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Ms. Foglia • AP Biology **Date** ______________________

LAB 2. FACTORS INFLUENCING ENZYME ACTIVITY

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.

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

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

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.

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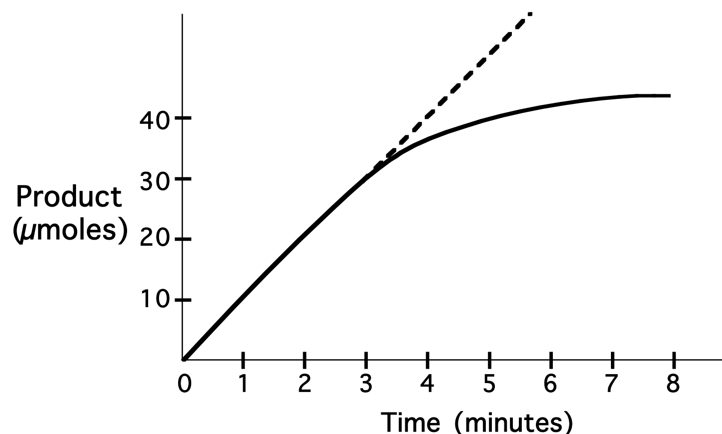
Much can be learned about enzymes by studying the kinetics (changes in rate) of enzyme-catalyzed reactions. For example, it is possible to measure the amount of product formed, or the amount of substrate used, from the moment the reactants are brought together until the reaction has stopped.

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.

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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, is measured.

Materials:

Obtain the following materials:

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

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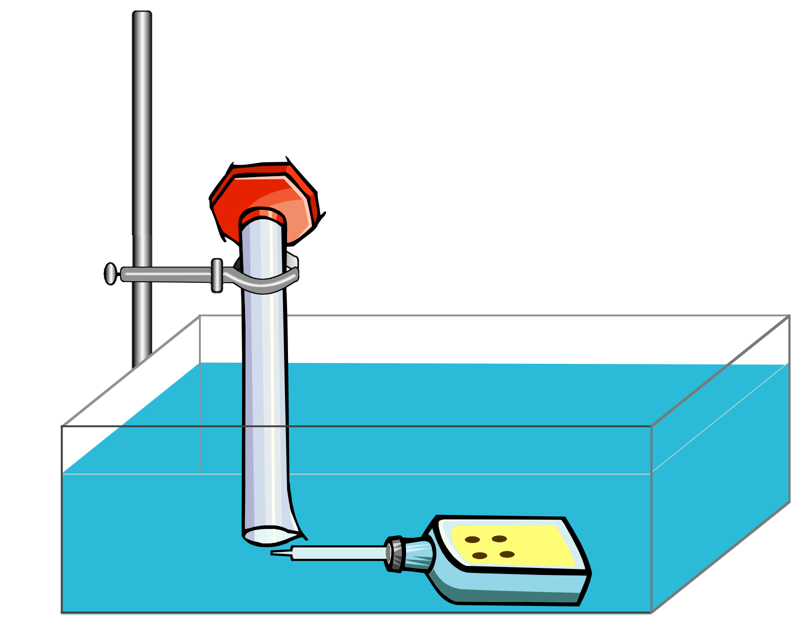
General Lab Procedure:

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 square bottle with a medicine dropper 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.



Catalase Extraction & Filter Paper Disk Instructions

1. Ground fresh liver has been provided for each lab group. It has been ground with water in a blender. Use this mixture to dip your filter paper disks into.
2. Collect paper disks by punching holes in the filter paper provided to you.

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FACTORS INFLUENCING THE ACTIVITY OF CATALASE

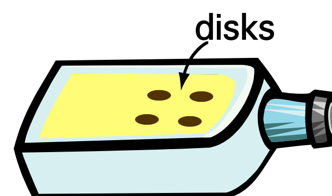
Several enzymatic variables will be examined in this lab. You will be using the protein enzyme, **catalase**. Catalase is found in most cells, however, liver is a particularly good source. In this lab, you will extract catalase from fresh beef or chicken liver and test its catalytic effectiveness on 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.

The breakdown of hydrogen peroxide, H_2O_2, is speeded up by catalase. The decomposition of hydrogen peroxide occurs as follows:

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

Part A. The Time Course of Enzyme Activity

1. Set up the experimental apparatus as described above.
2. Obtain a small amount of stock catalase solution in a 50mL beaker.
3. Obtain a reaction chamber and a number of filter paper disks.
4. Prepare 4 disks for use in the reaction chamber. Prepare each disk, one at a time, by holding each by its edge with a pair of forceps and dipping it into the stock catalase solution for a few seconds. ***Helpful Hint:* Stir your catalase solution (liver extract) before every disk is dipped.** Remove the excess liquid from the disk by blotting the disk on a Kimwipe or paper towel. With another Kimwipe or paper towel dry the tip of the forceps, so it doesn't drip in the next step. Next, transfer the dampened disks to the top interior wall of the reaction vessel. The wet filter paper disks will stick to the side wall of the glass. Position the disks in the **upper half** of the reaction vessel (the half near the opening). Repeat this procedure with the other disks, placing all disks on the same surface of the reaction vessel. ***Helpful Hint:* One person in each group should soak and handle all disks for all experiments. In this way, the techniques will remain similar and key operations will be performed consistently.**



5. Place four catalase-soaked filter paper disks high on one interior sidewall of the reaction
6. chamber.
7. Stand the reaction chamber upright and carefully add 10mL of 3% hydrogen peroxide (H_2O_2) solution. ***Do NOT allow the peroxide to touch the filter paper disks.***
8. Tightly stopper the chamber.
9. Retrieve the water bath and graduated cylinder. Turn the graduated cylinder upside down into an upright position, keeping its mouth underwater at all times.

10. Carefully place the reaction chamber and its contents on its side in the water bath. ***Make certain that the side with the disks faces upward.***

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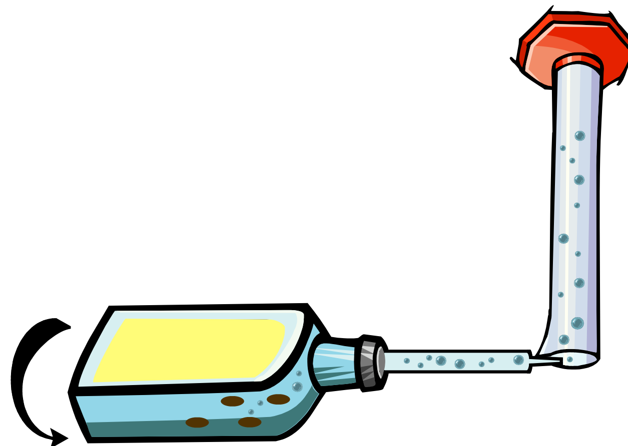
11. Move the graduated cylinder into a position such that its mouth comes to lie directly over the tip of the mouth of the reaction chamber, so that any O_2 released from the reaction chamber will collect in the graduated cylinder. ***One member of the team should hold it in this position for the duration of the experiment.***
12. Rotate the reaction chamber 180° onto its side so that the hydrogen peroxide solution comes into contact with the catalase-soaked disks. ***Note the time. This is time "zero".***
13. 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.
14. 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 **3_** catalase-soaked disks in the reaction chamber, instead of 4.
   b. **50% concentration**: Follow the procedure from Part A, but use **2_** catalase-soaked disks in the reaction chamber, instead of 4.
   c. **25% concentration**: Follow the procedure from Part A, but use **1_** catalase-soaked disks in the reaction chamber, instead of 4.
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.

Part C. The Effect of Temperature on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 4 disks), 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)**: Boil catalase for 5 minutes then let cool. Run the rest of the experiment at room temperature.

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 for 4 disks 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 4 disks), 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.

Part E. The Effect of Substrate Concentration on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 4 disks), 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 distilled water.
   b. **0.3%**: Prepare this by adding 3mL of H_2O_2 to 7mL of distilled water.
   c. **1.5%**: Prepare this by adding 3mL of H_2O_2 to 3mL 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.

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Part F. The Effect of Ionic Concentration on Enzyme Activity

1. Repeat the experiment from Part A (5 minute runs with 4 disks), 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.

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QUESTIONS

1. Describe the effect of enzyme concentration on enzyme activity.

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2. Describe the effect of temperature on enzyme activity. Be sure to discuss, in detail, the effect of boiling the enzyme

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3. Describe the effect of pH on enzyme activity.

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4. Describe the effect of substrate concentration on enzyme activity.

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5. Describe the effect of ionic concentration on enzyme activity.

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6. 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

d. What is the initial rate of this enzymatic reaction? Show your calculation.

e. What is the rate after 50 seconds? Show your calculation. Why is it different from the initial rate?