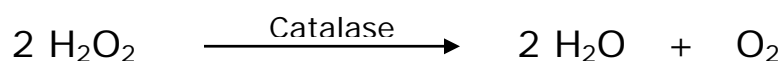


## CATALASE LAB

### INTRODUCTION

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a poisonous byproduct of metabolism that can damage cells if it is not removed. Catalase is an enzyme that speeds up the breakdown of hydrogen peroxide into water (H<sub>2</sub>O) and oxygen gas (O<sub>2</sub>).



Remember: A **catalyst** is a substance that lowers the activation energy required for a chemical reaction, and therefore increases the rate of the reaction without being used up in the process. CATALASE is an enzyme, a biological (organic) catalyst. Hydrogen peroxide is the substrate for catalase.

\*\*\*You will be working with hot water, acids and bases in this laboratory\*\*\*  
\*\*\*\*Use Extreme Caution!!!\*\*\*\*

The general procedure for the lab is outlined below, and specific details for each variable follow.

### GENERAL DIRECTIONS:

The assay system used in this lab consists of a filter paper disc coated with the enzyme and then pushed to the bottom of a beaker of substrate (hydrogen peroxide). As the catalase breaks down the hydrogen peroxide into water and oxygen gas, the bubbles of oxygen collect underneath the disc and make it rise to the surface of the hydrogen peroxide. The time it takes for the filter paper disc to rise is an indication of the rate of enzyme activity.

### RATE:

Rate of enzyme activity is equal to the distance (depth of hydrogen peroxide in mm) divided by the time (in sec) for the disc to rise to the surface. We will assume that each filter is coated with the same amount of catalase (except in the investigation of the effect of enzyme concentration on enzyme activity).

The enzyme has been prepared for you as follows: 50g of peeled potato was mixed with 50 ml cold distilled water and crushed ice and homogenized in a blender for 30 seconds. This extract was filtered through cheesecloth and cold distilled water was added to a total volume of 100 ml. Extract concentration is arbitrarily set at 100 units/ml. **The enzyme should be kept on ice at all times!!**

## **MATERIALS:**

Catalase on ice	50 mL beakers
Hydrogen peroxide 3% and 1.0%	Marker
Forceps	Stopwatch or timer
Filter paper	Craft punch
Water	Test tubes
Distilled water	Graduated cylinder
Water baths (0°C, 37°C, 100°C)	Pipet, calibrated

## **PROCEDURE:**

Each group will investigate and report on two of the following questions. Suggested procedures for each question are given below. **Every student is responsible for recording the results of all four experiments** on this activity.

1. What is the effect of enzyme concentration on enzyme activity?
2. What is the effect of substrate concentration on enzyme activity?
3. What is the effect of pH on enzyme activity?
4. What is the effect of temperature on enzyme activity?

### **1. What is the effect of enzyme concentration on enzyme activity?**

- a. Set up five beakers containing 40 mL of 3% hydrogen peroxide each. Measure and record the depth of the hydrogen peroxide in the beakers.
- b. Dilute the enzyme as follows. Make each dilution in a separate paper cup.

100 units/mL	=	20 ml	100 units/ml
80 units/mL	=	12 ml	100 units/ml + 3 ml cold dH <sub>2</sub> O
50 units/mL	=	10 ml	100 units/ml + 10 ml cold dH <sub>2</sub> O
20 units/mL	=	3 ml	100 units/ml + 12 ml cold dH <sub>2</sub> O
0 units/mL	=	20 ml	cold dH <sub>2</sub> O

- c. Using forceps, dip a disc into the enzyme solution at 100 units/ml, then remove it and drain it on a paper towel.
- d. Use the forceps to push the disc to the bottom of a beaker hydrogen peroxide. Time how long it takes the disc to rise to the surface.
- e. Repeat this procedure for each of the other enzyme dilutions, and be sure to use a FRESH vial of substrate for each filter.
- f. Record your results in the appropriate data chart.

**2. What is the effect of substrate concentration on enzyme activity?**

- a. Obtain 1 vial of catalase at 100 units/mL
- b. Dilute the substrate (hydrogen peroxide) as described below. Each dilution should be made in a separate labeled beaker. Measure and record the depth of the hydrogen peroxide.  
  

3.0% H <sub>2</sub> O <sub>2</sub> :	40 mL 3% H <sub>2</sub> O <sub>2</sub>
1.5% H <sub>2</sub> O <sub>2</sub> :	20 mL 3% H <sub>2</sub> O <sub>2</sub> + 20 ml distilled water
0.75% H <sub>2</sub> O <sub>2</sub> :	10 mL 3% H <sub>2</sub> O <sub>2</sub> + 30 ml distilled water
0.38% H <sub>2</sub> O <sub>2</sub> :	5 mL 3% H <sub>2</sub> O <sub>2</sub> + 35 ml distilled water
0.0% H <sub>2</sub> O <sub>2</sub> :	40 mL distilled water
- c. Dip a disc into the catalase, drain on a paper towel.
- d. Use the forceps to push the disc to the bottom of a beaker of peroxide. Time how long it takes the disc to rise to the surface.
- e. Repeat this procedure for each of the substrate dilutions. Record your results in the appropriate data chart.

**3. What is the effect of pH on enzyme activity?**

- a. Obtain 1 vial of catalase at 100 units/mL.
- b. Set up 5 beakers of 40 mL 1% H<sub>2</sub>O<sub>2</sub>. Measure and record the depth of the hydrogen peroxide.
- c. Label 5 paper cups as follows: pH3, pH5, pH7, pH9, pH11 and dilute catalase into the appropriate vial as directed below:  
  

pH 3:	5 mL catalase + 5 mL pH 3 Buffer
pH 5:	5 mL catalase + 5 mL pH 5 Buffer
pH 7:	5 mL catalase + 5 mL pH 7 Buffer
pH 9:	5 mL catalase + 5 mL pH 9 Buffer
pH 11:	5 mL catalase + 5 mL pH 11 Buffer
- d. Dip a filter into the catalase at pH 3, drain on a paper towel.
- e. Use the forceps to push the disc to the bottom of a beaker of peroxide. Time how long it takes the disc to rise to the surface.
- f. Repeat this procedure for each pH.
- g. Record your results in the appropriate data chart.

#### 4. What is the effect of temperature on enzyme activity?

- a. Obtain 1 vial of catalase at 100 units/mL.
- b. Set up an ice bath (0°C), a room temp water bath, a 37°C bath and a boiling water bath
- c. Place 5 ml of catalase at 100 units/mL in each of 4 test tubes. Place 1 test tube in each of the water baths.
- d. Place 40 mL 1% H<sub>2</sub>O<sub>2</sub> in each of 4 beakers. Measure and record the depth of the H<sub>2</sub>O<sub>2</sub>. Place 1 beaker in the 0°C bath and leave 3 at room temperature. This is necessary because heat will destroy the hydrogen peroxide.
- e. Allow the catalase and substrate to incubate at each temperature for about 5 minutes.
- f. Test the 0 °C catalase.
  - Pour the 0 °C catalase into a paper cup.
  - Dip a filter paper disc into the 0 °C catalase and drain the disc on a paper towel.
  - Use forceps to push the 0 °C disc to the bottom of the 0 °C hydrogen peroxide.
  - Time how long it takes the disc to rise to the top. Record your results in the appropriate data table.
- g. Test the 22 °C (room temperature) catalase.
  - Pour the 22 °C catalase into a paper cup.
  - Dip a filter paper disc into the 22 °C catalase and drain the disc on a paper towel.
  - Use forceps to push the 22 °C disc to the bottom of the room temperature hydrogen peroxide.
  - Time how long it takes the disc to rise to the top. Record your results in the appropriate data table.
- h. Test the 37 °C catalase.
  - Pour the 37 °C catalase into a paper cup.
  - Dip a filter paper disc into the 37 °C catalase and drain the disc on a paper towel.
  - Use forceps to push the 37 °C disc to the bottom of the room temperature hydrogen peroxide.
  - Time how long it takes the disc to rise to the top. Record your results in the appropriate data table.

- i. Test the boiled (100 °C) catalase.
- Pour the 100 °C catalase into a paper cup.
  - Dip a filter paper disc into the 100 °C catalase and drain the disc on a paper towel.
  - Use forceps to push the 100 °C disc to the bottom of the room temperature hydrogen peroxide.
  - Time how long it takes the disc to rise to the top. Record your results in the appropriate data table.

**DATA TABLES WITH RESULTS**

**1. What is the effect of enzyme concentration on enzyme activity?**

<b>Enzyme Conc.</b>	<b>Distance disc traveled</b>	<b>Time</b>	<b>Rate</b>	<b>Rate: Class Ave.</b>
0 units/mL				
20 units/mL				
50 units/mL				
80 units/mL				
100 units/mL				

**2. What is the effect of substrate concentration on enzyme activity?**

<b>H<sub>2</sub>O<sub>2</sub> Conc.</b>	<b>Distance disc traveled</b>	<b>Time</b>	<b>Rate</b>	<b>Rate: Class Ave.</b>
0 %				
0.38 %				
0.75 %				
1.5 %				
3.0 %				

3. **What is the effect of pH on enzyme activity?**

pH	Distance disc traveled	Time	Rate	Rate: Class Ave.
3				
5				
7				
9				
11				

4. **What is the effect of temperature on enzyme activity?**

Temp.	Distance disc traveled	Time	Rate	Rate: Class Ave.
0				
22				
37				
100				

**ANALYSIS:**

For EACH variable, use the class average rates to construct a graph of the independent variable vs. the dependent variable. This means you will have four graphs:

- Rate (y axis) vs. Enzyme Concentration (x axis)
- Rate (y axis) vs. Substrate Concentration (x axis)
- Rate (y axis) vs. pH (x axis)
- Rate (y axis) vs. Temperature (x axis)

Attach your graphs to this lab.

**CONCLUSION:**

Answer the following questions:

1. What is the effect of enzyme concentration on enzyme activity? Explain how enzyme activity changes as enzyme concentration decreases, and discuss why this occurs (on a molecular level).

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2. What is the effect of substrate concentration on enzyme activity? How does enzyme activity change as substrate concentration decreases? Explain your observations by discussing this reaction on a molecular level.

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3. How does temperature affect the activity of catalase? Explain your observations by discussing the effect of temperature on protein structure. Discuss both high and low temperature effects.

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4. How does pH affect the activity of catalase? Consider both high and low pH, and explain your observations by discussing the effect of pH on protein structure.

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5. Ectothermic organisms have body temperatures that vary with the temperature of their surroundings. Discuss the effect this variation might have on the functioning of enzymes in these organisms. Suggest some ways ectothermic organisms might cope with this problem.

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