AP Biology Lab
Factors That Influence Enzyme Activity

Essential Knowledge
- **2.C.1**: Organisms use feedback mechanisms to maintain their internal environments and respond to external environmental changes.
- **4.B.1**: Interactions between molecules affect their structure and function.
- **4.B.2**: Cooperative interactions within organisms promote efficiency in the use of energy and matter.

Learning Objectives
- **L.O. 2.15** The student can justify a claim made about the effect(s) on a biological system at the molecular, physiological or organismal level when given a scenario in which one or more components within a negative regulatory system is altered.
- **L.O. 2.16** The student is able to connect how organisms use negative feedback to maintain their internal environments.
- **L.O. 2.17** The student is able to evaluate data that show the effect(s) of changes in concentrations of key molecules on negative feedback mechanisms.
- **L.O. 2.18** The student can make predictions about how organisms use negative feedback mechanisms to maintain their internal environments.
- **L.O. 2.19** The student is able to make predictions about how positive feedback mechanisms amplify activities and processes in organisms based on scientific theories and models.
- **L.O. 2.20** The student is able to justify that positive feedback mechanisms amplify responses in organisms.
- **L.O. 4.17** The student is able to analyze data to identify how molecular interactions affect structure and function.
- **L.O. 4.18** The student is able to use representations and models to analyze how cooperative interactions within organisms promote efficiency in the use of energy and matter.

Science Practices
- **SP 1:** The student can use representations and models to communicate scientific phenomena and solve scientific problems.
- **SP 2:** The student can use mathematics appropriately.
- **SP 3:** The student can engage in scientific questioning to extend thinking or to guide investigations within the context of the AP course.
- **SP 4:** The student can plan and implement data collection strategies appropriate to a particular scientific question.
- **SP 5:** The student can perform data analysis and evaluation of evidence.
- **SP 6:** The student can work with scientific explanations and theories.

Background

Enzymes are biological catalysts capable of speeding up chemical reactions. One benefit of enzyme catalysts is that the cell can carry out complex chemical activities at a relatively low temperature.

Most enzymes are proteins and their 3-dimensional shape is important to their catalytic activity. Two specific regions on the enzyme structure play an important role in catalytic activity: the active site and the allosteric site. The active site is the area of the enzyme which binds to the substance(s) (substrate) and aids in the chemical reaction. The allosteric site is involved in forming the proper 3-dimensional shape when linked with specific cofactors. As a result of the unique characteristics of these sites, enzymes are highly specific in terms of the reactions they will catalyze and the condition under which they work best.

In biochemical reactions, the enzyme, E, combines reversibly with its specific substrate, S, to form an enzyme-substrate complex, ES. 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 of the reaction, P, are formed.

This can be summarized in the equation:

$$E + S \rightarrow ES \rightarrow E + P$$

Note that the enzyme is not consumed in the reaction and can recycle to work with additional substrate molecules. Each enzyme is specific for a particular reaction because its amino acid sequence is unique, which causes it to have a unique 3-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.

In practice, this specificity permits one to mix a purified substrate with crude preparations of enzyme that might contain many other substances and obtain a quantitative assay (analysis) of the amount of enzyme present.

We will be working in this lab with a representative enzyme – catalase. Catalase has a molecular weight of approximately 240,000 Daltons and contains 4 polypeptide chains, each composed of more than 500 amino acid monomers. This enzyme occurs universally in aerobic organisms. One function of catalase within cells is to prevent the accumulation of toxic levels of hydrogen peroxide (H_2O_2) formed as a by-product of metabolic processes. Catalase might also take part in some of the many oxidation reactions going on in all cells. Catalase is found in most cells; however, liver is a particularly good source. In this lab, you will extract catalase from fresh beef liver and test its catalytic on hydrogen peroxide.

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

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

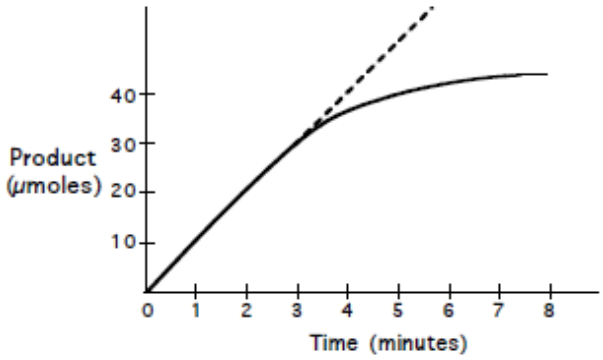
In the absence of catalase, this reaction occurs spontaneously, but very slowly. Catalase speeds up the reaction considerably. In this experiment, a rate for this reaction will be determined.

Much can be learned about enzymes by studying the kinetics (change in rate) of enzyme-catalyzed reactions. For example, it is possible to measure the amount of product formed, or the amount of substrate used, from the moment the reactants are brought together until the reaction has stopped.

So let's look at a hypothetical example:

An enzyme and its substrate are mixed in a reaction vessel. If the amount of product formed is measured at 30 second intervals and this quantity plotted on a graph, a curve like the one in Figure 1 is obtained:

Figure 1. Enzyme Activity



Observe the solid line for this reaction. At time 0 there is no product. After 30 seconds, 5 µmoles have been formed; after 1 minute, 10; after 2 minutes, 20. The rate of reaction could be given as 10 µmoles of product formed per minute for this initial period. Note, however, that by the 3rd and 4th minutes only about 5 additional µmoles of product have been formed. During the first 3 minutes, the rate is constant. From the 3rd minute through the 8th minute, the rate is changing – it is slowing down. For each successive minute after the first 3 minutes, the amount of product formed in the interval is less than in the preceding minute. From the 7th minute onward, the reaction rate is very slow.

In the comparison of kinetics of one reaction with another, a common reference point is needed. For example, suppose you wanted to compare the effectiveness of catalase obtained from potato with that of catalase obtained from liver. Would you want to compare the two reactions during the first few minutes when the rate is constant or later when the rates are changing?

Answer: It is best to compare the reactions when the rates are constant.

In the first few minutes of an enzymatic reaction such as this, the number of substrate molecules is usually so large compared to the number of enzyme molecules that the enzyme is constantly having successful collisions with

substrate. Therefore, during this early period, the enzyme is acting on substrate molecules at a constant rate (as fast as it can). The slope of a graphed line during this early period is called the **initial velocity** of the reaction. The initial velocity (or rate) of any enzyme-catalyzed reaction is determined by the characteristics of the enzyme molecules. It is always the same for a specific enzyme and its substrate as long as temperature and pH are constant and the substrate is present in excess.

The initial rate of the reaction, therefore, is the slope of the linear portion of the curve. To determine a rate, pick any two points on the straight-ling portion of the curve. Divide the difference in the amount of product formed between these two points by the difference in time between them. The result will be the rate of the reaction, which, if properly calculated, can be expressed as µmoles of product/second. This equation is:

$$\frac{\mu moles_2 - \mu moles_1}{t_2 - t_1}$$

In the graph shown as Figure 1:

$$\frac{30 - 20}{180 - 120} = \frac{10}{60} = 0.17 \text{ µmoles/second}$$

The rate of a chemical reaction may be studied in a number of ways, including the following:

1. Measuring the rate of disappearance of substrate, in this example, H_2O_2.
2. Measuring the rate of appearance of product, in this example, O_2, which is given off as a gas.
3. Measuring the heat released (or absorbed) during the reaction.

In this experiment, the disappearance of substrate, H_2O_2, and the generation of product, O_2, is measured. 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 from the bottom of the cup is an indication of the rate of enzyme activity.

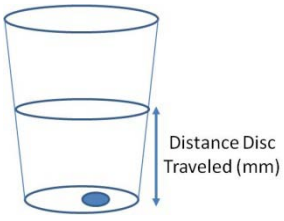
RATE ENZYME ACTIVITY = DISTANCE DEPTH OF HYDROGEN PEROXIDE IN mm/TIME IN sec

We will assume that each filter disc is coated with the same amount of catalase (except in the investigation of the effect of enzyme concentration of enzyme activity).

Materials

- 50 mL beaker or cup containing fresh catalase solution
- Reaction chamber
- Filter paper
- Hole punch
- Forceps
- Pipet
- Ring stand & clamp (or hands)
- 10mL graduated cylinder
- 100 mL graduated cylinder
- 3% hydrogen peroxide (H_2O_2)
- Pan (water bath)
- Hot plate
- Ice
- Thermometer
- Balance
- Water
- You inquiry materials

Part A: General Lab Procedure



1. Prepare a beaker or clear cup with 40 mL of 3% H_2O_2. Measure and record the depth of the hydrogen peroxide.
2. Using a single hole punch cut individual single layer discs 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 disc with forceps and dip the disc in your catalase enzyme solution in the cup.

5. Using the forceps push the disc to the bottom of the cup containing the H_2O_2. Watch the disc carefully. Start timing as soon as the disc hits the bottom of the cup; stop timing when the disc reaches the surface.
6. Run 4 timed trials with the H_2O_2 at room temperature to perfect your technique. Record your results.
7. When you are cleaning up make sure the filter discs do NOT go down the drain.

Data:

Table 1. Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 0 mL of H_2O_2. (Practice)		
Trial	**Distance Disc Traveled (mm)**	**Time for Disc to Rise (sec)**
1		
2		
3		
4		

Part B: The Effect of Enzyme Concentration on Enzyme Activity

1. Set up five beakers or cups containing 40 mL of 3% hydrogen peroxide.
2. Measure and record the depth of the hydrogen peroxide in the beakers.
3. 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 diH2O

   50 units/mL = 10 ml 100 units/ml + 10 ml cold diH2O

   20 units/mL = 3 ml 100 units/ml + 12 ml cold diH2O

   0 units/mL = 20 ml cold diH2O
4. Using forceps, dip a disc into the enzyme solution at 100 units/ml, then remove it and drain it on a paper towel.
5. Use the forceps to push the disc to the bottom of the beaker with hydrogen peroxide. Time how long it takes the disc to rise to the surface.
6. Repeat this procedure for each of the other enzyme dilutions.
7. Record your results. Your data table should include enzyme concentration, distance disc traveled, time to rise to the top, and calculated rate.
8. Record class data and calculate class averages for the rate at different concentrations.

Data: Make the necessary graphs required to record the data.

Part C: Inquiry

You are going to change one of the following aspects about your catalase solution. Each of the conditions below will be assigned to two groups. Design a procedure that will allow you to observe and measure the effects of one of the environmental conditions below on the rate of the enzymatic reaction. You will also need to determine what data to collect and make the necessary data tables.

1. pH
2. Temperature
3. Ionic condition
4. Substrate concentration

You will then create a mini-poster to share your experimental design and data with the class. Instructions will follow.