Pre-lab AP Lab: Enzyme Catalysis Name _____

Pre-Lab Questions (25 points) These questions must be done before you begin your lab!

1. Define the following terms IN YOUR OWN WORDS: **(2 pts each)**
   a. Enzyme

   b. Substrate

   c. Active Site

   d. Denaturation

2. What is the effect of enzyme concentration or substrate concentration on enzyme activity? **(2 pts)**

3. Explain the effect of increase pH on enzyme activity. **(2 pts)**

4. Explain the effect of increase AND decrease temperatures on enzyme activity. **(4 pts)**

5. What are the enzyme, the substrate, and the product(s) that will be used in this lab? **(3 pts)**

6. Describe how you will be measuring the rate of enzyme activity during lab. See the general and specific lab procedures for the control set-up at the end of this packet. **(6 pts)**

Name _____ **Period** _____

AP Biology **Date** _____

LAB _____. FACTORS INFLUENCING ENZYME ACTIVITY

Background

Enzymes are biological catalysts capable of speeding up chemical reactions by lowering activation energy. 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**, combines reversibly with its specific **substrate**, to form an enzyme-substrate complex. 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**, are formed.

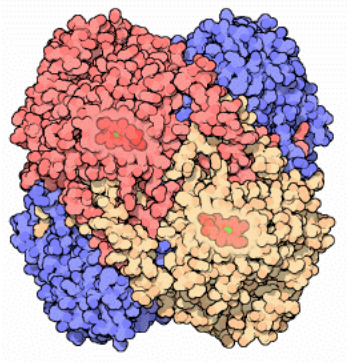
This can be summarized in the equation:

Enzyme + Substrate → Enzyme-Substrate Complex → Enzyme + Product

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.



The catalase that works in liver and in red blood cells.

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

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

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

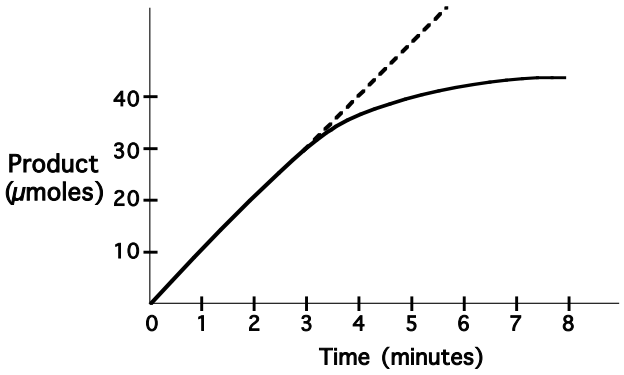
Name _____ Period _____

Much can be learned about enzymes by studying the kinetics (changes 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 that 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 molecule. 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-line 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:

$$\frac{\mu moles_2 - \mu mole_1}{t_2 - t_1}$$

In the graph shown as Figure 1:

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

You will calculate the rate using Milliliters of Oxygen collected during 30 second intervals.
The above calculation will use milliliters instead of μmoles in your calculation of reaction rate.
The above calculations may easily be seen on the AP EXAM.

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 you will measure the rate of oxygen (product) produced.

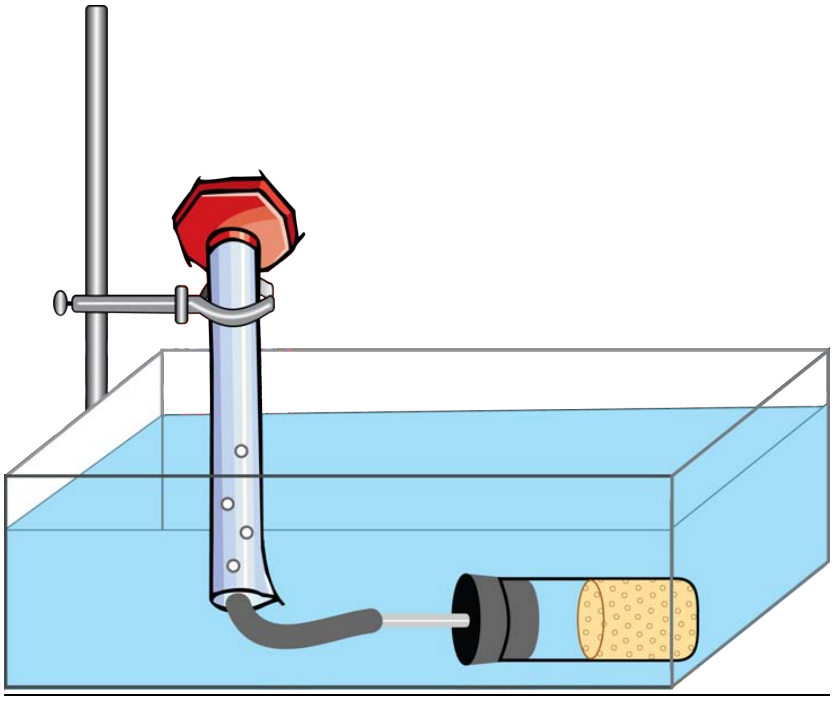
A description of several ways enzyme action may be affected follows:

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 the highest in acidity and 14 the 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, have a low pH optimum.
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. Many proteins are **denatured** by temperatures around 40-50°C, but some are still active at 70-80°C, and a few even withstand boiling.
4. **Activation 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.

Name _____ Period _____

General Lab Procedure:

1. Work as a lab group of 3-4 members. ***Each*** lab group will complete **Part A** and **Part B** of the lab. Your teacher will assign on an additional activity to each group form **Parts C, D, E & F**.
2. At your lab bench you will find a round vial with a rubber stopper top. This is called the **reaction chamber**. You will also find a 100mL graduated cylinder, ring stand and clamp, and a plastic pan which will be used as a water bath. All of this equipment needs to be assembled into our experimental apparatus, as described and illustrated below.
3. Fill the pan 3/4 full of tap water. Allow the water to come to room temperature.
4. Submerge the 100mL graduated cylinder to fill it with water. Turn the graduated cylinder upside down, keeping the open end under water, so as to keep it filled with water. Suspend it upside down in the clamp on the ring stand. Adjust the height of the clamp on the ring stand so the open end of the graduated cylinder is about 3cm above the bottom of the pan. See diagram below.
5. Place a thermometer in the pan and record the temperature of the water, **during Part A of the lab**. _____°C
6. When all sections of the lab are complete, share the data with the class from your group's section. Each person must plot the data for all parts of the lab on his/her own graph paper.



Specific Lab Procedure:

Part A. The Time Course of Enzyme Activity (Control Group)
Design a Data Table for this experiment before beginning.

1. Set up the experimental apparatus as illustrated and described on the previous page in the General Lab Procedures section.
2. Obtain a reaction chamber.
3. Obtain a bottle of 3% hydrogen peroxide (H_2O_2) solution and a 10 cc syringe.
4. Obtain a small amount of stock catalase (yeast) solution in a 50mL beaker. ***Keep the catalase on ice!!!!!*** You will need 1.0mL of yeast solution for each trial. When you are ready, you will add it to the vial with a 1cc syringe.
5. Use the **10cc syringe** to place 10mL of hydrogen peroxide (H_2O_2) into the reaction chamber. Place **1.0mL of stock catalase solution** (use the **1cc syringe)** into the reaction chamber and ***IMMEDIATELY*** stopper the reaction chamber tightly, submerge it in the water bath and place the plastic tubing into the bottom of the graduated cylinder. All the bubbles formed in the reaction chamber should be captured by the inverted graduated cylinder. **If the reaction is so quick that the bubbles can't be caught or that the stopper is popped off of the reaction chamber, you must dilute the catalase solution. Make a note in your lab book that explains this change in procedure.**
6. Measure the gas levels in the graduated cylinder at **30-second intervals** for 5 minutes. Record the levels in a **data table of your own design.**
7. **Plot the data on a graph. Don't forget to label your axes and title your graph.**

Part B. Inquiry Design (Experimental Group)

Prior to experimentation, you must have teacher approval/feedback before you are allowed to begin.
These are the things you must consider when designing your experiment:

- Question: What do you want to know about enzymes and what affects enzymatic rates?
- Constants: What conditions will you keep constant?
- Materials and Equipment: Choose materials from the list your teacher will provide and list them here. If there is something not listed that your teacher may have, ask.
- Experimental Set-Up: make a diagram of what you think your experimental setup will look like. You need to complete 3+ trials.
- Safety considerations: what safety precautions will you take? If you are working with an acid or base, ALL of you and your lab mates will need to where goggles.
- Procedure: write your proposed procedure. BE SPECIFIC.
- You will write a formal lab report for this experiment. See the Lab Report Rubric in order to include all components.

Materials you will have available to you for Part B

Catalase	Hydrogen peroxide of different concentrations	Water Baths
Distilled water	Ice	Salt
pH Buffers	Timers	Thermometer