Name_____ Date_____ Period_____

Enzyme Action: Testing Catalase Activity

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. Under ideal conditions, the 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 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 it before it can do much damage. H_2O_2 can be converted to oxygen and water as follows:

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

Although this reaction occurs spontaneously, enzymes increase the rate considerably. At lease 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 enzymes-catalyzed reactions.

In this experiment, you will measure the rate of enzyme activity under various conditions, such as different concentrations of enzyme, pH values and temperatures. This experiment, like the last, collects the gas released from the reaction. However, instead of measuring the mL of gas produced, we will measure the pressure that the gas exerts on the reaction chamber as the H_2O_2 is destroyed.

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.

Procedure:

NOTE: In all experiments, make certain that your reaction chamber is extremely clean. Catalase is a potent enzyme, and if the chamber is not washed thoroughly, enough will adhere to the sides to make subsequent tests inaccurate.

Part I: Testing the Effect of Enzyme Concentration.

1. Obtain all materials.

2. Measure out 20 mL of hydrogen peroxide with a graduated cylinder and add to the reaction chamber (baby bottle).

The next steps need to be performed quickly but safely in order to produce accurate results. The goal is to capture the air bubbles produced from the reaction chamber into the graduated cylinder as early as possible after the reaction begins.

3. Using a pipette, add 1.5 mL of enzyme solution to the reaction chamber. **NOTE: Be sure not to let the enzyme fall against the side of the test tube.**

4. Put the lid onto the reaction chamber and hit the **COLLECT** button. NOTE: It may take two people to put the lid on the chamber. Have one person hold the chamber still and the other twist the lid onto the chamber.

5. Allow data to collect for 3 minutes.

6. Find the rate of enzyme activity for this run. Record this data in the table provided.

7. Repeat procedure using 2.0 mL of enzyme solution for trial 2 and 1.0 mL of enzyme solution for trial 3.

Table 1 - Solutions Added to Chamber Part I

Run	Volume of H_2O_2 (mL)	Volume of Enzyme (mL)
1	20	1.5
2	20	2.0
3	20	1.0

Data Table 1: Concentration of Enzyme vs Rate of Reaction ***(rough draft)***

Volume of Enzyme (mL)	Rate (atm/min) - Slope
1.0	
1.5	
2.0	

Part II: Testing the Effect of Temperature

1. Add 20 mL of H_2O_2 solution to each of four reaction chambers. Place one reaction chamber in an ice water bath (0-5°C). Place the second into a warm water bath (30-35°C). Place the third into a hot water bath (50-55°C). Leave the fourth at room temperature.

2. Repeat procedure from Part I using 20 mL of H_2O_2 for each trial and 2.0 mL of enzyme solution. For each run use **H_2O_2** solution of a different temperature.

Table 2 - Solutions Added to Chamber Part II

Run	Volume of H_2O_2 (mL)	Volume of Enzyme (mL)
1	20	2.0
2	20	2.0
3	20	2.0
4	20	2.0

Data Table 2: Temperature of Solution vs Rate of Reaction ***(rough draft)***

Temperature of Solution (°C)	Rate (atm/min) - Slope
0-5°C	
20-25°C	
30-35°C	
50-55°C	

Part III: Testing the Effect of pH

1. Repeat procedure from Part I using 20 mL of H_2O_2 and 2.0 mL of enzyme solution. For each run use **H_2O_2** solution of a different pH. H_2O_2 of pH 4, pH 7 and pH 10 will be prepared for you.

Table 3 - Solutions Added to Chamber Part III

Run	Volume of H_2O_2 (mL)	Volume of Enzyme (mL)
1	20 of pH 4	2.0
2	20 of pH 7	2.0
3	20 of pH 10	2.0

Data Table 2: pH of Solution vs Rate of Reaction ***(rough draft)***

pH of Solution	Rate (atm/min) - Slope
pH 4	
pH 7	
pH 10	

Name_____ Date_____ Period_____

Rubric for Enzyme Action: Testing Catalase Activity Lab Report

Checklist

1. Title Page (this page)
2. Completed Pre-Lab Questions
3. Completed Data Table
4. 3 Graphs on 3 Separate Sheets of Graph Paper
5. Completed Analysis Questions

Pre-Lab

1	2	3	4
Student completed one question on pre-lab			Student completed all questions on pre-lab

Data Table

1	2	3	4
Student collected one variable of data, or data inappropriate			Student collected and displayed all variables appropriately

Graph

1	2	3	4
Student presented one graph or graphs are inaccurate			Student accurately presented all three graphs

Analysis Questions

Question	Points Possible	
2.	1 point for answer	2 points for explanation
3.	1 point for answer	2 points for explanation
4.	1 point for answer	2 points for explanation
5.	1 point for answer	2 points for explanation
6.	1 point for answer	2 points for explanation

Name_____ Date_____ Period

Enzyme Action: Testing Catalase Activity Lab Report

Prelab Questions:

Directions - Read the entire introduction and lab procedure and answer the following questions in the space provided.

1. What is a catalyst and how does it affect a reaction?

2. How quickly do enzymes work?

3. What might happen to an enzyme if it is in an environment outside of its optimum range for temperature and pH?

4. Why is it necessary that living things decompose H_2O_2? Why is an enzyme needed?

5. Will the decomposition of H_2O_2 occur spontaneously? What does spontaneous mean?

6. Based on your response to number 5, is this an exothermic or endothermic reaction?

7. Can enzymes ever catalyze endothermic (nonspontaneous) reactions? Why?

8. What three variables will we be adjusting in this lab?

9. What are we measuring in this experiment as we attempt to determine rate of enzyme activity?

10. What is meant by slope? What does the slope of the line indicate in this lab?

11. Our graphs will begin to level off by the end of the runs. Why do you think this might occur?

Data Table - Final Copy (must be neat)

Treatment	Rate of Reaction (atm/min)
Enzyme Concentration	
1.0 mL	
1.5 mL	
2.0 mL	
Temperature of Solution	
0-5°C range Actual Temperature_____	
20-25°C range Actual Temperature_____	
30-35°C range Actual Temperature_____	
50-55°C range Actual Temperature_____	
pH of Solution	
pH 4	
pH 7	
pH 10	

Analysis Questions

Directions - Answer the following questions in the space provided.

1. Plot all your data on a **three separate** graphs on a **three separate sheets of graph paper**. Use an entire sheet of graph paper and spread out your graph **for each graph**.

2. What effect does changing the concentration of enzyme have on the rate of decomposition of H_2O_2? (1 point) Fully explain and support your answer. Refer to data and/or graph or class discussion when necessary. (2 points)

3. If the amount of enzyme was increased to 5 mL, how should this effect the rate of the reaction? Give a prediction of the rate at 5 mL (1 point) Fully explain and support your answer. Refer to data and/or graph or class discussion when necessary. (2 points)

4. In general, what happens to the rate of the reaction as temperature increases? (1 point) Using the kinetic molecular theory explain why this pattern should be expected. (2 points)

5. What happened to the rate of the reaction once the temperature exceeded 50°C? (1 point) Explain why this may have occurred. (2 points)

6. At what pH is the rate of enzyme activity the highest? (1 point) Explain why this pattern should be expected. (2 points)