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Adapted from: AP Biology Labs; Brad Williamson of Olathe, KS; Gen Nelson, Access Excellence Activities Exchange; and NABT [Biology on a Shoestring](#)

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 and then test the impact of 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; and
- 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: $E + S \rightarrow ES \rightarrow E + P$

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 $-NH_2$ 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°C denature many proteins, but some are still active at 70-80°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.

INTRODUCTION:

The **enzyme used in lab 2A and 2B - catalase** - has four polypeptide chains, each composed of more than 500 amino acids. **This enzyme is found throughout aerobic organisms.** 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). Catalase may also take part in some of the many oxidation reactions that occur in all cells.

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

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

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 cup) is an indication of the rate of enzyme activity.

$$\text{RATE}_{\text{ENZYME ACTIVITY}} = \text{DISTANCE}_{\text{DEPTH OF HYDROGEN PEROXIDE IN MM}} / \text{TIME}_{\text{IN SEC}}$$

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.

EXERCISE 2A PROCEDURE:

1. Prepare a beaker or clear cup with old H₂O₂ that is 4 cm deep.
2. Using a single whole punch cut individual single layer disks of coffee filter paper.
3. Pour a small amount of 100% catalase in a second cup. **Shake the bottle of solution BEFORE pouring** to make sure it is well mixed.
4. Pick up a single disk with forceps and dip the disk in your catalase enzyme solution in the cup.
5. Still using the forceps, toss the disk into the H₂O₂. Watch the disk carefully and start timing when the disk hits the bottom of the cup, stop timing when the disk reaches the surface.
6. Run 5 timed trials with the old H₂O₂ at room temperature to perfect your technique.
7. **When you cleaning up** make sure the filter disks do **NOT go down the drain**.

Practice - Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂

Trial	Seconds to Rise
1	
2	
3	
4	
5	

- a. What is the **enzyme** in this reaction? _____
 - b. What is the **substrate** in this reaction? _____
 - c. What is/are the **product(s)** in this reaction? _____
 - d. What is the gas you see bubbling up? _____
8. Now get a small amount of the boiled catalase and run 2 timed trials.

Practice - Number of Seconds Required For a Boiled Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂

Trial	Seconds to Rise
1	
2	

- a. What happened? _____
- b. Why? _____

EXERCISE 2B PROCEDURE:

For this part of the lab you will work in 8 groups of two to four people each. **Each group** will test and report data on **one** of the following questions (two groups will do each question and their data will be pooled for the class use). EVERY STUDENT will be responsible for getting the class data for **all four** questions off Blackboard, answering **all questions** related to all four situations, and **evaluating all four** in his/her lab report with the help of box and whiskers graphs.

Investigation questions:

- What is the effect of enzyme concentration on enzyme activity?
- What is the effect of substrate concentration on enzyme activity?
- What is the effect of acid pH on this enzyme's activity?
- What is the effect on basic pH on this enzyme's activity?

Groups A1 and A2: What is the effect of enzyme concentration on enzyme activity?

- Set up 3 fresh cups of 1% H_2O_2 that is 4 cm deep.

PRE-LAB QUESTION: Why should these be *fresh* cups of H_2O_2 ? _____

- Begin with the double strength enzyme solution.** Make 2 serial dilutions of the enzyme so you have 3 strengths of enzyme: one of 200%, one of 100% strength, one of 50% strength.
- MIX WELL** then put a small amount of each into a cup to dip your disks in.
- Run 10 trials using each of the 3 catalase solutions (200%, 100%, and 50%).
- Record your data in the table provided then report your group's data to your instructor. **You will use CLASS data** to calculate your box and whisker values. Your **graph** will then help you answer the analysis questions.
- When cleaning up** make sure the filter disks do **NOT** go down the drain.

Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H_2O_2

Group A1	Seconds to Rise in Catalase Concentration			Group A2	Seconds to Rise in Catalase Concentration			
	Trial	200%	100%		50%	Trial	200%	100%
	1				1			
	2				2			
	3				3			
	4				4			
	5				5			
	6				6			
	7				7			
	8				8			
	9				9			
	10				10			

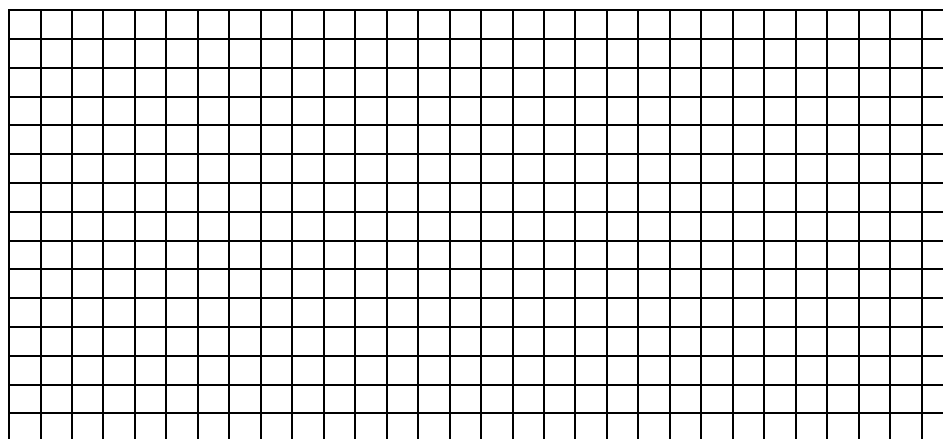
→ Record only **YOUR** data in the table above, in the unused portion of the table write "See Attached Class Data". You will use **CLASS** data to calculate values for the table on the next page **AND** for your **graph** ← (Don't forget to use **all 20 pieces of data** for each concentration and put them in order from smallest to largest in order to find the box and whisker values.)

**Table of Box and Whisker Values for the Number of Seconds Required
For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂**

Box and Whisker Values	Seconds to Rise in Catalase Concentration		
	200%	100%	50%
1 st Quartile			
Median			
3 rd Quartile			
Max, Min Values			
Inner Quartile Range (Q3 - Q1)			

- Now graph the three sets of data from this table on the grid on the following page.
Don't forget to label your units on your number line.

Graph Comparing the Number of Seconds Required For 3 Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂ in 3 Different Concentrations of Catalase



- Summarize the impact of enzyme concentration on the rate of reaction illustrated by the above graph: _____

- What is the independent variable? _____
- What is the dependent variable? _____
- Of the three conditions tested which is most logically the control group in this experiment?

Groups B1 and B2: What is the effect of substrate concentration on enzyme activity?

- Take a fresh amount of 1% H₂O₂ and dilute it to 0.5%, swirl so the solution mixes well then set up a cup that is 4 cm deep with this 0.5% solution.
PRE-LAB QUESTION: How much H₂O₂ should you use for every mL of water for the dilution?

- Set up a second cup with 4 cm of fresh 1% H₂O₂ and a third with 4 cm of the 2% H₂O₂.

- Run 10 trials using each of the 3 substrate solutions (0.5%, 1%, and 2% H_2O_2). **Again, make sure you mix the catalase solution** before you pour it into your cup for dipping the disks.
- Record your data in the table provided then report your group's data to your instructor. **You will use CLASS data** to calculate your box and whisker values. Your **graph** will then help you answer the analysis questions.
- **When cleaning up** make sure the filter disks do **NOT** go down the drain.

**Number of Seconds Required For a Catalase-coated
Filter Paper Disk to Rise to the Top of 4 cm of H_2O_2**

Group B1	Seconds to Rise in H_2O_2 Concentration			Group B2	Seconds to Rise in H_2O_2 Concentration		
	Trial	0.5%	1%		2%	Trial	0.5%
1				1			
2				2			
3				3			
4				4			
5				5			
6				6			
7				7			
8				8			
9				9			
10				10			

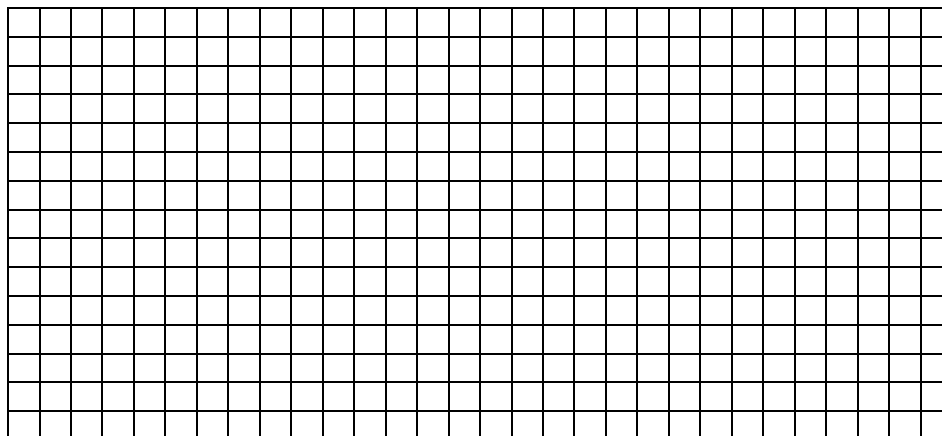
→ Record only **YOUR** data in the table above, in the unused portion of the table write "**See Attached Class Data**". You will use **CLASS** data to calculate values for the table below **AND** for your graph ← (Don't forget to use all 20 pieces of data for each concentration and put them in order from smallest to largest in order to find the box and whisker values.)

**Table of Box and Whisker Values for the Number of Seconds Required
For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H_2O_2**

Box and Whisker Values	Seconds to Rise in H_2O_2 Concentration		
	0.5%	1%	2%
1 st Quartile			
Median			
3 rd Quartile			
Max, Min Values			
Inner Quartile Range (Q3 - Q1)			

- Now graph the three sets of data from the above table on the following grid. **Don't forget to label your units on your number line.**

Graph Comparing the Number of Seconds Required For 3 Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂ in 3 Different Concentrations of H₂O₂



- Summarize the impact of substrate (H₂O₂) concentration on the rate of reaction illustrated by the graph: _____

- What is the independent variable? _____
- What is the dependent variable? _____
- Of the three conditions tested which is most logically the control group in this experiment?

Groups C1 and C2: What is the effect of acid pH on enzyme activity?

- Set up 3 fresh cups of 1% H₂O₂ that is 4 cm deep.
- Label 3 additional small cups pH 3, pH 5, and pH 7.
In the pH 3 cup put 5 ml double strength (200%) catalase (after shaking the bottle to mix it well) with 5 ml pH 3 buffer. Swirl the cup to MIX well.
In the pH 5 cup put 5 ml double strength catalase with 5 ml pH 5 buffer. Swirl the cup to MIX well.
In the pH 7 cup put 5 ml double strength catalase with 5 ml pH 7 buffer. Swirl the cup to MIX well.

PRE-LAB QUESTION: Why did you need to begin with double strength catalase when making these solutions? _____

- Run 10 trials using each of the 3 catalase solutions (pH 3, pH 5, and pH 7). **Again, make sure you mix the catalase solution** before dipping the disks.
- Record your data in the table provided then report your group's data to your instructor. **You will use CLASS data** to calculate your box and whisker values. Your **graph** will then help you answer the analysis questions.
- **When cleaning up** make sure the filter disks do **NOT go down the drain**.

Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂

Group C1	Seconds to Rise in pH			Group C2	Seconds to Rise in pH		
	3	5	7		3	5	7
Trial 1				Trial 1			
2				2			
3				3			
4				4			
5				5			
6				6			
7				7			
8				8			
9				9			
10				10			

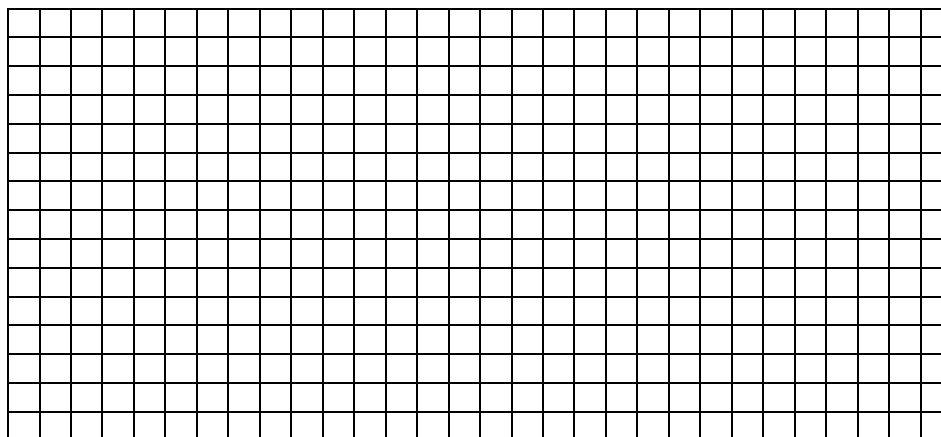
→ Record only YOUR data in the table above, in the unused portion of the table write "See Attached Class Data". You will use CLASS data to calculate values for the table below AND for your graph ← (Don't forget to use all 20 pieces of data for each concentration and put them in order from smallest to largest in order to find the box and whisker values.)

Table of Box and Whisker Values for the Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂

Box and Whisker Values	Seconds to Rise in pH		
	3	5	7
1 st Quartile			
Median			
3 rd Quartile			
Max, Min Values			
Inner Quartile Range (Q3 - Q1)			

- Now graph the three sets of data from this table on the grid below. Don't forget to label your units on your number line.

Graph Comparing the Number of Seconds Required For 3 Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂ in 3 Acidic pH Solutions



- Summarize the impact of acidic pH concentration on the rate of reaction illustrated by the above graph: _____
- What is the independent variable? _____
- What is the dependent variable? _____
- Of the three conditions tested which is most logically the control group in this experiment? _____

Groups D1 and D2: What is the effect of basic pH on enzyme activity?

- Set up 3 fresh cups of 1% H₂O₂ that is 4 cm deep.
- Label 3 additional small cups pH 7, pH 9, and pH 11.
In the pH 7 cup put 5 ml double strength (200%) catalase (after shaking the bottle to mix it well) and 5 ml pH 7 buffer. Swirl the cup to MIX well.
In the pH 9 cup put 5 ml double strength catalase and 5 ml pH 9 buffer. Swirl the cup to MIX well.
In the pH 11 cup put 5 ml double strength catalase and 5 ml pH 11 buffer. Swirl the cup to MIX well.
- Run 10 trials using each of the 3 catalase solutions (pH 7, pH 9, and pH 11). Again, make sure you mix the catalase solution before you pour it into your cup for dipping the disks
- Record your data in the table provided then report your group's data to your instructor. You will use CLASS data to calculate your box and whisker values. Your graph will then help you answer the analysis questions.
- When cleaning up make sure the filter disks do NOT go down the drain

Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂

Group D1	Seconds to Rise in pH			Group D2	Seconds to Rise in pH		
	7	9	11		Trial	7	9
Trial				Trial			
1				1			
2				2			
3				3			
4				4			
5				5			
6				6			
7				7			
8				8			
9				9			
10				10			

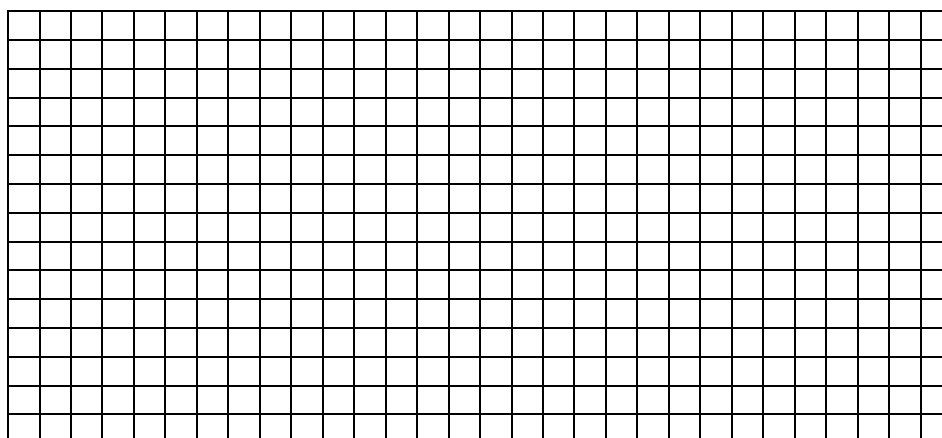
→ Record only YOUR data in the table above, in the unused portion of the table write "See Attached Class Data". You will use CLASS data to calculate values for the table on the following page AND for your graph ← (Don't forget to use all 20 pieces of data for each concentration and put them in order from smallest to largest in order to find the box and whisker values.)

Table of Box and Whisker Values for the Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂

Box and Whisker Values	Seconds to Rise in pH		
	7	9	11
1 st Quartile			
Median			
3 rd Quartile			
Max, Min Values			
Inner Quartile Range (Q3 - Q1)			

- Now graph the three sets of data from this table on the grid on the following page. **Don't forget to label your units on your number line.**

Graph Comparing the Number of Seconds Required For 3 Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H₂O₂ in Basic pH Solutions



- Summarize the **impact of basic pH concentration on the rate of reaction** illustrated by the above graph: _____

- What is the **independent** variable? _____
- What is the **dependent** variable? _____
- Of the three conditions tested which is most logically the **control** group in this experiment?

Teacher Instructions:

Catalase preparation:

Option 1 - Use dried yeast, simply dissolve a 7 g package of yeast in 1000 ml warm (not hot) water and wait, at room temperature, for an hour or so. Strain off the liquid using cheesecloth and discard the solids. Keep the liquid on ice (or refrigerated) until needed.

Option 2 - Use fresh potatoes. For each gram of potato add 1 ml of water and mix in a blender briefly. Strain off the liquid using cheesecloth and discard the solids. Keep the liquid on ice until needed.

Option 3 - Use fresh or frozen liver. For each gram of liver use 10 ml water and mix in blender briefly. Use the same technique as in option 1.

Whichever option is used for preparing the catalase, the same general technique should be applied to make a double strength solution.

Have students mark all data tables other than theirs with the following: "**See Attached Class Data**". I prefer to create the class data tables in Excel and provide these data tables to the students on my website.