# Lab 6: Enzymes

*BIOL-8*

**Name:** _____ **Date:** _____

**Name:** **Date:**

## Objectives

By the end of this lab, you will be able to:

- **Define enzymes** and explain their role as biological catalysts
- **Describe the enzyme-substrate interaction** using the lock-and-key or induced fit model
- **Investigate factors affecting enzyme activity** (temperature, pH, concentration)
- **Measure enzyme reaction rates** using qualitative and quantitative methods
- **Interpret graphical data** showing enzyme activity under different conditions
- **Apply enzyme concepts** to human physiology and medicine

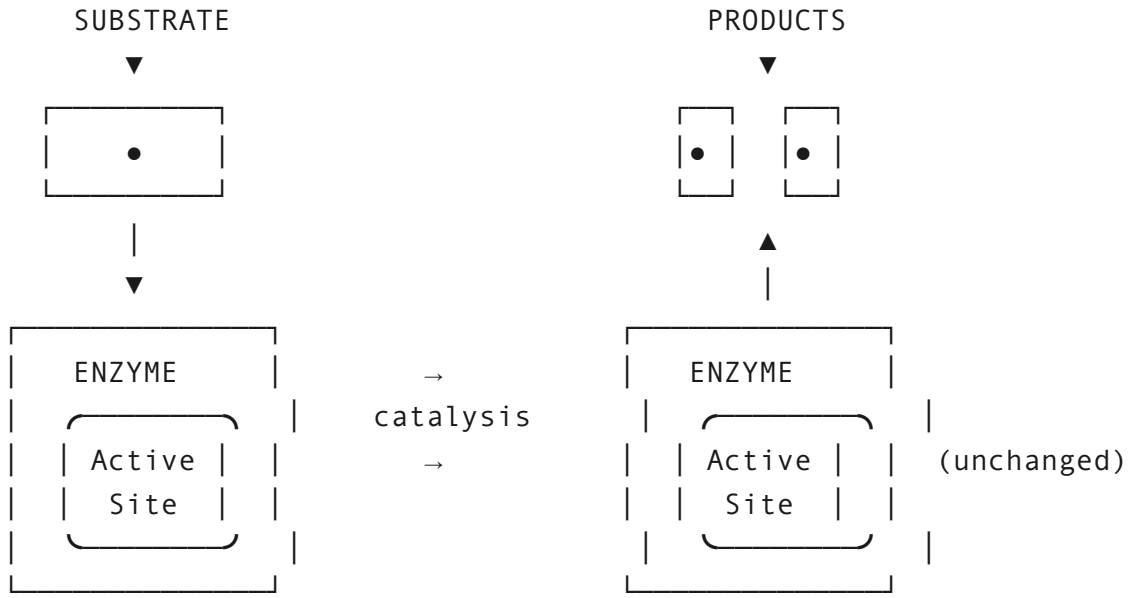
## Introduction

Enzymes are biological catalysts — proteins that speed up chemical reactions without being consumed in the process. Without enzymes, most biological reactions would occur too slowly to sustain life.

**Key Concepts:**

| Term | Definition |
|---|---|
| **Enzyme** | Protein that catalyzes (speeds up) a specific reaction |
| **Substrate** | The molecule(s) that an enzyme acts upon |
| **Active Site** | The region of the enzyme where the substrate binds |
| **Product** | The molecule(s) produced by the reaction |
| **Catalyst** | A substance that speeds a reaction without being consumed |

**The Enzyme-Substrate Model:**




**Factors Affecting Enzyme Activity:**

1. **Temperature** — Enzymes have an optimal temperature; too hot denatures them
2. **pH** — Each enzyme has an optimal pH range
3. **Substrate concentration** — More substrate = faster reaction (until saturation)
4. **Enzyme concentration** — More enzyme = faster reaction
5. **Inhibitors** — Molecules that reduce enzyme activity

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## Part 1: Catalase Activity

> ***Learning Goal:*** *Observe enzyme activity and measure reaction rates using catalase.*

### Background

**Catalase** is an enzyme found in nearly all living organisms. It breaks down hydrogen peroxide ($H_2O_2$), a toxic byproduct of cellular metabolism, into water and oxygen:

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2 \uparrow$$

(hydrogen peroxide) (water) (oxygen gas — bubbles!)

The production of oxygen gas (bubbles) provides a visible indicator of enzyme activity.

## Materials

- Fresh liver, potato, or yeast (source of catalase)
- 3% hydrogen peroxide ($H_2O_2$)
- Test tubes
- Graduated cylinder
- Ruler
- Timer/stopwatch
- Safety goggles

## Procedure

1. Place a small piece of liver (or potato, or yeast suspension) in a test tube
2. Add 5 mL of 3% hydrogen peroxide
3. Immediately observe and time the reaction
4. Measure the **height of the foam/bubbles** produced after 30 seconds
5. Rate the reaction intensity (-, +, ++, +++)

## Baseline Observations

### Catalase Activity — Baseline

| # | Enzyme Source | $H_2O_2$ Added | Foam Height at 30 sec (cm) | Intensity Rating | Observations |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |

**What evidence indicates that a chemical reaction occurred?**

**Why is $H_2O_2$ dangerous to cells, and why is catalase important?**

## Part 2: Effect of Temperature on Enzyme Activity

> ***Learning Goal:*** *Investigate how temperature affects the rate of an enzyme-catalyzed reaction.*

### Background

Like all proteins, enzymes are sensitive to temperature:

- **Low temperature:** Molecules move slowly, fewer collisions, slow reaction
- **Optimal temperature:** Maximum enzyme activity
- **High temperature:** Enzyme denatures (loses shape), activity decreases or stops

Human enzymes typically work best around 37°C (body temperature).

### Procedure

1. Prepare 4 test tubes, each with equal amounts of enzyme source (liver)
2. Pre-incubate each tube at a different temperature for 5 minutes:
3. Ice bath (~5°C)
4. Room temperature (~20-25°C)
5. Warm water bath (~37°C)
6. Hot water bath (~60-80°C)
7. Add 5 mL of hydrogen peroxide (also pre-warmed to the same temperature)
8. Measure foam height at 30 seconds

## Hypothesis

**Predict which temperature will show the highest enzyme activity. Explain your reasoning:**

## Data Collection

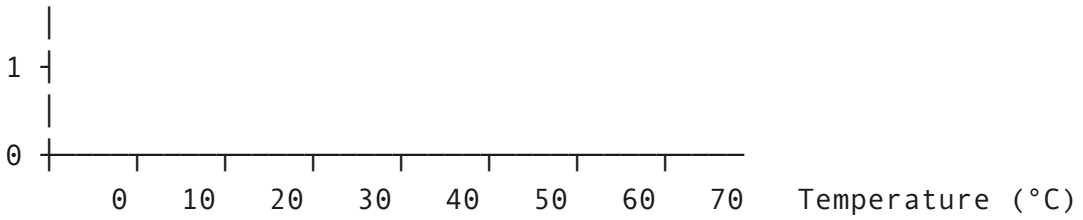
### Effect of Temperature on Catalase Activity

| # | Temperature (°C) | Foam Height (cm) | Intensity Rating | Observations |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |

## Graph Your Results

Plot temperature (x-axis) vs. foam height (y-axis):

```
Foam Height (cm)
    |
  6 ┤
    |
  5 ┤
    |
  4 ┤
    |
  3 ┤
    |
  2 ┤
```




## Analysis

**1. At which temperature was enzyme activity greatest?**

**2. What happened to enzyme activity at high temperature? Why?**

**3. If an enzyme is denatured by high temperature, can it recover when cooled? Explain:**

**4. Why do you think human enzymes work best around 37°C?**

**5. A high fever (40°C+) can be dangerous. How might this relate to enzyme function?**

## Part 3: Effect of pH on Enzyme Activity

> ***Learning Goal:*** *Investigate how pH affects the rate of an enzyme-catalyzed reaction.*

### Background

Each enzyme has an **optimal pH** — the pH at which it works best. Extreme pH values can denature enzymes, just like extreme temperatures.

**Examples of Enzyme Optimal pH:**

| Enzyme | Location | Optimal pH |
|---|---|---|
| Pepsin | Stomach | 1.5 - 2.0 |
| Catalase | Most cells | 7.0 - 7.5 |
| Trypsin | Small intestine | 7.5 - 8.5 |
| Lipase (pancreatic) | Small intestine | 8.0 |

### Procedure

1. Prepare 4 test tubes, each with equal amounts of enzyme source
2. Pre-treat each sample with a buffer of different pH for 2 minutes:
3. pH 2 (acidic)
4. pH 5 (slightly acidic)
5. pH 7 (neutral)
6. pH 10 (basic)
7. Add 5 mL of hydrogen peroxide
8. Measure foam height at 30 seconds

### Hypothesis

**Predict which pH will show the highest catalase activity. Explain:**

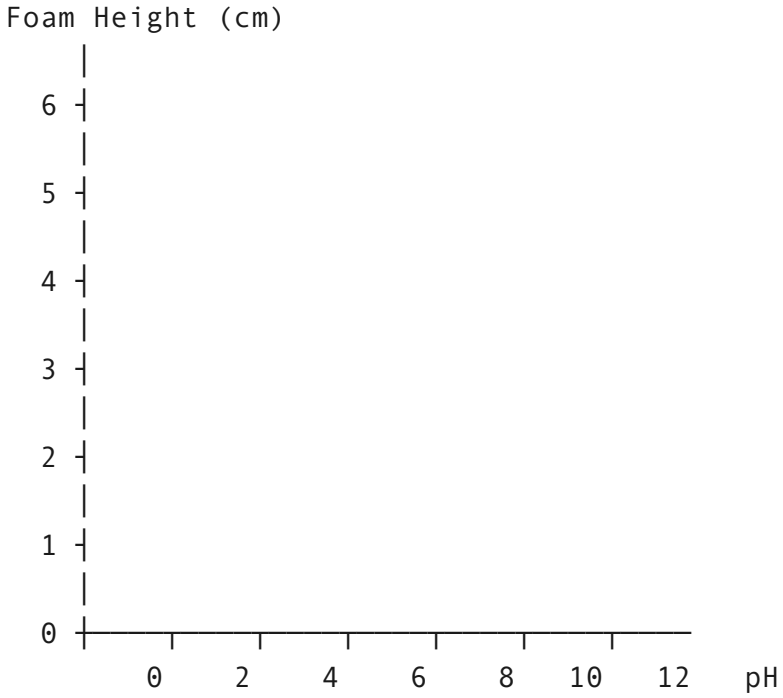
## Data Collection

### Effect of pH on Catalase Activity

| # | pH | Foam Height (cm) | Intensity Rating | Observations |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |

### Graph Your Results

Plot pH (x-axis) vs. foam height (y-axis):




### Analysis

**1. At which pH was enzyme activity greatest?**

**2. Why does extreme pH (very acidic or very basic) reduce enzyme activity?**

**3. Pepsin (a stomach enzyme) works best at pH 2. Why would this enzyme be inactive in the small intestine (pH 8)?**

**4. How do the results relate to the importance of pH buffers in the body?**

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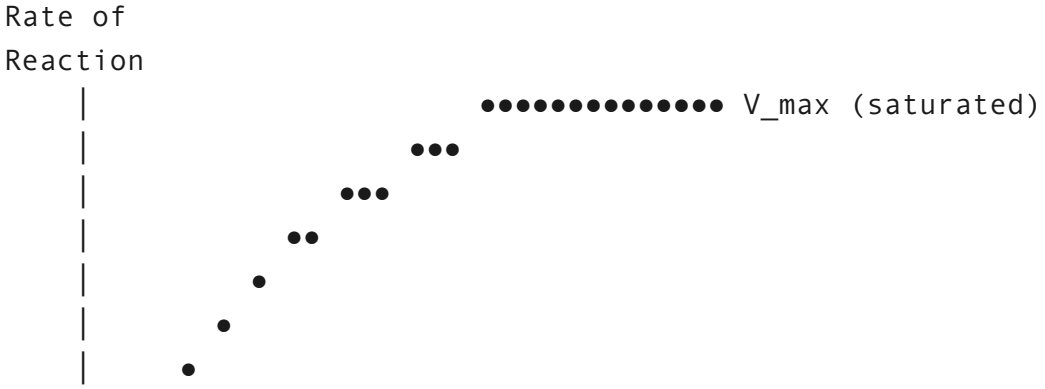
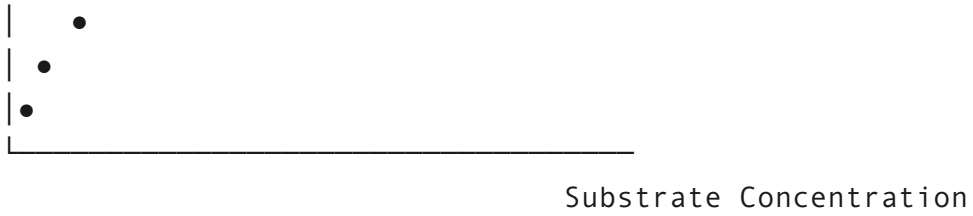
## Part 4: Effect of Substrate Concentration

***Learning Goal:*** *Investigate how substrate concentration affects reaction rate.*

### Background

As substrate concentration increases, the reaction rate increases — up to a point. When all enzyme active sites are occupied, the reaction rate reaches a maximum (**V_max**). Adding more substrate beyond this point will not increase the rate.






## Procedure

1. Prepare 4 test tubes with equal amounts of enzyme (liver)
2. Add different concentrations of $H_2O_2$:
3. 0.5% $H_2O_2$
4. 1.5% $H_2O_2$
5. 3.0% $H_2O_2$
6. 6.0% $H_2O_2$ (if available)
7. Measure foam height at 30 seconds

## Hypothesis

**Predict how increasing $H_2O_2$ concentration will affect reaction rate:**

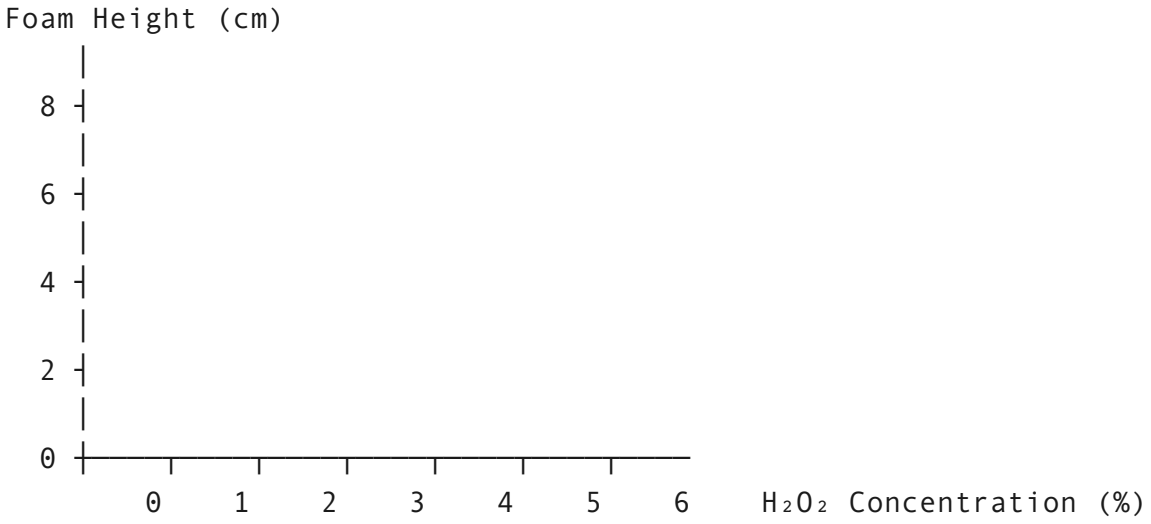
## Data Collection

### Effect of Substrate Concentration on Catalase Activity

| # | $H_2O_2$ Concentration (%) | Foam Height (cm) | Intensity Rating | Observations |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |

### Graph Your Results

Plot substrate concentration (x-axis) vs. foam height (y-axis):




### Analysis

**1. Did reaction rate continue to increase as substrate concentration increased?**

**2. Explain the concept of enzyme saturation in your own words:**

**3. If you wanted to increase the reaction rate beyond V_max, what would you need to do?**

## Part 5: Summary Data and Comparisons

### Combined Results Summary

### Summary of Optimal Conditions

| # | Factor Tested | Condition with Maximum Activity | Predicted Optimal | Match? (Y/N) |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |

## Conclusions

**1. Summarize the role of enzymes in biological systems:**

**2. Explain why enzymes are described as "specific." What determines which substrate an enzyme will bind?**

**3. A patient has a fever of 40°C (104°F). How might this affect enzyme function in their body?**

**4. Some medications work by inhibiting specific enzymes. Give an example and explain how this relates to treatment:**

**5. What would happen to catalase activity if you boiled the liver first, then added $H_2O_2$? Explain:**

## Quick Reference

### Enzyme Kinetics Terms

| Term | Definition |
|---|---|
| V_max | Maximum reaction rate (when enzyme is saturated) |
| Optimal temperature | Temperature at which enzyme activity is highest |
| Optimal pH | pH at which enzyme activity is highest |
| Denaturation | Permanent loss of protein shape/function |

### Factors Affecting Enzyme Activity Summary

| Factor | Effect of Increase |
|---|---|
| Temperature | ↑ activity until optimal, then ↓ (denaturation) |

| Factor | Effect of Increase |
|---|---|
| pH | ↑ activity until optimal, then ↓ |
| Substrate concentration | ↑ activity until saturation (V_max) |
| Enzyme concentration | ↑ activity (proportional) |

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**Connection to Module 06:** Enzymes are central to metabolism — every chemical reaction in your body is catalyzed by an enzyme. From digesting food (amylase, pepsin, lipase) to generating energy (ATP synthase) to building muscle (protein synthesis enzymes), understanding enzymes is essential for understanding how your body works. Enzyme disorders and enzyme-targeting drugs are major topics in medicine.

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*Lab adapted for BIOL-8: Human Biology, Spring 2026*