into test tube **pH 1**. Immediately stopper the test tube. The reaction should begin. Stop data collection after the reaction begins to slow down.
24. When data collection has finished, disconnect the plastic tubing connector from the rubber stopper. Rinse off the rubber stopper and reconnect it to the plastic tubing.
25. If the reaction was so strong that the data cannot all be seen at once, change the range of the Y axis so that you can see all of it.
26. Find the rate of enzyme activity:
    a. Move the mouse pointer to the point where the data values begin to increase. Hold down the mouse button. Drag the mouse pointer to the point where the pressure values no longer increase and release the mouse button.
    b. Click the Linear Fit button, , to perform a linear regression. A floating box will appear with the formula for a best-fit line.
    c. Record the slope of the line, *m*, as the rate of enzyme activity.
    d. Close the linear regression floating box.
27. Repeat steps 23-26 for test tubes **pH 4**, **pH 7**, **pH 10**, and **pH 12**.

RESULTS

Data Table #1: Effect of Enzyme Concentration on Chemical Reaction Rate

# of Drops of Liver	Expected Results	Rate (kPa/min)
1 drop		
2 drops		
3 drops		
4 drops		

Data Table #2: Effect of Temperature on Chemical Reaction Rate

Temperature	Expected Results	Rate (kPa/min)
0-5°C range: _____°C		
20-25°C range: _____°C		
35-40°C range: _____°C		
95–100°C range: _____°C		

Data Table #3: Effect of pH on Chemical Reaction Rate

pH	Expected Results	Rate (kPa/min)
pH = 1		
pH = 4		
pH = 7		
pH = 10		
pH = 12		

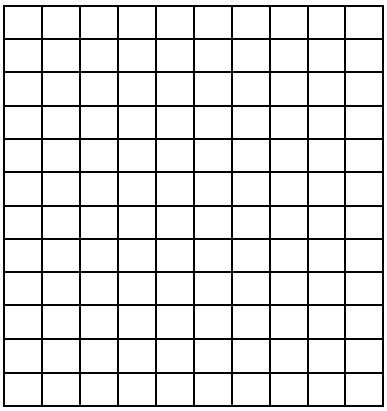
PROCESSING THE DATA

1. For Part I of this experiment, make a graph of the rate of enzyme activity *vs*. enzyme concentration. Plot the rate values from Data Table #1 on the y-axis and the number of drops of enzyme on the x-axis.

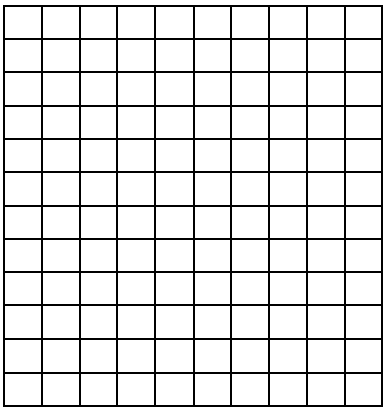
2. For Part II of this experiment, make a graph of the rate of enzyme activity *vs*. temperature. Plot the rate values from Data Table #2 on the y-axis and the temperature on the x-axis.

3. For Part III of this experiment, make a graph of the rate of enzyme activity *vs*. pH. Plot the rate values from Data Table #3 on the y-axis and the pH on the x-axis.

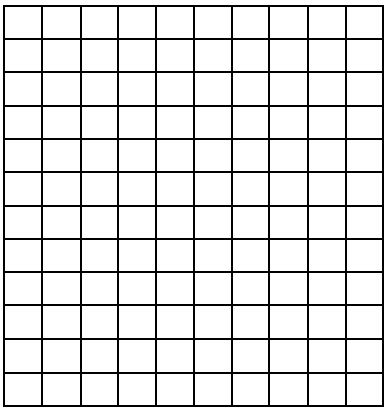
Data Table #4:
Enzyme Activity vs. Number of Drops



Data Table #5:
Enzyme Activity vs. Temperature



Data Table #6:
Enzyme Activity vs. pH



POST-LAB QUESTIONS

Part I – Testing the Effect of Enzyme Concentration

1. How does changing the concentration of enzyme affect the rate of decomposition of H_2O_2? Explain using CLAIM → EVIDENCE → REASONING.

2. What do you think will happen to the rate of reaction if the concentration of enzyme is increased to five drops? Predict what the rate would be for 5 drops.

Part II – Testing the 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? Explain using CLAIM → EVIDENCE → REASONING.

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

Part III – Testing the Effect of pH

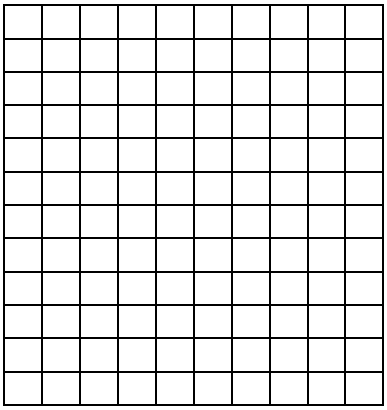
6. At what pH is the rate of enzyme activity the highest? Lowest?

7. How does changing the pH affect the rate of enzyme activity? Does this follow a pattern you anticipated? Explain using CLAIM → EVIDENCE → REASONING.

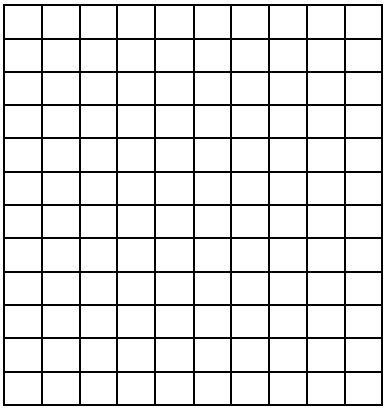
8. Why might the enzyme activity decrease at very high or very low pH values?

EXTRA DATA TABLES IN CASE YOU MAKE ANY MISTAKES

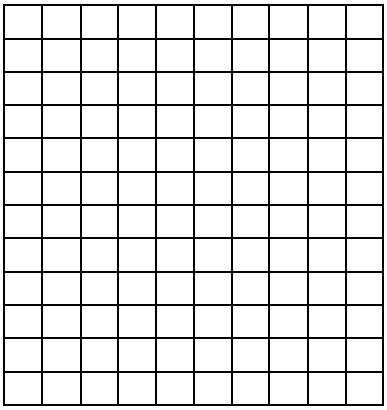
Data Table #4:
Enzyme Activity vs. Number of Drops



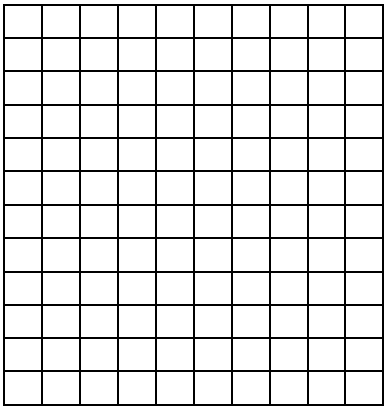
Data Table #5:
Enzyme Activity vs. Temperature



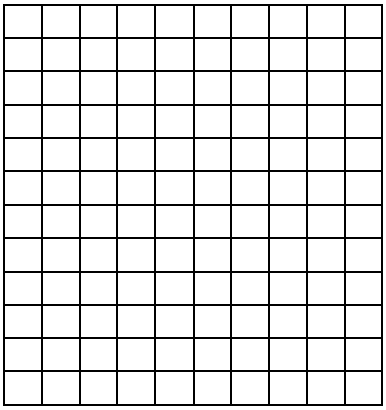
Data Table #6:
Enzyme Activity vs. pH



Data Table #4:
Enzyme Activity vs. Number of Drops



Data Table #5:
Enzyme Activity vs. Temperature



Data Table #6:
Enzyme Activity vs. pH