Enzyme Lab: The Breakdown of H_2O_2 by Catalase

OVERVIEW: In this lab you will observe the conversion of hydrogen peroxide (H_2O_2) to water and oxygen gas by the enzyme catalase. You will measure the rate of the enzyme-catalyzed reaction for substrate concentration, pH, enzyme concentration, and temperature on the efficiency of the enzyme.

OBJECTIVES:

Before doing this lab you should understand:

- The general functions and activities of enzymes;
- The relationship between the structure and function of enzymes;
- The concept of initial reaction rates of enzymes;
- How the concept of free energy relates to enzyme activity; and
- In general, how environmental changes (changes in temperature, pH, enzyme concentration, salt concentration, and substrate concentration) might affect the reaction rates and efficiency of enzyme-catalyzed reactions.

After doing this lab you should be able to:

- Measure and explain the effects of changes in enzyme and substrate concentration on reaction rates of an enzyme catalyzed reaction in a controlled experiment and
- Measure and explain the effects of pH on the reaction rates and efficiency of enzymes.

INTRODUCTION: In general, enzymes are proteins produced by living cells; they act as catalysts in biochemical reactions. A catalyst affects the rate of a chemical reaction. One consequence of enzyme activity is that cells can carry out complex chemical activities at relatively low temperatures.

In an enzyme-catalyzed reaction, the substance to be acted upon (the substrate = S) binds reversibly to the active site of the enzyme (E). One result of this temporary union is a reduction in the energy required to activate the reaction of the substrate molecule so that the products (P) of the reaction are formed.

In summary: **E + S → ES → E + P**

Note that the enzyme is not changed in the reaction and can be recycled to break down additional substrate molecules. Each enzyme is specific for a particular reaction because its amino acid sequence is unique and causes it to have a unique three-dimensional structure. The **active site** is the portion of the enzyme that interacts with the substrate, so that any substance that blocks or changes the shape of the active site affects the activity of the enzyme. A description of several ways enzyme action may be affected follows.

1. **Salt concentration** – If the salt concentration is close to zero, the charged amino acid side chains of the enzyme molecules will attract each other. The enzyme will denature and form an inactive precipitate. If, on the other hand, the salt concentration is very high, normal interaction of charged groups will be blocked, new interactions will occur, and again the enzyme will precipitate. An intermediate salt concentration such as that of human blood (0.9%) or cytoplasm is the optimum for many enzymes.

2. **pH** – pH is a logarithmic scale that measures the acidity or H+ concentration in a solution. The scale runs from 0 to 14 with 0 being highest in acidity and 14 lowest. When the pH is in the range of 0-7, a solution is said to be acidic; if the pH is around 7, the solution is neutral; and if the pH is in the range of 7-14, the solution is basic. Amino acid side chains contain groups such as –COOH and –NH2 that readily gain or lose H+ ions. As the pH is lowered an enzyme will tend to gain H+ ions, and eventually enough side chains will be affected so that the enzyme's shape is disrupted. Likewise, as the pH is raised, the enzyme will lose H+ ions and eventually lose its active shape. Many enzymes perform optimally in the neutral pH range and are denatured at either an extremely high or low pH. Some enzymes, such as pepsin, which acts in the human stomach where the pH is very low, work best at a low pH.

3. **Temperature** – Generally, chemical reactions speed up as the temperature is raised. As the temperature increases, more of the reacting molecules have enough kinetic energy to undergo the reaction. Since enzymes are catalysts for chemical reactions, enzyme reactions also tend to go faster with increasing temperature. However, if the temperature of an enzyme-catalyzed reaction is raised still further, a temperature optimum is reached; above this value the kinetic energy of the enzyme and water molecules is so great that the conformation of the enzyme molecules is disrupted. The positive effect of speeding up the reaction is now more than offset by the negative effect of changing the conformation of more and more enzyme molecules. Temperatures around 40-50^{o}C denature many proteins, but some are still active at 70-80^{o}C, and a few even withstand boiling.

4. **Activators and Inhibitors** – Many molecules other than the substrate may interact with an enzyme. If such a molecule increases the rate of the reaction it is an activator, and if it decreases the reaction rate it is an inhibitor. These molecules can regulate how fast the enzyme acts. Any substance that tends to unfold the enzyme, such as an organic solvent or detergent will act as an inhibitor. Some inhibitors act by reducing the –S-S- bridges that stabilize the enzyme's structure. Many inhibitors act by reacting with side chains in or near the active site to change its shape or block it. Many well-known poisons such as potassium cyanide and curare are enzyme inhibitors that interfere with the active site of critical enzymes.

The enzyme used in this lab – **catalase** – has four polypeptide chains, each composed of more than 500 amino acids. This enzyme is found in the tissue of aerobic organisms (animals, plants, fungi, etc). It is found in the peroxisomes of liver and kidney cells in animals, but is especially abundant in plant storage organs such as potatoes and fleshy parts of fruits. One function of catalase within cells is to prevent the accumulation of toxic levels of hydrogen peroxide (formed as a by-product of metabolic processes). Hydrogen peroxide is a strong oxidizing agent, which tends to disrupt the delicate balance of cell chemistry. If too much hydrogen peroxide accumulates, it will kill the cell.

The primary reaction catalyzed by catalase is the decomposition of H_2O_2 to form water and oxygen:

$$\mathbf{2H_2O_2 \rightarrow 2H_2O + O_2\ (gas)}$$

In the absence of catalase, this reaction occurs spontaneously, but very slowly. Catalase speeds up the reaction considerably. In this experiment, the rate of reaction will be indirectly measured. The assay system used in this lab consists of a filter paper disk that is coated with the enzyme and then dropped into a cup of substrate (hydrogen peroxide). As the catalyst breaks down the hydrogen peroxide into water and oxygen gas, the bubbles of oxygen collect underneath the filter paper disk and make it rise to the surface of the hydrogen peroxide. The time it takes for the filter paper disk to rise (from the bottom of the test tube) is an indication of the rate of enzyme activity.

We will assume that each filter disk is coated with the same amount of catalase (except in the investigation of the effect of enzyme concentration of enzyme activity).

SAFETY: You'll be using glass, hot water, acids, and bases. Use caution and wear goggles.

PART 1: Testing the Effect of Temperature on Catalase Activity

This part of the lab will be conducted as a class demonstration. You will observe the effect of extreme heat on enzyme function as well as get an understanding of controls. The enzyme catalase is present in potatoes and will catalyze the hydrolysis reaction of hydrogen peroxide into water and oxygen.

DATA TABLE 1: TEMPERATURE

	Paper Disk + Water	**Paper Disk + H_2O_2 Substrate**	**Paper Disk soaked in Enzyme + Water**	**Paper Disk soaked in Enzyme + H_2O_2 Substrate**	**Paper Disk soaked in Boiled Enzyme + Water**	**Paper Disk soaked in Boiled Enzyme + H_2O_2 Substrate**
Observations						
Conclusions	Purpose is…	Purpose is…	Purpose is…	Purpose is…	Purpose is…	Purpose is…
Type of Control (circle)	Negative Positive	Negative Positive	Negative Positive	Negative Positive	Negative Positive	This is the Experimental Group

PART 2: Testing the Effect of Enzyme Concentration on Catalase Activity

INVESTIGATION QUESTION: How does _____ affect the rate of _____?

HYPOTHESIS: ("If…then…because…")

VARIABLES:

Independent Variable = _____

Dependent Variable = _____

Experimental Controls = _____

PROCEDURE: ENZYME CONCENTRATION

1. Obtain 7 clean beakers.
2. Label the beakers with tape and pen: 100% catalase, 80% catalase, 60% catalase, 40% catalase, 20% catalase, and 0% catalase. Label the last beaker "reaction beaker."
3. Create the appropriate enzyme concentrations following the protocol in the table here below. Use a graduated cylinder to measure each amount. Be sure to rinse the cylinder well when switching between enzyme and water. Stir all solutions well.

Test	Catalase Enzyme Concentration	Volume of Enzyme	Volume of Water
# 1	100%	40 ml	0 ml
# 2	80%	32 ml	8 ml
# 3	60%	24 ml	16 ml
# 4	40%	16 ml	24 ml
# 5	20%	8 ml	32 ml
# 6	0%	0 ml	40 ml

4. Obtain the 3% hydrogen peroxide substrate.
5. Pour 30 ml of the 3% H_2O_2 solution into a clean beaker, labeled "reaction beaker.
6. Pick up a paper disk with a clean forceps (or tweezers) and dunk the disk into the enzyme extract for 5 seconds, until the disk is uniformly moistened but not beaded with drops of liquid. Gently tap it on a paper towel for about 5 seconds to remove excess enzyme.
7. Use the forceps to place the filter disk (containing enzymes) onto the **BOTTOM** of the "reaction beaker" containing the H_2O_2 liquid substrate.
8. One person should watch the clock/stopwatch, another watch the rising disk. Under "Trial 1," record the number of seconds that it takes for the disk to "lift off" in a flat orientation**. Remove the disk from the substrate immediately after it floats to the top** and discard it. This is to prevent more substrate being catalyzed.
9. Watch the filter disk to see tiny bubbles of oxygen being released. Record the time in seconds in data table 2.
10. Repeat steps 5-8 for all of the Test #s. Remember to use a clean disk each time.
11. You will record data from 3 other groups (under the other trials columns) to obtain results for multiple trials and then calculate the average enzymatic rate for each enzyme concentration.

DATA TABLE 2: ENZYME CONCENTRATION

Test #	Enzyme Concentration	Trial 1 (seconds)	Trial 2 (seconds)	Trial 3 (seconds)	Trial 4 (seconds)	Average (seconds)	1/t
1	100%						
2	80%						
3	60%						
4	40%						
5	20%						
6	0%						

GRAPH PART 2: CONCENTRATION OF ENZYME VS. TIME OF REACTION

Plot the **averages only**. The **x-axis** should be designated as enzyme concentration (%), starting at point 0. The **y-axis** should be 1/t (sec), so you have to do the math on this before you graph your data. Record these values in Data Table 2. All graphs must be on graph paper with a title, labeled axes, units included, correct averages plotted, and correct line drawn.

PART 3: Testing the Effect of Substrate Concentration on Catalase Activity

INVESTIGATION QUESTION: How does _____ affect the rate of _____?

HYPOTHESIS: ("If…then…because…")

VARIABLES:

Independent Variable = _____

Dependent Variable = _____

Experimental Controls = _____

PROCEDURE: H_2O_2 CONCENTRATION

1. Obtain 6 clean beakers.
2. Label the beakers with tape and pen: 3.0% H_2O_2, 2.0% H_2O_2, 1.5% H_2O_2, 1.0% H_2O_2, 0.5% H_2O_2, and 0.3% H_2O_2.
3. Create the appropriate substrate concentrations following the protocol in the table here below. Use a graduated cylinder to measure each amount. Be sure to rinse the cylinder well when switching between H_2O_2 substrate and water. Stir all solutions well.

Test	H_2O_2 Substrate Concentration	Volume of H_2O_2	Volume of Water
# 1	3.0%	30 ml	0 ml
# 2	2.0%	20 ml	10 ml
# 3	1.5%	15 ml	15 ml
# 4	1.0%	10 ml	20 ml
# 5	0.5%	5 ml	25 ml
# 6	0.3%	3 ml	27 ml

4. For this part of the lab, use a 60% catalase solution (from Part 2 if available).
5. Pick up a paper disk with a clean forceps (or tweezers) and dunk the disk into the 60% enzyme extract for 5 seconds, Gently tap it on a paper towel for about 5 seconds to remove excess enzyme.
6. Use the forceps to place the filter disk (containing enzymes) onto the **BOTTOM** of the "reaction beaker" containing the H_2O liquid substrate. (Start with the 3.0% H_2O_2).
7. Time how long it takes the disk to "lift off" and become horizontal in the solution. Be sure that the disk is placed at the bottom of the hydrogen peroxide before you start the timer. Remove the disk as soon as lift off is achieved.
8. Record the time in seconds in data table 3.
9. Repeat steps 5-8 for all of the Test #s. Remember to use a clean disk each time.
10. You will record data from 3 other groups (under the other trials columns) to obtain results for multiple trials and then calculate the average enzymatic rate for each enzyme concentration.

DATA TABLE 3: SUBSTRATE CONCENTRATION

Group Number	Substrate Concentration	Trial 1 (seconds)	Trial 2 (seconds)	Trial 3 (seconds)	Trial 4 (seconds)	Average (seconds)	1/t
1	2.0%						
2	1.5%						
3	1.0%						
4	0.5%						
5	0.3%						

GRAPH PART 3: SUBSTRATE CONCENTRATION VS. REACTION TIME

Plot the **averages only**. The **x-axis** should be designated as substrate concentration (%), starting at point 0. The **y-axis** should be 1/t (sec), so you have to do the math on this before you graph your data. Record these values in Data Table 3. All graphs must be on graph paper with a title, labeled axes, units included, correct averages plotted, and correct line drawn.

PART 4 – Testing the Effect of pH on Catalase Enzyme Activity

INVESTIGATION QUESTION: How does….?

HYPOTHESIS: ("If, then, because…")

VARIABLES:

Independent Variable = _____

Dependent Variable = _____

Experimental Controls = _____

PROCEDURE: IMPACT OF pH

1. Obtain 5 clean test tubes.
2. Label them according to your teacher's directions:

   pH = _____ pH = _____ pH = _____ pH = _____ pH = _____

3. Using a plastic pipette or dropper, measure 4 ml of each of the pH solutions provided and transfer into the corresponding test tube. Be sure to use separate pipettes for each solution!
4. To each test tube, add 2 ml of 60% catalase solution using a plastic pipette. Swirl the test tube to mix the pH solution with the catalase. Allow it to sit for about 5 minutes.
5. Use a pipette to measure 1 ml of 3% hydrogen peroxide into each of the test tubes. Allow the reaction to occur for 5 minutes. Foam should form on the top of the solutions.

DATA TABLE 3: PH

pH					
Foam Height (mm) (from bottom of test tube to top of foam)					

GRAPH PART 4: PH VS. HEIGHT OF SOLUTION/FOAM (MM)

Create a bar graph of your data. The **x-axis** should be pH. The **y-axis** should be height of solution/foam (mm). This is an indirect measurement of enzyme activity. All graphs must be on graph paper with a title, labeled axes, units included, correct bar heights.

FINAL LAB REPORT

For this lab you will submit a typed (or neatly hand-written in ink) formal report. The report should include the following headings/sections with appropriate sentence format, grammar, and spelling. All hand-drawn graphs should be on graph paper.

1. **Background Information – This should be ~2 well-organized paragraphs that address all of these questions.**
    a. What is an enzyme?
    b. What do they do?
    c. What are several properties/characteristics of enzymes?
    d. Why are they important for living organisms?
    e. What is catalase?
    f. Why is it important for living organisms?
    g. What is denaturation?
    h. Which levels of protein structure are impacted by denaturation and how?
    i. Discuss 4 factors that can cause denaturation.
    j. What is the impact of denaturation for a cell/organism?
    k. What did you learn from the demonstration in Part 1 about catalase and temperature?
    l. What did you learn from the demonstration in Part 1 about experimental controls?

YOU WILL REPORT ON PARTS 2-4 OF THE LAB, ONE AT A TIME.

FOR <u>EACH</u> SECTION, INCLUDE:

2. **Research Questions**

3. **Hypothesis** (written in correct "if…then…because" format)

4. **Chart with Variables**
    a. Independent variables (with units)
    b. Dependent variable (with units)
    c. Controlled variables (with units)

5. **Data Tables** – 1 data table per procedure with individual group results, class results, and appropriate calculations or conversions

6. **Graphs**
    a. One graph per procedure
    b. Add a title and label axes appropriately

7. **Conclusion**
    a. For paragraph 1 - Use the Claim, Evidence, Reasoning model.
        i. State a claim that answers the investigation question.
        ii. Provide evidence that supports the claim. Evidence = specific data/averages and/or trends in the graph. Be specific!
        iii. Propose scientific reasoning that links your data to your claim. This is the "Why."

For paragraph 2 –

iv. Tell what your hypothesis was and whether you were correct or incorrect.
v. Tell something specific you learned from the investigation.
vi. Tell how your new learning applies to real life/organisms.
vii. Discuss any errors made during the procedure OR discuss 1 specific improvement to the lab procedure that could be made.

EACH STUDENT IS RESPONSIBLE FOR TURNING IN THEIR OWN, UNIQUELY WRITTEN LAB REPORT.
DO **NOT** SHARE YOUR DOCUMENT WITH YOUR LAB PARTNERS OR FRIENDS!!
IF ANY LABS SHARE ANY SECTIONS, ALL STUDENTS INVOLVED WILL RECEIVE A **ZERO**.

REPORT GRADING RUBRIC

LAB REPORT COMPONENT	**CRITERION** (to earn maximum points, all criteria should be met for each component) (# in parentheses indicates maximum # of points per criterion)	**POINTS**
Background Information	• Paragraph format (2) • No/minimal errors in grammar, punctuation, spelling, or sentence structure (2) • ALL questions A-L are thoroughly answered (12)	_____ / 16
Research Questions	All 3 questions written appropriately with proper punctuation. (6)	_____ / 6
Hypotheses	• Correct "If, then…because" format (3) • Testable and Measurable (3) • Clear indication of IV and DV (3)	_____ / 9
Variables Charts	• 3 charts (3) • Correct variables indicated (6)	_____ / 9
Data Tables	• 3 detailed tables with a title (6) • Units are included (6)	_____ / 12
Graphs	• Correct format (line, bar) (3) • Title, labeled axes, units, legends (15) • Data plotted correctly (and lines if line graph) (3) • Appropriate use of color (3)	_____ / 24
Conclusions	• 2 paragraphs for each investigation (6) • Paragraph 1: Good use of the ClEvR model (3 x3 = 9) • Paragraph 2: Detailed answers for all 4 questions (4 x 3 = 12) • No/minimal errors in grammar, punctuation, spelling, or sentence structure (3)	_____ / 30
Format	• Appropriately labeled sections for each lab component (2) • Clear Headings (2) • Correct Sequence (2) • Ink or Typed (2) • Appropriate use of fonts and color (1)	_____ / 9
Final Score	*Comments*:	**_____ / 115**