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Enzyme Activity
Measuring the Effect of Enzyme Concentration

Objective
Students will measure the length of time it takes for various concentrations of catalase-soaked filter paper disks to float to the top of a cup filled with hydrogen peroxide. Students will perform dilutions to produce the various enzyme concentrations.

Additionally, students will measure the effect of the competitive inhibitor hydroxylamine hydrochloride on the catalase reaction.

Level
Biology I

Common Core Standards
TBD

Connections to AP*
AP Biology: I. Molecules and cells A. Chemistry of life 4. Enzymes

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Materials
For a class of 28 working in pairs

2 L hydrogen peroxide solution, 1.5%
150 mL hydroxylamine hydrochloride solution, 10%
beef liver
distilled water

168 filter paper disks
42 small beakers or medicine cups
14 small disposable cups
14 small dispensing cups
14 graduated cylinders, 50 mL
14 syringes, 10 cc
14 marking pens
14 stopwatches
14 forceps
28 aprons
28 pairs of gloves
28 goggles
blender
paper towels

TEACHER

Teacher Notes

Prepare the filter disks by using a standard hole punch to punch holes in the filter paper. Be careful to separate the disks if you punch through multiple layers of filter paper.

Paper towels should be placed at each lab table. Students should be encouraged to wipe any excess catalase from the disks and forceps in an effort to apply a consistent amount of catalase to the disk.

Hydrogen Peroxide Solution

Hydrogen peroxide 3% can be purchased locally at any drug store or supermarket. Add equal volumes of Hydrogen peroxide 3% and distilled water to produce a 1.5% solution. Store this solution in a brown bottle.

Hydroxylamine Hydrochloride Solution

Hydroxylamine hydrochloride can be purchased from a chemical supply company. Prepare a 10% solution by adding 15 g hydroxylamine hydrochloride to 135 mL distilled water.

Catalase Stock Solution

Place a 1 cm^3 slice of beef liver in 500 mL distilled water and liquefy in a blender. Before using this stock with students, test the catalase solution for activity by placing a few drops into 10 mL hydrogen peroxide. Bubbles should form immediately. If you do not see bubbles, add more liver solution.

Once the catalase solution is prepared, it should be refrigerated. Keep it on ice throughout the day if you need to leave it out in the lab room. The exact concentration and reactivity of the catalase is not significant for this activity. However, you may need to dilute the stock solution so that the disks will not immediately float to the surface.

Answer Key

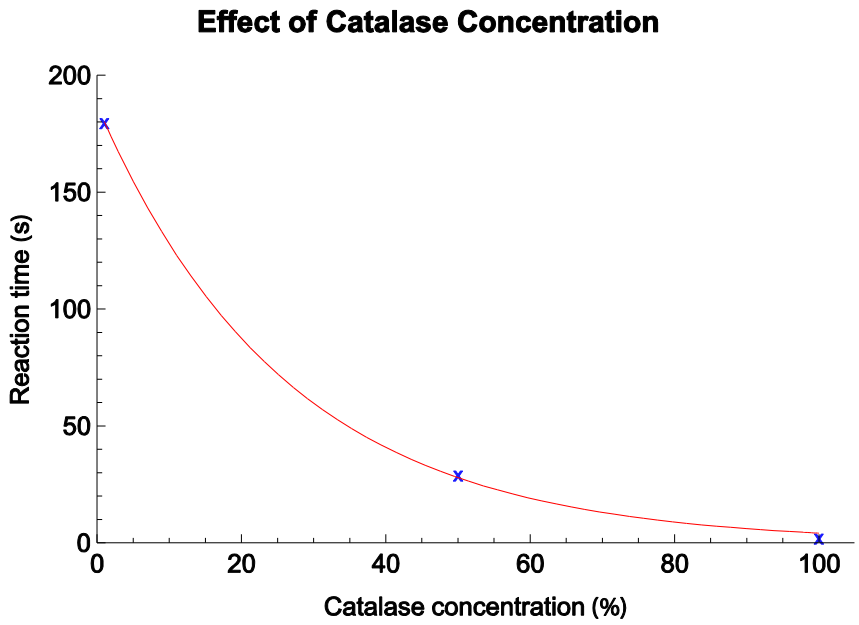
Data and Observations

Table 2: Catalase and Hydrogen Peroxide				
Percent Catalase	**Time (s)**			
	Trial 1	**Trial 2**	**Trial 3**	**Average**
100	1	2	4	2.34
50	27	22	39	29.3
0	180+	180+	180+	180+
100% plus hydroxylamine	50	35	42	42.3

Analysis

a. The *independent* variable: catalase concentration

b. The *dependent* variable: reaction time



TEACHER

Answer Key (continued)

Conclusion Questions

1. The oxygen produced in the reaction accumulates under the disk, causing the disks to float.

2. The 100% solution had the shortest reaction time.

3. The 50% solution had the longest reaction time.

4. Catalase is a protein.

5. It temporarily binds with the hydrogen peroxide, causing it to be broken apart. The hydrogen peroxide decomposes into water and oxygen gas, hence the bubbles in the liquid.

6. As the concentration of enzyme decreases, the reaction time increases.

7. The disk would not float to the top.

8. Hydroxylamine hydrochloride acts as an inhibitor, slowing the decomposition of hydrogen peroxide.

9. The reaction time for that trial will be shorter than expected because there are additional catalase molecules introduced into the solution from the forceps.

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Enzymes are proteins that serve as biological catalysts in a wide variety of life-sustaining chemical reactions that take place in cells. As catalysts, enzymes lower the amount of energy required to make a reaction occur. We call this energy the **activation energy**. By lowering the activation energy, enzymes serve to speed up the rate at which the reactions occur.

Enzymes are said to be substrate-specific. A **substrate** is a molecule that temporarily binds with the enzyme at an area on the enzyme called the **active site**. Each enzyme catalyzes one specific reaction because there is only one type of substrate molecule with the exact shape that will fit into the enzyme's active site.

For example, the enzyme amylase will only act on the starch called amylose. The enzyme sucrase will only act on the sugar called sucrose because it is the only substrate that can fit into the active site of the sucrase enzyme. The enzyme and substrate temporarily join to form the enzyme-substrate complex. The substrate is then converted to its products, and the enzyme is free to repeat the process with another substrate molecule (Figure 1).

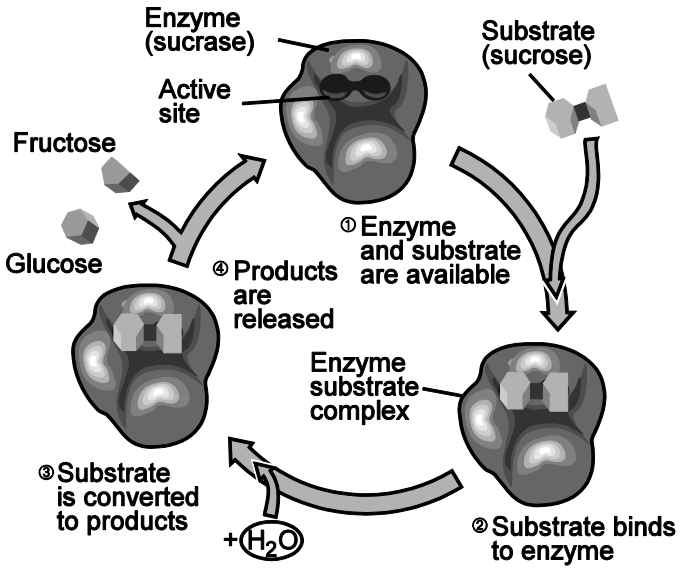


Figure 1. Enzymatic process

Your cells and the cells of most living organisms contain an enzyme called *catalase*. Cells use the enzyme catalase to break down hydrogen peroxide, H_2O_2, a poisonous byproduct of cell reactions. You have probably seen evidence of this reaction if you have ever poured hydrogen peroxide on a cut. The catalase decomposes hydrogen peroxide into water and oxygen. The oxygen gas is released as bubbles. The rate at which this occurs depends on the number of catalase molecules that are available.

The activity of enzymes is controlled in many ways. One of the simplest ways is through the action of inhibitors. **Inhibitors** compete with the substrate molecule for the active site of the enzyme. If the inhibitor gets to the active site before the substrate, it will block the substrate from binding and prevent the reaction from taking place. Hydroxylamine hydrochloride, $(NH_2OH)HCl$, is a known competitive inhibitor of the catalase/hydrogen peroxide reaction.

Purpose

In this activity, you will measure the time it takes for a disk of filter paper soaked with varying concentrations of the enzyme catalase to float to the top of a cup filled with hydrogen peroxide. The disk will float as oxygen produced in the catalase/hydrogen peroxide reaction accumulates under the paper disk.

Additionally, you will measure the effect of hydroxylamine hydrochloride on the catalase reaction.

Materials

hydrogen peroxide solution, 1.5%
hydroxylamine hydrochloride solution, 10%
catalase stock solution
distilled water

12 filter paper disks
3 small beakers or medicine cups
small disposable cup
graduated cylinders, 50 mL
syringe, 10 cc
marking pens
stopwatch
forceps
aprons
gloves
goggles
blender
paper towels

Safety Alert!

- Wear goggles at all times.
- Do not eat or drink in the laboratory.
- Avoid unnecessary contact with chemicals.

Procedure

Part I: The Effect of Catalase Concentration on the Decomposition of Hydrogen Peroxide

1. In the space provided on your student answer page, write an "if-then" statement that answers the following question: What effect does increasing the concentration of catalase have on the rate of decomposition of hydrogen peroxide?
2. Using small beakers or cups, prepare the catalase solutions as listed in Table 1. Use a marking pencil to label the enzyme solutions as 100%, 50%, and 0%.

Table 1: Catalase Solutions

Final Quantity Needed	Concentration of Final Solution	Quantity of Catalase (mL)	Quantity of Water (mL)
10 mL	100%	10	0
10 mL	50%	5	5
10 mL	0%	0	10

3. Pour 40 mL of 1.5% hydrogen peroxide into a clean cup or beaker.
4. Using forceps, pick up one filter paper disk and submerge it in the 100% enzyme solution for 5 seconds. Do not let go of the disk.
5. Remove the disk from the solution and use a paper towel to blot it dry for 5 seconds. Be sure to also dry the tips of the forceps.
6. Use forceps to place the disk on the bottom of the cup with the hydrogen peroxide (Figure 2). Begin timing as soon as the disk touches the surface of the hydrogen peroxide.

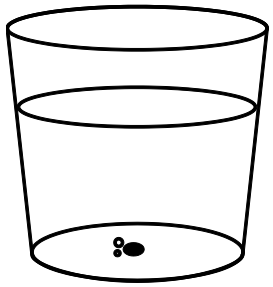


Figure 2. Disk at bottom of cup

Procedure (continued)

7. Record the time required for the disk to float to the surface of the hydrogen peroxide (Figure 3) in Table 2.

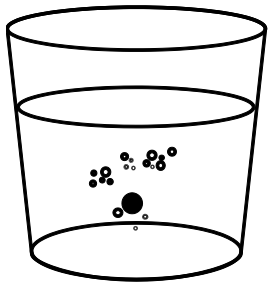


Figure 3. Disk floating to surface

8. Conduct two additional trials with the 100% enzyme solution. Use a different filter paper disk for each trial. Use a fresh 40 mL of hydrogen peroxide for each trial.
9. Repeat Steps 3 to 8 for the 50% and 0% catalase solutions. Remember to use clean filter paper each time you test. Record the times for the trials of the remaining solutions in the appropriate columns of Table 2. Note: If any disk takes longer than 180 seconds (3 minutes) to float to the surface, simply record this time as "180+" in the data table.
10. Prepare a line graph of the average reaction time it takes for the disk to float to the top versus the percent concentration of enzyme.

Part II: The Effect of Hydroxylamine Hydrochloride on Catalase

1. Pour 10 mL of the hydroxylamine hydrochloride into a clean cup or beaker. Pour 40 mL of 1.5% hydrogen peroxide into another clean cup or beaker.
2. Using forceps, pick up one filter paper disk and submerge it in the 100% enzyme solution for 5 seconds. Do not let go of the disk.
3. Remove the disk from the solution and use a paper towel to blot it dry for 5 seconds. Be sure to also dry the tips of the forceps.
4. Dip the disk with catalase into the hydroxylamine hydrochloride solution for 5 seconds. Remove the disk from the solution and blot it and the forceps dry using a paper towel.
5. Use forceps to place the disk on the bottom of the cup with the hydrogen peroxide. Begin timing as soon as the disk touches the surface of the hydrogen peroxide.
6. Record the time required for the disk to float to the surface of the hydrogen peroxide in Table 2.
7. Repeat Steps 2 to 6 for a total of three trials with hydroxylamine hydrochloride. Remember to use clean filter paper and a fresh 40 mL of hydrogen peroxide for each trial. Record the times for the three trials of the remaining solutions in the appropriate columns of Table 2.

Hypothesis

Data and Observations

Table 2: Catalase and Hydrogen Peroxide				
Percent Catalase	**Time (s)**			
	Trial 1	**Trial 2**	**Trial 3**	**Average**
100				
50				
0				
100% plus hydroxylamine				

Analysis

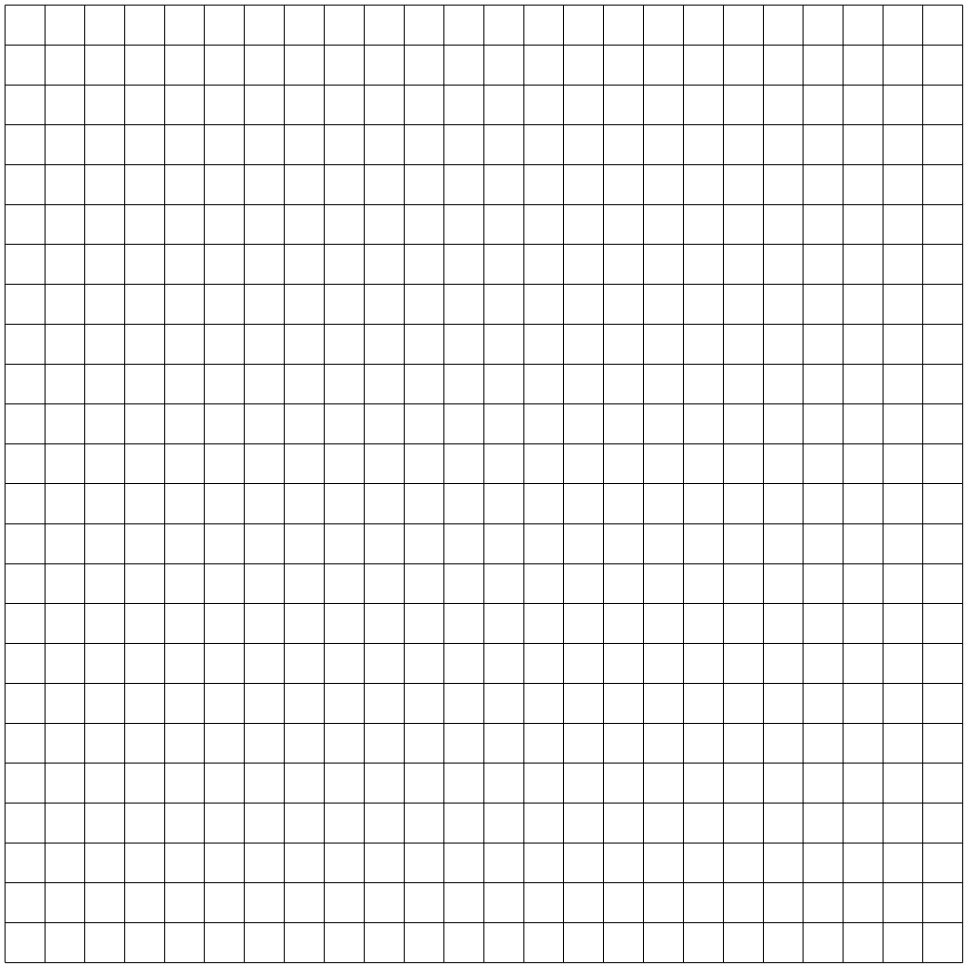
Graph

For this graph, you will need to determine the following:

a. The *independent* variable: _____
Use this value to label the horizontal *x*-axis.

b. The *dependent* variable: _____
Use this value to label the vertical *y*-axis.

Graph 1: _____
(*title*)



Conclusion Questions

1. What causes the disks to float to the surface?

2. Which concentration of catalase had the shortest reaction time?

3. Which concentration of catalase had the longest reaction time?

4. What type of biological molecule is catalase?

5. What does catalase do to hydrogen peroxide?

6. Based on the graph and overall slope of the line, what can you conclude about the effect of enzyme concentration on reaction time?

Conclusion Questions (continued)

7. How would the results be different if you repeated Part I of this experiment using water instead of hydrogen peroxide?

8. Describe the effect hydroxylamine hydrochloride has on the reaction time.

9. A student forgets to dry the tip of the forceps after dipping the disk in catalase solution. What effect will this error have on the reaction time for that trial? Explain.

