Biology
ENZYME ACTIVITY

This activity is an alternative to the titration proposed for Enzyme Catalysis (AP Bio Lab #2, *Biology Lab Manual*). There are numerous alternative lab activities that measure the rate of enzyme activity (i.e. Gen Nelson, *Catalase Lab*, http://www.accessexcellence.org). This one is Erol Altug's, which has been modified from the one developed by Tom Carroll of St. Alban's School. The introduction and some questions are from *Biology Lab Manual.*

Introduction

Enzymes are **biological catalysts** that carry out thousands of chemical reactions, which occur in living cells. They are generally large proteins made up of several hundred amino acids, and often contain a non-proteinaceous group that is important in the actual catalysts.

In an enzyme-catalyzed reaction, the substance to be acted upon, the **substrate**, binds in the **active site** of the enzyme. The enzyme and substrate are held together in an enzyme-substrate complex by hydrophobic bonds, hydrogen bonds, and ionic bonds.

The enzyme then converts the substrate to the **reaction products** in a process that often requires several chemical steps, and may involve covalent bonds. Finally, the products are released into solution and the enzyme is ready to form another enzyme-substrate complex. As is true of any catalyst, the enzyme is not used up as it carries out the reaction, but it recycled over and over. One enzyme molecule can carry out thousands of reaction cycles every minute.

Each enzyme is specific for a certain reaction because its amino acid sequence is unique and causes it to have a unique three-dimensional structure. The "business" end of the enzyme molecule, the active site, also is specific so that only one or a few of the thousands of compounds present in a cell can interact with it. If there is a **prosthetic group** on the enzyme, it will form part of the active site. *Any substance that blocks or changes the shape of the active site will interfere with the activity and efficiency of the enzyme.*

If these changes are large enough, the enzyme can no longer act at all, and has become **denatured**.

There are several factors that are especially important in determining the enzyme's shape, and these are closely regulated both in the living organism and in laboratory experiments to give the optimum or most efficient enzyme activity:

1. **pH** – pH is a negative logarithmic scale that measures the acidity of H^+ concentration in a solution. The scale runs from 0 to 14 with 0 being the highest in acidity and 14 the lowest. Neutral solutions have of pH of 7. Acid solutions have a pH less than 7; basic solutions have a pH greater than 7. 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 enzymes have an optimum in the neutral pH range and are denatured at either extremely high or low pH. Some have an appropriately low pH optimum. A buffer is a compound that will gain or lose H^+ ions so that the pH changes very little.

2. **SALT CONCENTRATION** – If the salt concentration is very low or zero, the charged amino acid side chains of the enzyme will stick together. 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 occur, and again the enzyme will precipitate. An intermediate salt concentration such as that of blood (0.9%) or cytoplasm is optimum for most enzymes.

3. **TEMPERATURE** – All chemical reactions speed up as temperature increases. 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, an optimum is reached. Above this optimum point the kinetic energy of the enzyme and water molecules is so great that the structure of the enzyme molecules starts to be disrupted. The positive effect of speeding up the reaction is now more than offset by the negative effect of denaturing more and more enzyme molecules. Many proteins are denatured by temperatures around $40^0 – 50^0$ C, but some are still remain active at $70^0 – 80^0$ C, and a few even withstand being boiled.

4. **SMALL MOLECULES** – Many molecules other than the substrate may interact with an enzyme. If such a molecule increases the rate of the reaction it is called an **activator**. If the molecule decreases the reaction rate it is called an **inhibitor**. The cell can use these molecules to 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 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 or block it. Others may damage or remove the prosthetic group. Many well-known poisons, such as potassium cyanide and curare, are enzyme inhibitors that interfere with the active site of a critical enzyme.

While not affecting the enzyme's shape, the reaction rate may be further affected by either the **enzyme concentration** and/or the **substrate concentration**. The more molecules of either that are present will increase the mathematical probability of random collisions and **enzyme- substrate couplings**.

The Reaction and the Assay

In this exercise you will study the enzyme **catalase**, which accelerates the breakdown of **hydrogen peroxide** (a common – but poisonous - byproduct of oxidative metabolism) into water and oxygen, according to the summary reaction:

$$2H_2O_2 + \text{catalase} \rightarrow 2H_2O + O_2 + \text{catalase}$$

This catalase-mediated reaction is extremely important in the cell because it prevents the accumulation of hydrogen peroxide (H_2O_2), a strong oxidizing agent that tends to disrupt the delicate balance of cell chemistry. This compound is broken down in the **peroxisome**.

Catalase is found in both animal and plant tissues. It is more easily found in animal tissue (especially bovine liver), but animal extract is too strong for this experiment and if used requires significant dilution prior beforehand. Catalase is significantly abundant in plant storage organs such as potato tubers, corns, and in the fleshy parts of fruits and those work much better for this activity.

You will isolate catalase from potato tubers and measure its rate of activity under different conditions. You will be conducting a **biological assay**, whereby a disc of filter paper will be immersed in the enzyme solution, and then placed in the hydrogen peroxide substrate. The oxygen produced from the subsequent reaction will become trapped in the disc, thus giving it buoyancy. The time measured from the moment the disc touches the substrate until it reaches the surface of the solution is a measure of the rate of the enzyme activity. (The disc should be flat on top of the solution at the end.)

Materials

- 100 mL graduated cylinders
- 2.1 cm filter paper discs (or uniform-cut discs made by identical paper punches)
- 50 mL beakers (or small disposable paper cups)
- Rulers (if paper cups are used)
- Blender
- Cheesecloth
- Distilled water
- Forceps
- Hot plate
- Hydrogen peroxide
- Ice
- Potatoes
- Thermometer

PROCEDURE

Extraction/Preparation of Potato Catalase

(Your instructor may do this in advance)

1. Peel a fresh potato tuber and cut the tissue into small cubes.
2. Weigh out 50 grams of tissue.
3. Place the tissue, 50 mL of cold distilled water, and a small amount of crushed ice in a pre-chilled blender.
4. Homogenize for 30 seconds at high speed.

FROM THIS POINT ON, THE ENZYME PREPARATION MUST BE CARRIED OUT IN AN ICE BATH.

5. Filter the potato extract, using cheesecloth.
6. Pour the filtrate into a 100 mL graduated cylinder and add cold distilled water to bring up the final volume to 100 mL.

NOTE

This extract will arbitrarily be labeled 100 units of enzymes per mL, or 100 units/mL, and will be used in the tests.

Independent Variables & Measurements & Recordings

You will be manipulating the independent variables. So as to be able to plot more than two data points (i.e. a line) any experiment needs a minimum of three measurements, which usually works out as: standard conditions, conditions above normal, conditions below normal. In these tests, you will work with five variations of any one condition.

You and your lab partner(s) will be responsible for conducting an experiment measuring rate changes for one of the conditions, which your instructor will assign. However, you need to observe how the other conditions are being manipulated and what can be learned about enzyme function.

All recordings are to be kept in the Data Table at the end of these instructions. Those results will be submitted along with your graphs and answers to questions.

EVERY STUDENT IS RESPONSIBLE FOR RECORDING AND GRAPHING THE CLASS AVERAGE RESULTS OF ALL THE EXPERIMENTS.

Part I
EFFECT OF CATALASE CONCENTRATION

This part of the lab exercise considers the impact of enzyme concentration to carry out biochemical reactions (rate of reactivity) while using a substrate concentration that is in access.

2. Label five 50 mL beakers as follows:
   - 100% 100 units/mL
   - 75% 75 units/mL
   - 50% 50 units/mL
   - 25% 25 units/mL
   - 0% 0 units/mL

3. Prepare **40 mL of enzyme for each of the above concentrations** in the following manner: **{Your instructor MAY have already prepared the catalase (enzyme) concentrations already.}**
   - Use the table below, combining **catalase (enzyme)** with water, in order to get the required enzyme concentrations.

Enzyme Concentration %	mL of **original** enzyme solution		mL of cold distilled water		units per ml
100%	40	ADD	0	=	100
75%	30	ADD	10	=	75
50%	20	ADD	20	=	50
25%	10	ADD	30	=	25
0%	0	ADD	40	=	0

4. **Keep your catalase preparations in the ice bath.**
5. Label an additional set of IDENTICAL beakers **for** the **substrate (hydrogen peroxide)**. Into each of the labeled beakers, place 40 ml of hydrogen peroxide in each.
   - Label an additional set of five 50 mL beakers as follows.
   - 100% 100 units/mL
   - 75% 75 units/mL
   - 50% 50 units/mL
   - 25% 25 units/mL
   - 0% 0 units/mL
6. Using forceps, immerse a 2.1 cm filter paper disc into the prepared catalase solution for 5 seconds.
7. Remove the disc and drain for 10 seconds on a paper towel.
8. Place the disc at the bottom of the first substrate solution—corresponding to the concentration of catalase in which the disk was placed in. The **oxygen** produced from the breakdown of the hydrogen peroxide by catalase becomes trapped in the fibers of the disc, thereby causing the disc to float to the surface of the solution.

9. Measure (using a stopwatch) the reaction time for the amount of time from when the disc was placed at the bottom of the beaker until the disc floats on top of the solution (rate will be measured in seconds).
    - **The rate (R) of the reaction is calculated as R = 1/t.**
10. Repeat this procedure twice for each enzyme concentration and average the results.
11. Record your results in Table 1 on the Data Tables.
12. Later, plot your data on a graph, which should be properly labeled.

Part 2
EFFECT OF SUBSTRATE CONCENTRATION

During this section of the activity, you will determine the effect of **substrate (hydrogen peroxide) concentration** on the rate of catalase activity.

1. Obtain **five 50 mL beakers** and label them as indicated **by the chart below**. Prepare the **serial solutions** using the instructions below and add them to the appropriate beaker.

    - Be certain to use room-temperature water.

2. Using ***only*** the 100 units/mL enzyme concentration solution, **100% catalase solution**, **repeat the assay procedure as outlined on page 5-6 (I, steps 3 – 8)** with the filter papers. **Keep the enzyme solution in an ice bath.**
3. Repeat the procedure for each of the substrate concentrations. Be sure to use the same enzyme every time.
4. Record your results in Table 2 in the Data Tables. Later, plot your data on a graph.

How to Prepare Serial Dilutions for Substrate (Hydrogen Peroxide)

As you did with the Enzyme Concentration, you will have five variations of the stock substrate concentrations (100%, 75%, 50%, 25%, and 0%). However, it is too dangerous to work with 100% hydrogen peroxide, so our maximum strength will be 3%, which is the standard concentration of H_2O_2 that can be purchased in grocery stores and pharmacies. You will end up with 3.0%, 2.25%, 1.5%, 0.75%, and 0.0%.

Take 3.0% H_2O_2 and mix with an equal volume of distilled water to create 1.5%. Mix equal volumes of 3.0% and 1.5% to create 2.25% and mix equal volumes of 1.5% and 0.0% to create your 0.75% solution of H_2O_2. You need equal amounts to do the assay.
Use this table as a guide to determine how much.

Beaker	Hydrogen Peroxide Dilution	mL of 3% H_2O_2	mL of water
0.0%	0.0% H_2O_2 (0% Concentration)	0.0	40.0
0.75%	0.75% H_2O_2 (25% Concentration)	10.0	30.0
1.5%	1.5% H_2O_2 (50% Concentration)	20.0	20.0
2.25%	2.25% H_2O_2 (75% Concentration)	30.0	10.0
3.0%	3.0% H_2O_2 (100% Concentration)	40.0	0.0

IMPORTANT: It is very important that your substrate solutions are accurately prepared.

Part 3
EFFECT OF TEMPERATURE

1. Use 40 mL of a 3.0% hydrogen peroxide solution as the substrate.
2. Use 100% (100 units/mL) enzyme concentration.
3. **Run the reactions in five water baths at five different temperatures**. **(Since the experiment will be ran FIVE times in FIVE different water baths, students will need FIVE BEAKERS of 3.0% hydrogen peroxide (100% concentration), each one containing 40 mL of the solution, AND FIVE BEAKERS of catalase at 100% concentration with EACH BEAKER containing 100 mL.)** Follow the same procedure of using standard conditions (i.e. room temperature) and then two temperatures above and two below. The following temperatures are suggested:
   - 4^0C
   - 10^0C
   - 22^0C (room temperature)
   - 40^0C
   - 65^0C +

   NOTE: At the time of each assay record the exact temperature of the water bath.

4. **Use the same assay procedure** as outlined on **page 5-6 (I, steps 3 – 8) for the five beakers with each one ran at one of the temperatures above.**

5. Record your results in Table 4. Plot your data on a graph.

DISCUSSION and/or QUESTIONS for REPORT

1. Basic questions of the experiment:
   a. What is the enzyme of this reaction? _____
   b. What is the substrate of this reaction? _____
   c. What is the product of this reaction? _____
   d. What is the gas produced and how could you demonstrate that?
      _____
2. How does enzyme activity vary with enzyme concentration?
3. How is the rate of enzyme activity affected by increasing the concentration of the substrate?
4. What do you think would happen if you increased the substrate concentration to 40.0% hydrogen peroxide?
5. How does changing the substrate concentration compare to changing the enzyme concentration in this experiment?
6. Explain the results when the hydroxylamine is added to the enzyme.
7. From the temperature data, what can you conclude about how temperature affects enzyme activity? How would you explain the results?
8. What is meant by **optimum temperature**? According to the class data, what is the optimum data for the potato catalase and why?
9. What would you predict if you had first mixed the catalase (100%) in serial solutions of varying pHs of 3, 5, 7, 9, 11? What would you predict would be **optimum pH**?

10. Design a controlled experiment to test the effect of varying pH, temperature, or enzyme concentration.

DATA TABLES

Name _____ Date _____

Lab partner(s) _____

Table 1 – Effect of Enzyme Concentration on Rate of Activity

Enzyme Concentration %	Enzyme Concentration (units/mL)	Time to Float Disc (in seconds) Trial 1	Trial 2	Average	Rate	**Class Average**
100	100					
75	75					
50	50					
25	25					
0	0					

Table 2 – Effect of Substrate Concentration on Enzyme Activity

Substrate Concentration (from serial dilutions)	Time to Float Disc (in seconds) Trial 1	Trial 2	Average	Rate	**Class Average**
0.0 % H_2O_2					
0.75% H_2O_2					
1.5% H_2O_2					
2.25% H_2O_2					
3.0% H_2O_2					

Table 3 – Effect of Temperature on Rate of Enzyme Activity

	Time to Float Disc (in seconds)				
Temperature (degrees C)	Trial 1	Trial 2	Average	Rate	**Class Average**
4					
10					
22					
40					
65+					

While you only worked on one of the variables, you are responsible for understanding all the factors manipulated (enzyme concentration, substrate concentration, temperature, inhibitor, and – if done – pH) and how that affects enzyme function.

Because you will need to graph the class average of multiple experiments, your instructor may allow you to use an electronic graphing program for this laboratory exercise.

Submit these tables and your graphs along with any questions the instructor asks you to answer.

COLLEGE BOARD – ENZYME CATALYSIS LABORATORY ACTIVITY

Overview

In this laboratory activity, students will observe the conversion of hydrogen peroxide to water and oxygen gas by the enzyme catalase. They will then measure the amount of oxygen generated and calculate the rate of enzyme-catalyzed reaction.

Objectives

Before doing this laboratory activity students 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
- how the concept of free energy relates to enzyme activity
- that changes in temperature, pH, enzyme concentration, and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions

After performing this laboratory activity students should be able to:

- measure the effects of changes of temperature, pH, enzyme concentration, substrate concentration on reaction rates of an enzyme-catalyzed reaction in a controlled experiment
- explain how environmental factors affect the rate of enzyme-catalyzed reactions