Interactions

Big Idea 4

Baker's Dozen Lab 13: Enzyme Catalysis

Objectives

Before doing this lab you should understand the functions and activities of enzymes, the relationship between structure and function of enzymes, the factors that affect the rate of enzyme activity, and the terms *catalyst, catalysis,* and *catalase.*

After doing this lab you should be able to measure the effects of changes in temperature, pH, enzyme concentration, and substrate concentration on reaction rates of an enzyme-catalyzed reaction in a controlled experiment and explain how environmental factors affect the rate of enzyme-catalyzed reactions.

Introduction

Enzymes are proteins produced by living cells; they act as **catalysts** in biochemical reactions. A catalyst affects the rate of a chemical reaction. Enzymes enable cells to carry out complex chemical activities at relatively low temperatures.

Enzymes form an enzyme-substrate complex that reduces energy required for the specific reaction to occur. Enzymes have specific shapes and structures that determine their functions. Each enzyme is specific for a particular reaction because its amino acid sequence is unique, which causes it to have a unique three-dimensional structure. The **active site** is the portion of the enzyme that interacts with the **substrate** (the substance to be acted upon). If the shape of an enzyme is changed in any way, or the protein **denatured**, then the active site also changes, thus disrupting enzymatic functions.

Enzymes are organized into a number of groups depending on their specific activities. Two common groups are **catabolic** enzymes ("*cata"* or *"kata-"* from the Greek "to break down") and **anabolic** enzymes (*"a-"* or *"an-"* from the Greek "to build up"). Some catabolic enzymes are amylase (breaks complex starches into simple sugars) and lactase (digests sugar found in milk – lactose). Some anabolic enzymes are ATP synthase (combines ADP and phosphate to make ATP) and rubisco (builds sugar molecules in photosynthesis).

When an enzyme assists in chemical reactions, it is not changed nor used up; it can be recycled to do the same job with more substrates. However, they can be affected by a variety of factors.

1. **Salt Concentration:** If the salt concentration is close to zero, the charged amino acid sidechains of the enzyme molecules will attract each other. The enzyme will denature and form an inactive precipitate. If the salt concentration is very high, normal interactions of charged groups will be blocked, new interactions will occur, and the enzyme will precipitate. An intermediate salt concentration, like that found in human blood or cytoplasm, is ideal.
2. **pH:** Amino acid chains contain –COOH and $–NH_2$ groups that readily gain or lose H^+ ions. As the pH is lowered, an enzyme will tend to gain H^+ ions, which will disrupt the enzyme's shape. As the pH is raised, the enzyme may lose H^+ ions, which will cause the active site to be lost. Many enzymes perform optimally in the neutral range and are denatured outside of that. However, pepsin, an enzyme that digests proteins in the stomach, have an optimum pH range which is very low.
3. **Temperature:** Chemical reactions usually speed up as the temperature increases due to an increase in kinetic energy and movement of molecules. Since enzymes are catalysts for chemical reactions, enzyme reactions will also go faster as the temperature increases. However, there is a range for optimum temperature since enzymes are proteins. Remember proteins will denature, disrupting the active site conformation, if temperatures get too high. Many proteins are denatured by temperatures around 40-50^{o}C, but some are active around 60-70^{o}C and some can even withstand boiling.

4. **Activations and Inhibitors:** Many molecules other than the substrate may interact with an enzyme. If such a molecule increases the rate of reaction, it is called an **activator**. If it decreases the rate of reaction, it is called an **inhibitor**. 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 disulfide bridges formed between cysteine amino acids. Poisons are inhibitors that interfere with the active site of the enzyme.

The enzyme used in this lab, **catalase**, has four polypeptide chains, each composed of more than 500 amino acids. Catalase occurs universally in aerobic organisms. This enzyme prevents the accumulation of hydrogen peroxide in cells, which is toxic in high levels but is formed as a byproduct of metabolic activities. The primary reaction catalyzed by catalase is the decomposition of H_2O_2 to form water and oxygen, as shown below.

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

In the absence of catalase, this reaction still occurs, albeit very slowly. In this experiment, you will determine the rate at which catalase speeds up this reaction. The rate of a chemical reaction may be studied in a variety of ways, as described below:

1. Measuring the rate of disappearance of the substrate (in this case, hydrogen peroxide)
2. Measuring the rate of appearance of a product (in this case, water or oxygen gas)
3. Measuring the heat released (or absorbed) during the reaction.

Video Demonstration: Test of Catalase Activity

1. Observe the reaction as shown in the video and answer the questions below.

What is the enzyme in this reaction? _____

What is the substrate in this reaction? _____

What are the products in this reaction? _____

How could you show that the gas is actually oxygen? _____

2. Observe the reaction using boiled catalase. How does the reaction compare to the one using unboiled catalase? Explain the reason for this difference.

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3. Observe the reaction using catalase from the potato or liver. What do you observe? What evidence is there for the presence of catalase in the macerated potato or liver?

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General Procedural Set Up

1. Working as a group of four (whole lab table), assemble the experimental apparatus as shown in the picture. The vial with the rubber stopper is **the reaction chamber**. The plastic bin will be used as a water bath.
2. Fill the bin ¾ full of tap water. Allow water to come to room temperature (25°C).
3. Submerge the 100 ml graduated cylinder to fill it with water. You might need to tilt it up under the water to ensure all air bubbles are removed.
4. Turn the graduated cylinder upside down, keeping the open end always under water so no air gets in. Suspend it upside down with the clamp on the ring stand. Adjust the height of the clamp so the open end of the graduated cylinder is about 3 cm above the bottom of the bin.
5. Place a thermometer in the bin.

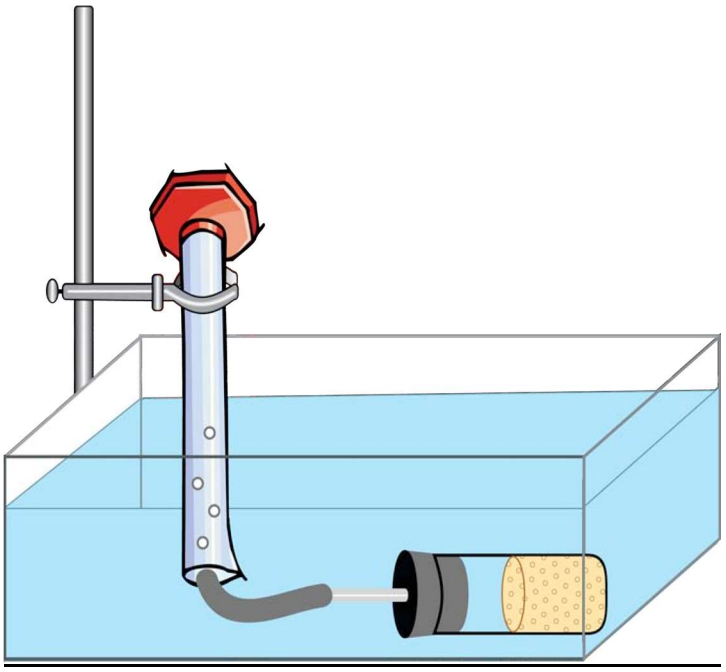
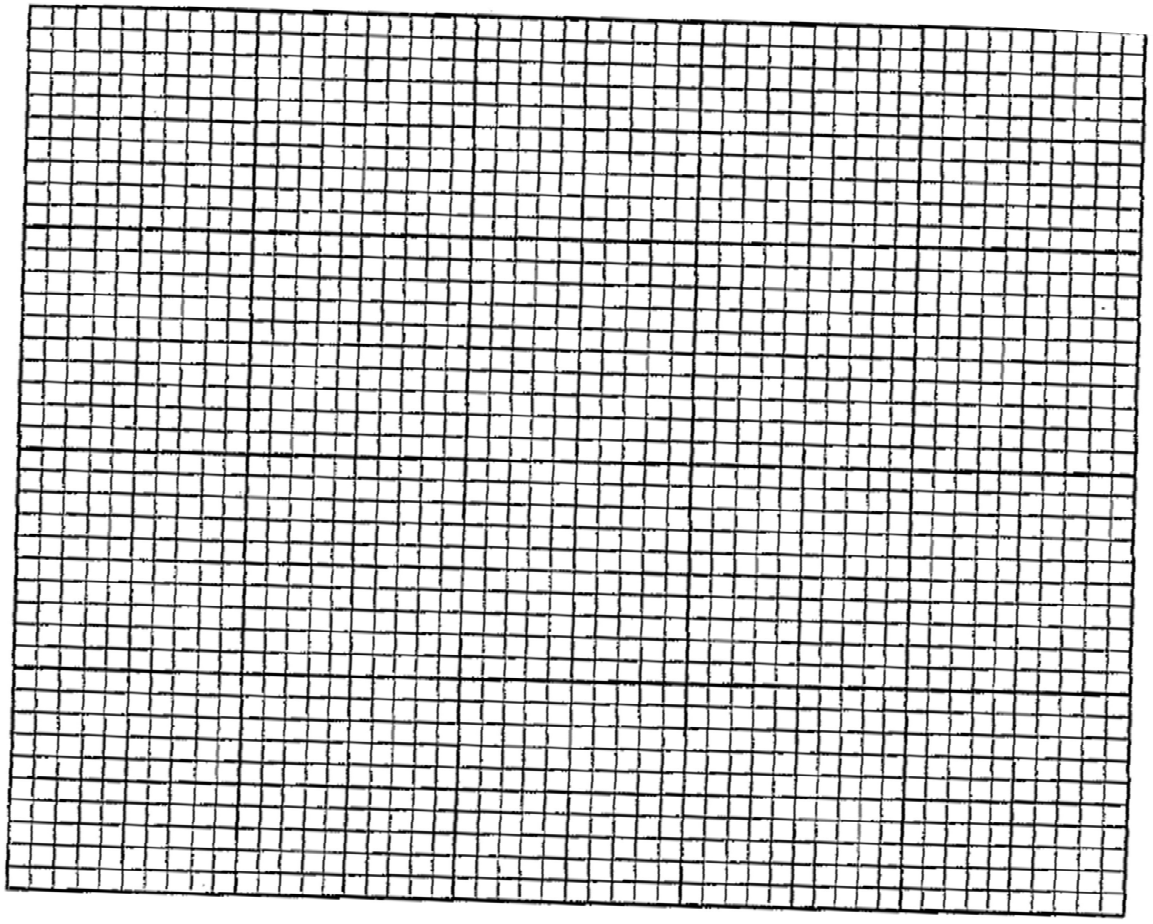


Figure 13.1

Procedure 13A: The Time Course of Catalase

1. Pour 10 mL of hydrogen peroxide (H_2O_2) into the reaction chamber.
2. Add 1 mL of catalase solution (yeast) and ***IMMEDIATELY*** stopper the reaction chamber, submerge it in the water and place the plastic tubing into the graduated cylinder.
3. Measure the gas levels in the graduated cylinder every 30 seconds for 5 minutes. Record your data in the table below, and graph your results.

Time Interval (min)	Gas Produced (in mL)	Total Gas Produced (in mL)
0		
0.5		
1		
1.5		
2		
2.5		
3		
3.5		
4		
4.5		
5		



Analysis of Results

1. Using the formula for slope (rate of change), determine the rate of the reaction for each time interval below. $\Delta y/\Delta x$

	Time Intervals (seconds)						
	0-10 sec	**10-30 sec**	**30-60 sec**	**60-120 sec**	**120 180 sec**	**180-240 sec**	**240-300 sec**
Rates							

2. When is the rate the highest? Explain why.

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3. When is the rate the lowest? For what reasons is the rate low?

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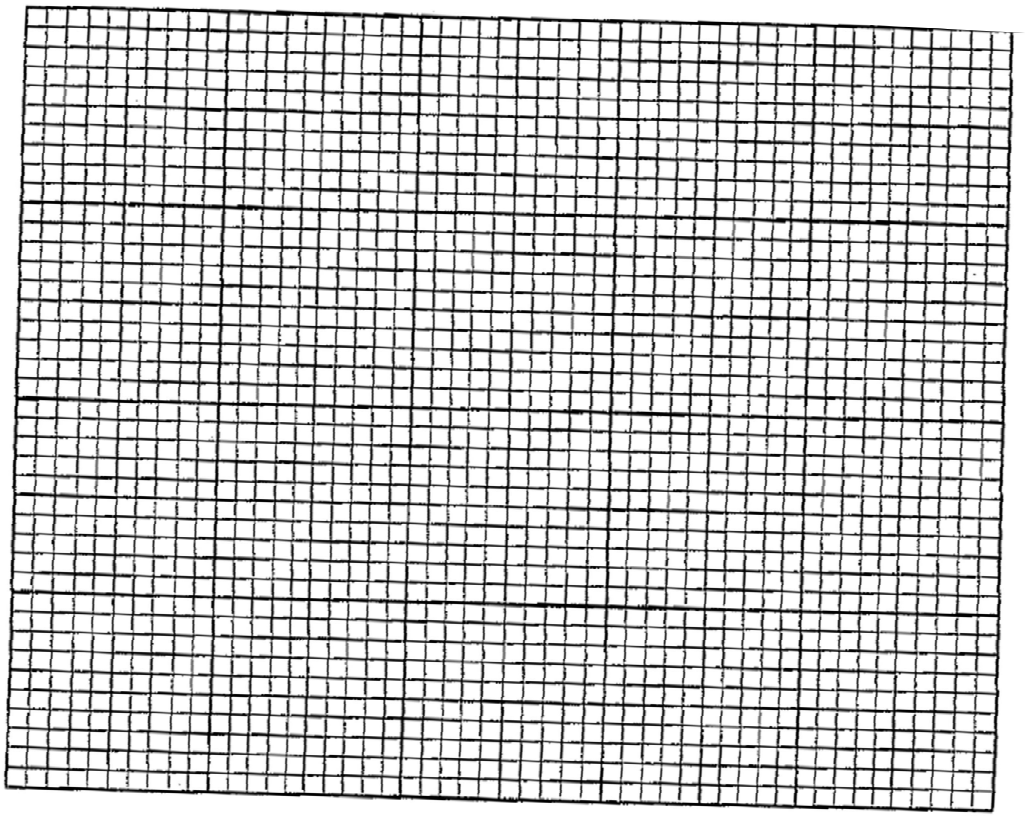
Procedure 13B: Effect of Enzyme Concentration on Rate of Reaction

1. Repeat the experiment from part A using three different levels of enzyme concentration.
    a. Use 0.75 mL catalase solution and 0.25 mL of distilled water in the reaction chamber.
    b. Use 0.50 mL catalase solution and 0.50 mL of distilled water in the reaction chamber.
    c. Use 0.25 mL catalase solution and 0.75 mL of distilled water in the reaction chamber.
2. Record the data below and graph it.

Time Interval	Total Gas (in mL) [**0.75**]	Total Gas (in mL) **[0.50]**	Total Gas (in mL) **[0.25]**
0			
0.5			
1			
1.5			
2			
2.5			
3			
3.5			
4			
4.5			
5			



Day Two: You will be assigned a factor to test (temperature, pH, salt concentration). Write your experimental design, including your hypothesis, DV, control group, and steps below. Be sure to include at least 3 different "levels" of factor.

Assigned factor: _____ DV: _____

Hypothesis: _____

Procedure: _____

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Data for Assigned Factor: Include your data table on a separate sheet of paper and attach it to this lab.

Graph for ALL FACTORS: Graph the class averages for EACH of the three factors (data will be on the class website).

Analysis of Results (Be sure to graph the class average data for all three factors and attach it to this lab)

1. Explain how changing the temperature affected the enzyme reaction. Relate this to enzyme structure and kinetics.

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2. Explain how changing the pH affected the enzyme reaction. Relate this to enzyme structure.

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3. Explain how changing the ionic concentration affected the enzyme reaction. Relate this to enzyme structure.

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