

Lab 2: Enzyme Activity: Measuring the Effect of Enzyme Concentration

I. Framing the Investigation:

Introduction:

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 in 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 in 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 freed to repeat the process with a substrate molecule. See Fig. 1.

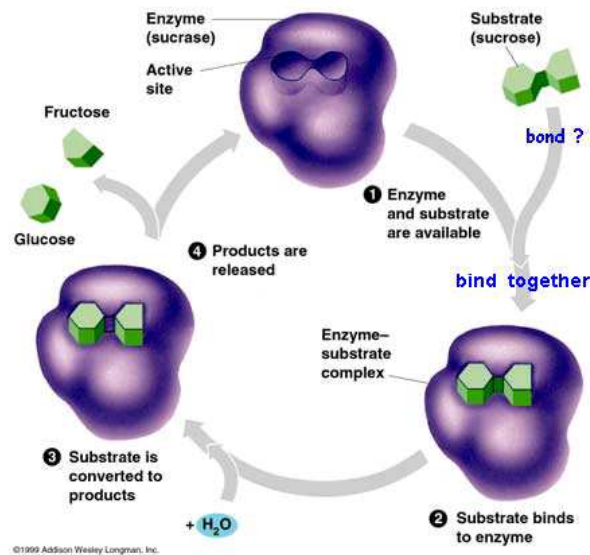


Fig. 1

Your cells, and the cells of most living organisms, contain an enzyme called catalase. Cells use an enzyme (**catalase**) to breakdown the poisonous substance (**hydrogen peroxide**) which is produced during 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 may 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 is a known competitive inhibitor of the catalase/hydrogen peroxide reaction.

Purpose:

In this lab you will measure the time it takes for a disc 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.

Hypothesis:

Using variable assigned, develop a hypothesis, using the **If**, and, **then** concerning the effects enzyme concentration has on the reaction rate.

II. Designing the Investigation:

Materials:

- 5 filter paper disks
- forceps
- scissors
- small beakers or medicine cups for dilutions
- 15 mL distilled water
- paper towel
- 10% hydroxylamine hydrochloride solution
- catalase stock solution
- small disposable cups
- timing device (seconds)
- 1.5% hydrogen peroxide (H₂O₂)
- gloves, aprons, and eye protection
- marking pen

Safety Alert

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

Procedure: Part I: The effect of catalase concentration on the decomposition of hydrogen peroxide

1. On your student answer page in the space marked Hypothesis, 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 following catalase solutions.

Final Quantity Needed	Concentration of Final Solution	ml of Catalase	ml of Water
10ml	100%	10	0
10ml	75%	7.5	2.5
10ml	50%	5	5
10ml	25%	2.5	7.5
10ml	0%	0	10

3. Use a marking pencil to label the enzyme solutions as 100%, 50% and 0%.

4. In a clean cup or beaker, pour 40 mL of 1.5% hydrogen peroxide.

5. Using your 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.

6. Remove the disk from the solution and use a paper towel to blot it dry for five seconds. Be sure to dry the tips of the forceps.

7. Use the forceps to place the disk on the bottom of the cup. See Figure 2. **Begin timing as soon as the disk touches the surface of the hydrogen peroxide.**



Fig. 2

8. Record the time required for the disk to float to the surface of the hydrogen peroxide cup in the data table.

9. Conduct two additional trials with the 100% enzyme solution. Use a different filter paper disk for each trial.

10. Repeat steps 1-9 for the 50% and 0% catalase solutions. Remember to use clean filter paper each time you test. Record the times for the three trials of the remaining solutions in the appropriate column of the data table.

11. Prepare a line graph of the average reaction time versus the enzyme concentration using the space.

Procedure: Part II: The effects of hydroxylaminehydrochloride on catalase

1. Using your 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.

2. Remove the disk from the solution and use a paper towel to blot it dry for five seconds. Be sure to dry the tips of the forceps.

III. Collecting and Presenting Data:

% Catalase	Trial 1	Trial 2	Trial 3	Average
100				
75				
50				
25				
0				
100% plus Hydroxylamine				

Data Table 1:

Graph Below

For this graph you will need to determine the following:

- a. The independent variable _____
Use this to label the horizontal x -axis.
- b. The *dependent* variable _____
Use this to label the vertical y -axis.

Graph 1 Title:

IV. Analyzing and Interpreting Results:

1. What causes the disks to float to the surface?
2. Which concentration of catalase had the fastest reaction time?
3. Which concentration of catalase had the slowest reaction time?
4. What type of biological molecule is catalase?
5. What does catalase do to hydrogen peroxide concentration on reaction time?
6. Based on the graph and overall slope of the line, what can you conclude about the effect of enzyme?
7. How would the results be different if you repeated Part I this experiment using water instead of hydrogen peroxide?

V. Reference:

Layering the Foundation: Biology, www.apstrategies.org

VI. TEKS/TAKS:

TEKS: 1A, 1B, 2A, 2B, 2C, 2D, 3A, 3D, 9A, 9C, 11A, 11C

TAKS: 1, 2