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.

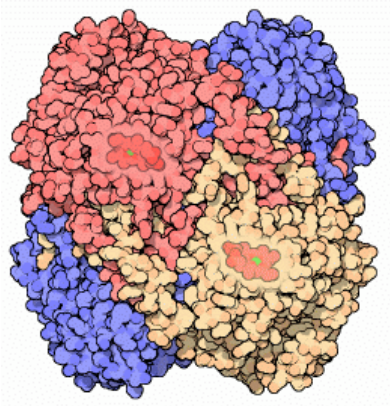
This can be summarized in the equation:

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.

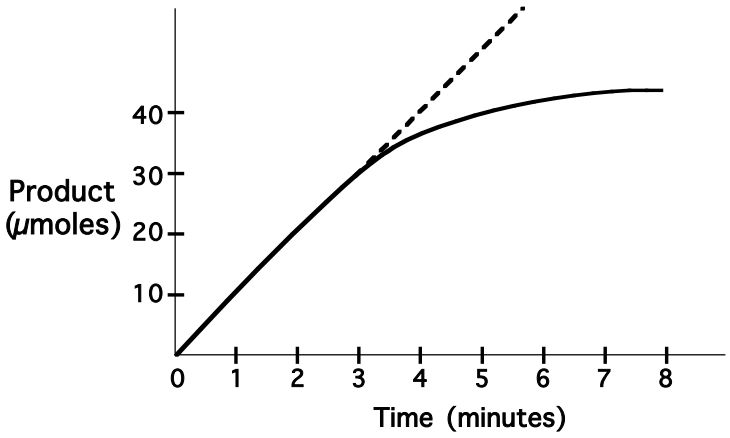
Name _____ Period _____

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.

Name _____ Period _____

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:

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

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, are measured.

Materials:

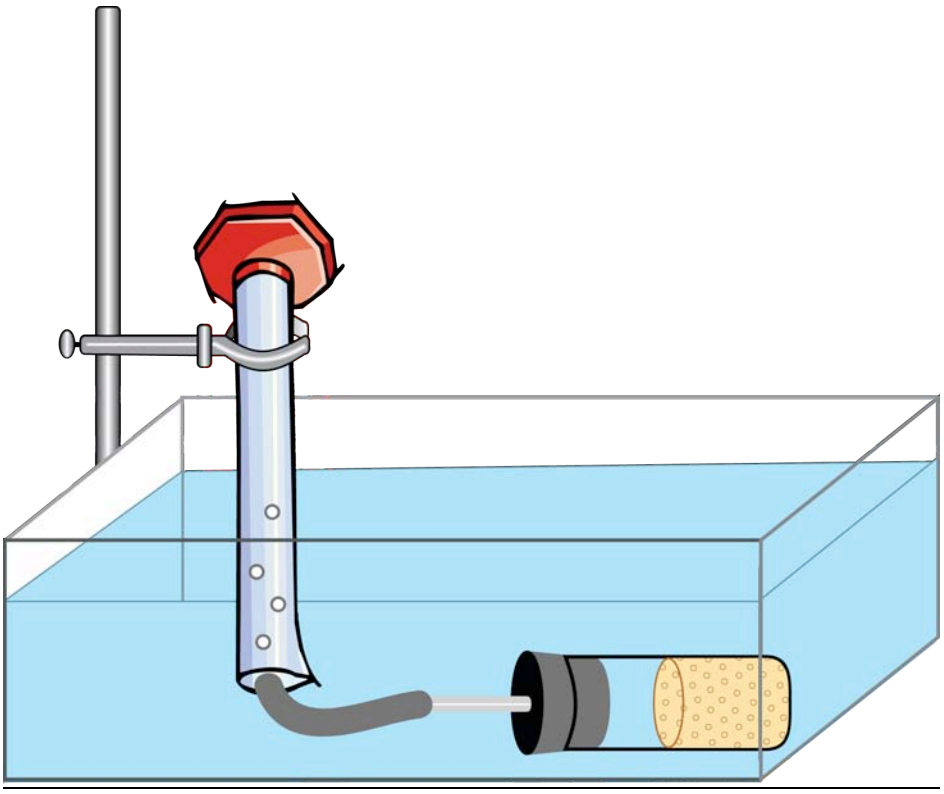
Obtain the following materials:

- 50mL beaker containing fresh catalase (yeast) solution
- reaction chamber
- ring stand & clamp
- 10mL graduated cylinder
- 100mL graduated cylinder
- 3% hydrogen peroxide (H_2O_2)
- pan (water bath)
- pipette
- hot plate
- ice
- thermometer
- boiled catalase
- buffers of varying pH: 4, 7, 10
- distilled water
- balance
- NaCl (salt)

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.



Name _____ Period _____

FACTORS INFLUENCING THE ACTIVITY OF CATALASE

Several enzymatic variables will be examined in this lab. You will be using the protein enzyme, **catalase**. Catalase is found in most cells, even in single-celled eukaryotes like yeast. In this lab, you will extract catalase from a yeast solution and test its catalytic effectiveness on hydrogen peroxide. Catalase speeds up the breakdown of peroxides which may form during respiration (metabolic energy production). This breakdown prevents the peroxide from causing unwanted oxidation of important biomolecules:

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

We will measure enzyme activity by measuring the generation of oxygen gas — a product in the reaction.

Part A. The Time Course of Enzyme Activity

1. Set up the experimental apparatus as illustrated and described above.
2. Obtain a reaction chamber.
3. Obtain a bottle of 3% hydrogen peroxide (H_2O_2) solution and a 10mL graduated cylinder.
4. Obtain a small amount of stock catalase (yeast) solution in a 50mL beaker. You will need 1.0mL of yeast solution for each trial. When you are ready, you will add it to the vial with a plastic pipette.
5. Pour 10mL of hydrogen peroxide (H_2O_2) into the reaction chamber. Pipette in **1.0mL of stock catalase solution** (yeast solution) 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, so all the bubbles formed in the reaction chamber are captured by the inverted graduated cylinder.
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. The Effect of Enzyme Concentration on Enzyme Activity

1. Repeat the experiment from Part A, using 3 different levels of enzyme concentration: 75%, 50%, and 25% concentration of enzyme solution. You may easily do this by using the following procedures:
    a. **75% concentration**: Follow the procedure from Part A, but use **0.75mL catalase** solution in the reaction chamber, instead of 1.0mL.
    b. **50% concentration**: Follow the procedure from Part A, but use **0.50mL catalase** solution in the reaction chamber, instead of 1.0mL.
    c. **25% concentration**: Follow the procedure from Part A, but use **0.25mL catalase** solution in the reaction chamber, instead of 1.0mL.
2. Record all data in a data table of your own design.
3. Plot the data on the same graph as Part A. Don't forget to clearly label the enzyme concentrations on your plotted lines.

Name _____ Period _____

Part C. The Effect of Temperature on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 1mL catalase solution), using 3 different temperatures: 5°C, 37°C, and 100°C (boiled catalase). You may easily do this by using the following procedures:
    a. **5°C**: Set up your reaction vessel and water bath and add ice to the water bath so that it is chilled to 5°C for 5 minutes before running the experiment. Keep adding ice to keep the temperature at 5°C or colder.
    b. **37°C**: Set up your reaction vessel and water bath with heated water so that it is warmed to 37°C for 5 minutes before running the experiment. Keep adding hot water to keep the temperature at 37°C.
    c. **100°C (boiled catalase)**: We can't use a water bath of boiling water since that may injure a student, so instead of keeping the reaction vessel in boiling water during the experiment, we will instead boil the catalase solution for 5 minutes. Then after the catalase solution has cooled you run the experiment in room temperature water (but with already boiled catalase).
2. Record all data in a data table of your own design.
3. Plot the data on a new graph. Also plot on this graph the **room temperature** data recorded in Part A. Don't forget to clearly label your axes and plotted lines, and title your graph.

Part D. The Effect of pH on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 1mL catalase solution), using 3 different hydrogen peroxide solutions at 3 different pH values: pH 4, pH 7, and pH 10. You may easily do this by using the following procedures
    a. **pH 4**: Make a 1.5% solution of H_2O_2 at pH4 by adding 5mL of H_2O_2 to 5mL of pH 4 buffer.
    b. **pH 7**: Make a 1.5% solution of H_2O_2 at pH7 by adding 5mL of H_2O_2 to 5mL of pH 7 buffer.
    c. **pH 10**: Make a 1.5% solution of H_2O_2 at pH10 by adding 5mL of H_2O_2 to 5mL of pH 10 buffer.
2. Record all data in a data table of your own design.
3. Plot the data on a new graph. Don't forget to clearly label your axes and plotted lines, and title your graph.

Name _____ Period _____

Part E. The Effect of Substrate Concentration on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 1mL catalase solution), using 4 different substrate concentrations: 0%, 0.3%, 1.5%, and 3.0%. You may easily do this by using the following procedures
    a. **0%**: Use 10mL distilled water only.
    b. **0.3%**: Prepare this by adding 3mL of H_2O_2 to 7mL of distilled water.
    c. **1.5%**: Prepare this by adding 5mL of H_2O_2 to 5mL of distilled water.
    d. **3.0%**: This is the concentration of substrate from the original experiment in Part A; just use this initial data.
2. Record all data in a data table of your own design.
3. Plot the data on a new graph. Don't forget to clearly label your axes and plotted lines, and title your graph.

Part F. The Effect of Ionic Concentration on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 1mL catalase solution), using 3 different ionic concentrations: 10% NaCl, 2% NaCl, and 0% NaCl. You may easily do this by using the following procedures
    a. **10% NaCl**: Make a 1.5% solution of H_2O_2 containing 10% NaCl by dissolving 5g of NaCl in 50mL of water then add 5mL of this solution to 5mL of H_2O_2.
    b. **2% NaCl**: Make a 1.5% solution of H_2O_2 containing 2% NaCl by dissolving 1g of NaCl in 50mL of water then add 5mL of this solution to 5mL of H_2O_2.
    c. **0% NaCl**: Prepare this by adding 5mL of distilled water to 5mL of H_2O_2.
2. Record all data in a data table of your own design.
3. Plot the data on a new graph. Don't forget to clearly label your axes and plotted lines, and title your graph.

Name _____ Period _____

QUESTIONS

1. What was the enzyme in this lab? _____
2. What was the substrate in this lab? _____
3. What was the product(s) in this lab? _____
4. What variable was measured in this lab? _____
5. If you wanted to determine rate of enzyme activity from the data collected in this lab, how would you do that?

   _____

   _____

6. What are some good sources of the human enzyme catalase? _____

   _____

7. **Enzyme concentration**
   a. Summarize the effect of enzyme concentration on enzyme activity. Explain the mechanisms that cause this effect.

      _____

      _____

      _____

   b. What would cause the rate of enzyme activity to level off on the enzyme concentration graph?

      _____

      _____

   c. Draw an "idealized" graph to illustrate the relationship between enzyme concentration and rate of enzyme activity.

Name _____ Period _____

8. Temperature

a. Summarize the effect of temperature on enzyme activity. Explain the mechanisms that cause this effect.

_____

_____

_____

b. Explain the specific effect of boiling the enzyme.

_____

_____

c. Draw an "idealized" graph to illustrate the relationship between temperature and rate of enzyme activity.

d. What is the optimal temperature for human enzymes? _____

9. pH

a. Summarize the effect of pH on enzyme activity. Explain the mechanisms that cause this effect.

_____

_____

_____

b. Draw an "idealized" graph to illustrate the relationship between pH and rate of enzyme activity.

c. Do all human enzymes function within the same optimal pH range, like they do with temperature? Explain.

_____

_____

_____

Name _____ Period _____

10. <u>Substrate concentration</u>

a. Summarize the effect of substrate concentration on enzyme activity. Explain the mechanisms that cause this effect.

_____

_____

_____

b. What would cause the rate of enzyme activity to level off on the substrate concentration graph?

_____

_____

c. Draw an "idealized" graph to illustrate the relationship between substrate concentration and rate of enzyme activity.

11. <u>Ion concentration</u>

a. Summarize the effect of ion concentration on enzyme activity. Explain the mechanisms that cause this effect.

_____

_____

_____

_____

b. Draw an "idealized" graph to illustrate the relationship between ion concentration and rate of enzyme activity.

Name _____ Period _____

12. After an enzyme is mixed with its substrate, the amount of product formed is determined at 10-second intervals for 1 minute. Data from this experiment are shown below.

Time (sec)	0	10	20	30	40	50	60
Product formed (mg)	0.00	0.25	0.50	0.70	0.80	0.85	0.85

a. What is the initial rate of this enzymatic reaction? _____

Show your calculation:

b. What is the rate after 50 seconds? _____

Show your calculation:

c. Why is the rate after 50 seconds different from the initial rate?

_____

_____

_____