UNIT 4: *Enzyme: The Action of Catalase*

Laboratory Objectives

After completing this lab unit, you should be able to:

1. Define *enzyme* and describe the activity of enzymes in cells.
2. Understand the substrate-specific action of enzyme
3. Discuss the effects of the following factors on the rate of enzyme reaction:
   - varying enzyme concentration
   - varying substrate concentration
   - varying pH

Introduction to Enzyme

Enzymes are biological **catalysts**, compounds that *speeds up a chemical reactions without being used up or altered in the reaction*. The substance with which the catalyst reacts, called the **substrate**, is modified during the reaction to form a new **product**. The basic enzymatic reaction can be represented as:

$$[E] + [S] \rightarrow [ES] \rightarrow [EP] \rightarrow [P] + [E]$$

where the enzyme [E] and substrate [S] form an enzyme-substrate complex [ES], which is modified to form an enzyme-product complex [EP], and then the product and the enzyme are released. The released enzyme participates another round of reaction, while the product may be used for a subsequent reaction (See Figure 4-1).

Since the enzyme itself emerges from the reaction unchanged and ready to bind with another substrate molecules, a small amount of enzyme can alter a relatively enormous amount of substrate.

Enzymes are, in part or in whole, **proteins** that are constructed from a specific sequence of amino acids and folded into a three-dimensional shape. It is this three-dimensional shape that allows enzymes to fit onto a specific *substrate*. This specific portion of an enzyme is called the **active site** (Figure 4-2). The active site fit together only with *a specific substrate like a lock and key*, and *it is where the catalysis takes place*.

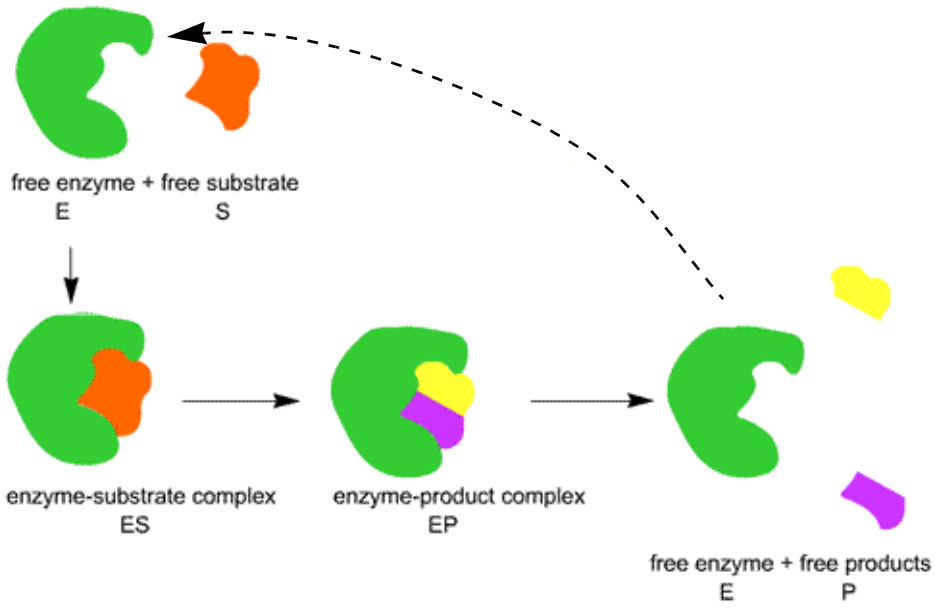

FIGURE 4-1. Summarization of an enzyme catalyzed reaction in which a compound split in two new compounds. A free substrate [S] binds to the active site of a free enzyme [E] available for reaction to form an enzyme-substrate complex [ES]. A catalytic reaction occurs at the active site forming enzyme-product complex [EP]. At the end of the reaction, products are released from the enzyme, which then becomes available for another round of reaction.

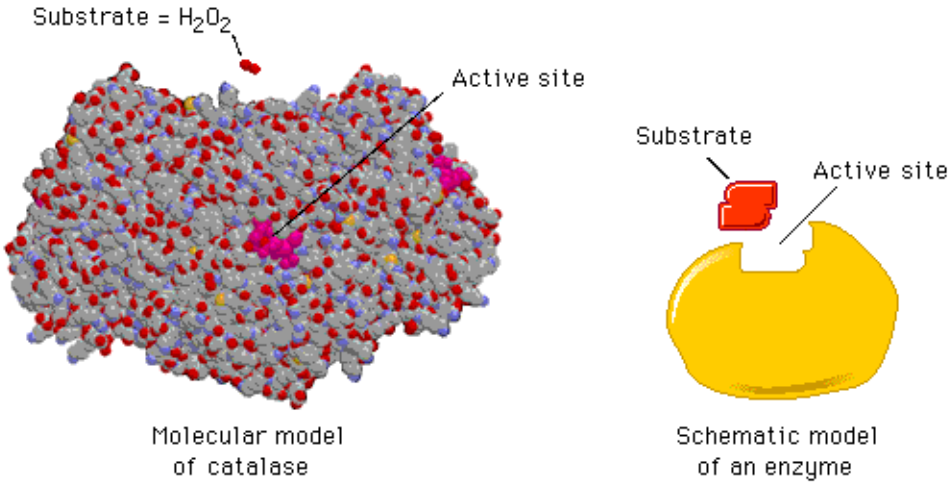

FIGURE 4-2. Schematic diagrams showing an active site of an enzyme; molecular model of catalase (left) and a schematic model of an enzyme and a substrate (right).

Factors Affecting Enzyme Activity

Enzyme activity is influenced by numerous physical and chemical factors that include **temperature**, **pH**, **ionic concentration**, and the **concentration of the enzyme and its substrate**. Temperature and pH may alter the three-dimensional shape of the enzyme which destroys its ability to act as a catalyst. When an enzyme's shape is changed it is **denatured and no longer functional**.

Enzyme activity increases with temperature until a maximum rate is reached, after which with further increases in temperature the reaction rate decreases. The temperature at which enzyme activity is at a maximum rate is called **optimum temperature**. Many enzymes are denatured by temperatures around 40-50°C, but some are more active at 70-80°C as found in microorganisms living in the hot springs.

For each specific enzyme there is an **optimal pH**. Most enzymes function best in a relatively neutral medium where the pH is between 6 and 8. Extremely high or low pH values generally result in complete loss of activity for most enzymes. Thus, an optimal pH is essential in enzyme stability and function.

Exercise 1. *Action of Catalase*

Each of enzymes is responsible for one particular reaction that occurs in the cell. In this exercise, you will study an enzyme that is found in the cells of many living tissues, which is **catalase**. It speeds up a reaction which breaks down hydrogen peroxide (H_2O_2), a toxic chemical, into two harmless substances – water and oxygen:

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

This is an important cellular reaction because it prevents the toxic buildup of **hydrogen peroxide** formed as a by-product of metabolic processes. Catalase occurs in animal and plant tissues, and is especially abundant in plant storage organs such as tubers, corms and in the fleshy parts of fruits.

In this exercise, you will use the enzyme catalase, which has been isolated from horseradish, to measure how fast it breaks down the substrate (3% H_2O_2) under different conditions. Briefly, you will:

1. immerse a filter paper disc in the catalase solution so that the spaces between fibers in the filter paper are filled with enzyme solution.
2. touch the filter paper disc on a piece of Kimwipes to remove excess of the enzyme solution
3. place the filter paper disc in the substrate H_2O_2. The oxygen (O_2) produced from the breakdown of H_2O_2 in the catalase reaction will push the enzyme solution out of the space; but the gas will be trapped in the space between fibers of the disc, causing the disc to float to the surface of the solution.
4. measure the time interval from the addition of the disc to the substrate solution until it reaches the surface of the solution. Measuring the rate of the O_2 generation allows a quantitative measurement of reaction rate.

Activity A: *Effects of Enzyme Concentration on Reaction Rate*

Before considering the factors which affect enzyme reactions, it is important to demonstrate that the enzyme actually follows accepted chemical principles. One way to demonstrate this is by determining the effect of varying enzyme concentration on the rate of activity, while using a substrate concentration which is in excess.

Question, Hypothesis & Prediction

Q 4-1. *Pose a question about catalase concentration and reaction rate.*

Q 4-2. *Hypothesize about the effect of changing catalase concentration on the rate of reaction.*

Q 4-3. *Predict the result of the experiment based on your hypothesis (if/then).*

Experimental Procedure

Measuring Reaction Rates

1. Obtain each bottle of catalase, 3% H_2O_2, deionized water, and dilution buffer.
2. Obtain a half piece of filter paper and a hole puncher. Punch out ~25 discs into the square plastic dish labeled 'Filter Paper Disc'.
3. Obtain three (3) disposable plastic transfer pipettes and label on the bulb using a permanent marker as follows: 'E' for enzyme, 'S' for substrate, 'B' for buffer.
4. Obtain six (6) small plastic cups and label as follows: '0', '25', '50', '75', and '100' to indicate different concentrations of catalase (see Table 4-1); one, 'H_2O_2' to indicate substrate.
5. In the cup labeled 'H_2O_2', add **~20** ml of 3% H_2O_2 solution. (Precise measurements are not required. You may carefully pour from the bottle to 20 ml mark on the side of the cup.)
6. Set aside the cup containing the substrate, and while letting the substrate warm up to room temperature, prepare a series of diluted catalase solutions as follows:
   - 6a. Using the plastic transfer pipette labeled 'B' (for buffer), add dilution buffer in each of 5 labeled plastic cups as shown in Table 4-1.
   - 6b. Using the plastic transfer pipette labeled 'E' (for enzyme), add catalase solution as shown in Table 4-1.
   - 6c. Gently mix your enzyme solutions by swirling DO NOT use a pipette for mixing as it will cross-contaminate the pipette.

TABLE 4-1. Preparation of catalase solutions.

Catalase Conc. (Units/ml)	Amt. of Catalase to add (ml)[a]	Amt. of Dil. Buffer to add (ml)
0	0	4
25	1	3
50	2	2
75	3	1
100	4	0

a. 100 Units/ml

7. When the temperature of buffer solution has equilibrated to room temperature, measure the rate of reaction for each concentration of catalase **one by one** as follows:

7a. Using forceps, pick up and hold a filter paper disc by the edge of the disc. Avoid folding or creasing.

7b. **Without releasing** into the solution, immerse the disc held by forceps in the catalase solution of the highest concentration (100 U/ml). Keep the disc immersed for 5 seconds to allow absorption of the enzyme solution.

7c. Remove the disc from the solution and quickly blot excess solution from the disc by touching the other end of the disc on a Kimwipe.

NOTE: IMPORTANT! The substrate solution contains adequate amount of H_2O_2 *so that the amount of* H_2O_2 *broken down by the enzyme contained in the filter paper discs would not affect much the concentration of* H_2O_2 *in the substrate solution. However, it is very important to remove the excess of catalase solution from the disc before dropping it into the substrate solution because the excessive amount of enzyme added to the substrate solution will break down extra amount of* H_2O_2 *and lower the concentration of the substrate causing inaccurate results.*

7d. Drop the disc into the corresponding cup of substrate solution. If the disc will not sink immediately into the solution, push it down using the tip of forceps. (Wipe forceps to remove residual catalase from the tip before touching the substrate solution)

7e. Measure the time (*t*) in seconds from when the disc touches the substrate until it floats to the surface of the solution.

7f. Repeat for each of the remaining enzyme concentrations in the order of decreasing concentration.

7g. Record your data in Table 4-2, filling the bottom row first.

7h. The rate of enzyme reaction (*R*) is inversely proportional to time taken for the disc to float and calculated by the following equation:

$$R = \frac{1}{t} \qquad \text{[EQ 1]}$$

TABLE 4-2. Time of disc floating and the reaction rate in different concentrations of enzyme catalase.

Enzyme Conc. (Units/ml)	Time (t)	Reaction Rate (R)
0		
25		
50		
75		
100		

8. Empty the used cups, rinse out, and place upside down on a piece of Kimwipe to drain and dry for the subsequent experiments.
9. Construct a graph to illustrate your results showing reaction rate as a function of enzyme concentration. **Remember to title your graph and label each axis including appropriate unit.**

Q 4-4. *What is the independent variable? Which is the appropriate axis for this variable?*

Q 4-5. *What is the dependent variable? Which is the appropriate axis for this variable?*

Q 4-6. *How did the change in enzyme concentration affect the reaction rate?*

Activity B: *Effect of Substrate Concentration on Reaction Rate*

In this activity, you will determine the effects of varying substrate concentration on the rate of catalytic reactions, while using an enzyme at high concentration.

Question, Hypothesis and Prediction

Q 4-7. *Pose a question about substrate concentration and reaction rate.*

Q 4-8. *Hypothesize about the effect of changing substrate concentration on the rate of reaction.*

Q 4-9. *Predict the result of the experiment based on your hypothesis (if/then).*

Experimental Procedure

1. Label five (5) clean plastic cups and label as follows: '0', '1.0', '2.0', and '3.0' to indicate different concentrations of 'H_2O_2' (see Table 4-3); and one 'E' to indicate the enzyme catalase solution.

NOTE: You may use the same cup labeled '100' in "Activity A:", but using fresh catalase solution.

2. Prepare a series of diluted substrate H_2O_2 solutions as follows:
   - **2a.** Using the plastic transfer pipette labeled 'S' for substrate, add substrate (4% H_2O_2) in each of 4 labeled plastic cups as shown in Table 4-3.
   - **2b.** Using the plastic transfer pipette labeled 'B' for buffer, add appropriate amount of dilution buffer as shown in Table 4-3.
   - **2c.** Gently mix your substrate solutions by swirling. DO NOT use a pipette for mixing.

TABLE 4-3. Preparation of substrate solutions.

H_2O_2 Conc. (%)	4% H_2O_2 to add (ml)	Dil. Buffer to add (ml)
0.0	0	8
1.0	2	6
2.0	4	4
4.0	8	0

3. Set aside cups containing the substrate, and while letting the substrate warm up to room temperature (if it is still cold), add 2-3 ml of the undiluted catalase (100 U/ml) into the remaining empty cup labeled 'E'. Precise measurement is not required. You may pour out of the tube of the enzyme just enough amount to soak filter discs.
4. When the temperature of buffer solution has equilibrated to room temperature, determine the rate of the reaction starting at the highest substrate concentration, and record the results filling the bottom row first in Table 4-4. Use the filter paper disc procedure described in "Activity A:" on page 3 as summarized below:
   - **4a.** Using forceps, immerse a filter paper disc in the catalase solution.
   - **4b.** Allow the disc to absorb the enzyme solution for 5 seconds.
   - **4c.** Remove and very lightly blot the disc on a Kimwipe.
   - **4d.** Drop the disc into the first substrate solution.
   - **4e.** Measure and record the time interval.
   - **4f.** Repeat Step 4a-4e.
5. Record your results in Table 4-4.

TABLE 4-4. Effect of substrate concentration on catalase activity.

Substrate Conc. (%)	Time (t)	Reaction Rate (R)
0.0		
1.0		
2.0		
4.0		

6. The rate of enzyme reaction (*R*) is inversely proportional to time taken for the disc to float and calculated by the following equation:

$$R = \frac{1}{t}$$ **[EQ 2]**

7. Empty the used cups, rinse out, and place upside down on a piece of Kimwipe to drain and dry for the subsequent experiments.
8. Construct a graph to illustrate your results showing reaction rate as a function of substrate concentration. **Complete the plot (Figure 4-4) with proper labeling.**

Q 4-10. *How did change in substrate concentration affect the reaction rate?*

Q 4-11. *What would happen if the substrate concentration was increased to 30%; i.e., excessive amount of substrate in the reaction solution? Would it be 10 times faster than in the 3% substrate solution? Explain your answer.*

Activity C: *Effect of pH on Reaction Rate*

In this activity, you will determine the effects of varying pH on the rate of catalytic reactions, while using the highest concentrations of enzyme and substrate.

Question, Hypothesis and Prediction

Q 4-12. *Pose a question about pH and reaction rate.*

Q 4-13. *Hypothesize about the effect of changing pH on the rate of reaction.*

Q 4-14. *Predict the result of the experiment based on your hypothesis (if/then).*

Experimental Procedure

1. Label four (4) clean plastic cups and label them as follows to indicate different pH's (see Table 4-5): 3, 5, 7, 9; and, one 'E' to indicate the enzyme catalase solution.

NOTE: You may use the same cup labeled '100' in "Activity A:", or the cup labeled 'E' in "Activity B:", but using fresh catalase solution.

2. Into each of 4 cups, obtain 10 ml of the appropriate buffered H_2O_2 solution from the dispenser bottles on the front tables (use the white plastic organizer tray on your table to carry the cups).

NOTE: This buffered substrate are ready for reaction. Do not add any other solution.

3. Set aside cups containing the buffered substrates, and while letting the substrate warm up to room temperature (if it is still cold), add 2-3 ml of the undiluted catalase (100 U/ml) into the cup labeled 'E'. Precise measurement is not required. You may pour out of the bottle of the enzyme just enough to soak the filter discs.

4. When the temperature of buffer solution has equilibrated to room temperature, determine the rate of the reaction starting at pH 4, and record the results in Table 4-5. Use the same procedure used in “Activity A:” and “Activity B:”)..

TABLE 4-5. Effect of pH on catalase activity.

pH	Time (t)	Reaction Rate (R)
3		
5		
7		
9		

5. The rate of enzyme activity (*R*) is inversely proportional to time taken for the disc to float and calculated by the following equation:

$$R = \frac{1}{t} \qquad \textbf{[EQ 3]}$$

6. Construct a graph to illustrate your results showing reaction rate as a function of pH. **Complete the plot (Figure 4-5) with appropriate title and labels on each axis.**

Q 4-15. *How did pH affect enzyme activity?*

Q 4-16. *What was the optimal pH for catalase?*

Wrap-up

1. **Show your graphs to your lab instructor for verification.**
2. **Empty and dispose of used plastic cups and disposable pipettes in a trash can.**
3. **Empty, rinse and return the catalase tube, bottles of 3% H_2O_2 and dilution buffer so that your lab instructor can refill them for the next lab session.**
4. **Rinse glass pipettes, drain water, and return them on your lab table.**

PLOT #1: ENZYME CONC.

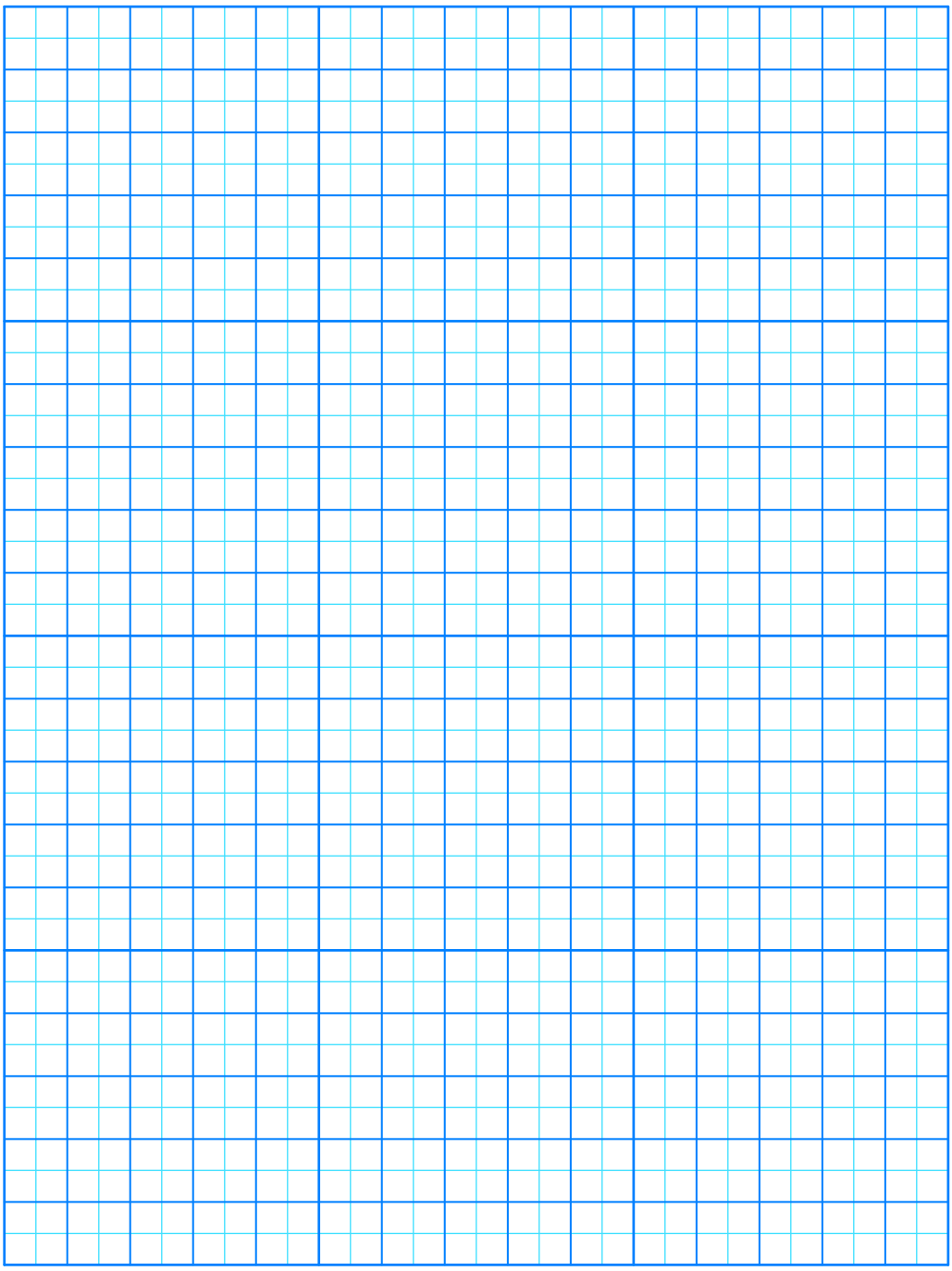

FIGURE 4-3. Enzyme reaction rate for different concentration of catalase.

PLOT #2: SUBSTRATE CONC.

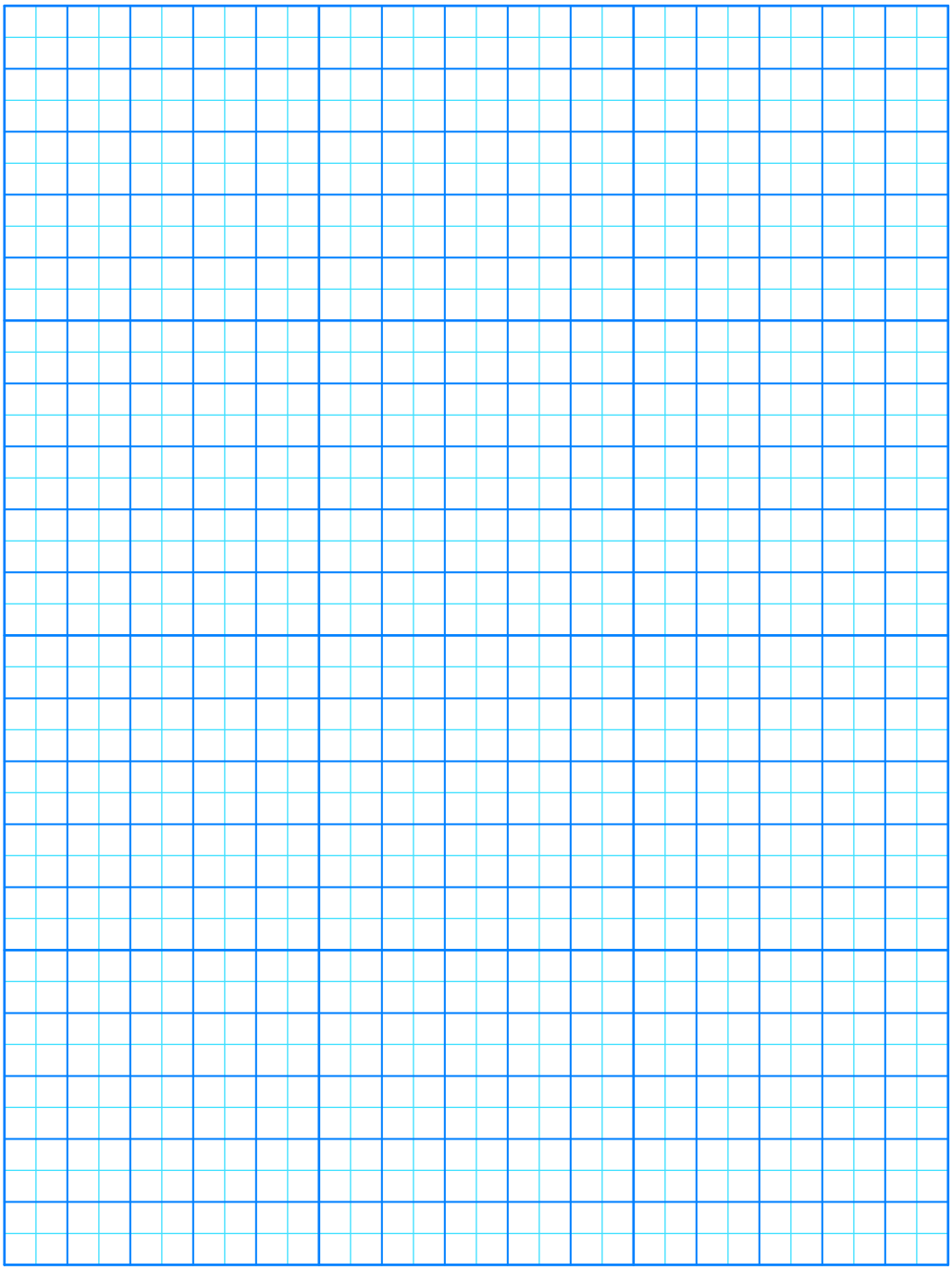

FIGURE 4-4. Enzyme reaction rate for different concentration of H_2O_2.

PLOT #3: pH

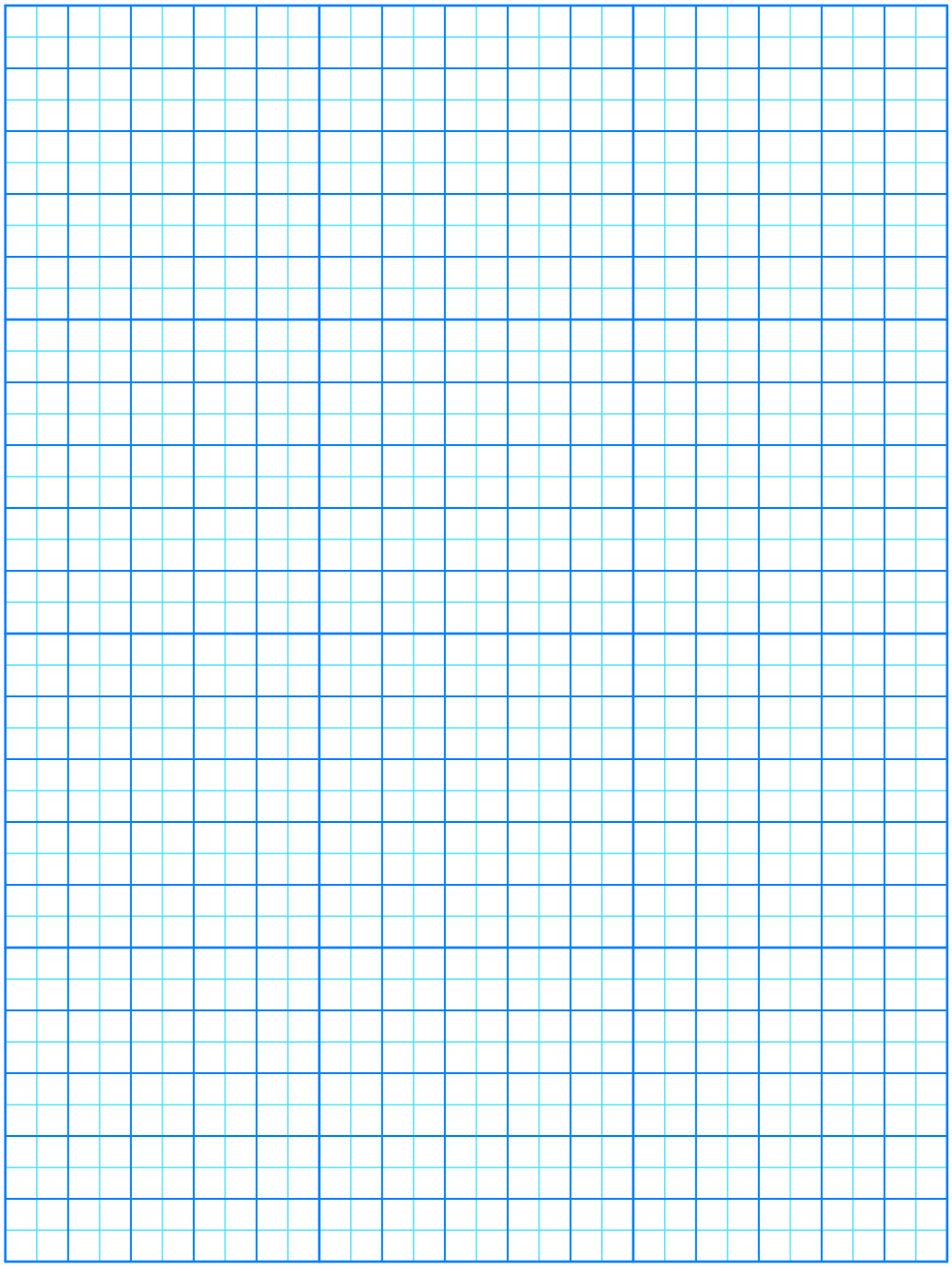

FIGURE 4-5. Enzyme reaction rate for different pH.

UNIT 4: Enzyme: The Action of Catalase

Lab Section #:_____ Lab Group #:_____

Name:_____

Q 4-4. What is the independent variable? Which is the appropriate axis for this variable? (p. 6)

Q 4-5. What is the dependent variable? Which is the appropriate axis for this variable? (p. 6)

Q 4-6. How did the change in enzyme concentration affect the reaction rate? (p. 6)

Q 4-10. How did change in substrate concentration affect the reaction rate? (p. 9)

Q 4-11. What would happen if the substrate concentration was increased to 30%; i.e., excessive amount of substrate in the reaction solution? Would it be 10 times faster than in the 3% substrate solution? Explain your answer. (p. 9)

Q 4-15. How did pH affect enzyme activity? (p. 11)

Q 4-16. What was the optimal pH for catalase? (p. 11)