Biology 2201 Investigation 3.B Factors Affecting Enzyme Activity Gillam Holy Heart Name: _____

Metabolism is the sum of all of the chemical reactions in the body that maintain life. Enzymes are the biological catalysts that these metabolic reactions specific and spontaneous. Without them, metabolic reactions would not occur within the normal range of body conditions. Enzymes are like molecular machines that work to change a specific substrate into a specific desired product. Like any other tool, it is their 3D shape that confers function to an enzyme. The complex folding of proteins relies heavily on hydrogen bonds which are sensitive to changes in temperature and pH. Disrupting hydrogen bonds may compromise the enzymes shape and therefore its function. For optimum enzyme activity, they also need to be flexible in order to undergo a shape change when forming the enzyme substrate complex.

In this lab, you will investigate how changes in temperature and pH affect the activity of the enzyme catalase from an animal the liver. Hydrogen peroxide is a natural by-product of cellular processes. It is generally regarded as toxic therefore cells produce catalase enzyme to convert it to harmless products. The liver, which is large responsible for detoxifying the blood, is particularly rich in this enzyme.

Like all enzymes, catalase is highly efficient. One molecule of catalase can degrade about six million molecules of hydrogen peroxide in one minute. This same reaction can be catalyzed by iron, however, to achieve the same speed you would need about six tons of iron.

The reaction is as follows: $2H_2O_2$ (aq) ----> $2H_2O$ (l) + O_2 (g)

Below is a list of useful information about enzymes that will assist you in understanding enzyme activity:

- They are specific in their action. Each enzyme controls one particular reaction, or type of reaction.
- They are not altered by the reaction they catalyse. This means that an enzyme molecule is reusable. They are neither reactants nor products.
- They are denatured by heat. Heat causes hydrogen bonds between amino acid side chains to break. As a result the proteins unfolds, losing its 3D shape.
- They are lose flexibility when they cool. A decrease in temperature may restrict the flexibility of an enzymes. Without flexibility are less able to catalyze reactions
- They are sensitive to pH. The term pH refers to the degree of acidity and alkalinity of a solution. Most intracellular enzymes work best in neutral conditions, i.e. conditions that are neither acidic nor alkaline.

Materials:

Test tube rack	9 Test tubes	10 ml graduated cylinder	Tweezers
2 Thermometers	50 ml beakers (3)	Ice	Liver
Hydrogen peroxide	Hydrochloric acid	Sodium hydroxide	Stop Watch
Hot plate	Water		

Part A: Effect of Temperature on catalase enzyme reaction rate

1) Label four test tubes 1 through 4. Put a small piece of liver in each of these test tubes.
2) Label two other test tubes P1 and P3. Put 2 ml of hydrogen peroxide in each of these test tubes.
3) Prepare an ice water bath (0 °C) and put test tubes 1 and P1 in it and let sit for 10 minutes.
4) Prepare a warm water bath (37 °C) and put test tubes 3 and P3 in it and let sit for 3 minutes.
5) Pour 2 ml of hydrogen peroxide into test tube 2 and measure the height in mm of the reaction. (Room temperature)
6) Put 1 ml of water in test tube 4 and put it in boiling water (100 °C) for 5 minutes.
7) After 5 minutes, remove test tube 4 and let sit till cool.
8) While test tube 4 is cooling, combine test tubes 1 and P1 and measure the height in mm of the reaction
9) While test tube 4 is cooling, combine test tubes 3 and P3 and measure the height in mm of the reaction
10) When test tube #4 is cool, pour in 2 ml of hydrogen peroxide and measure the height in mm of the reaction
11) Clean up all materials very well. No liver should be put down the drain.

Table 1.0 _____

Test Tube #	Temperature	Height	Observations
1			
2			
3			
4			

Construct a line graph based on the data. **Label the horizontal axis temperature and the vertical axis rating**.

Figure 1.0 _____

Part B: Effect of PH on catalase enzyme reaction rate

1) Label three test tubes A, N, and B
2) Obtain three small equal size pieces of liver and add each piece of liver to a test tube
3) Add just enough Hydrochloric acid to test tube A to cover the liver
4) Add just enough water to test tube N to cover the liver
5) Add just enough sodium hydroxide to B to cover the liver
Let stand for 5 minutes, then pour off excess liquid into the designated waste container.
6) **One at a time,** add 3mL of hydrogen peroxide to each test tube and using a ruler, record the height of the bubbles (in mm) at 15, 30, 45, 60 and 75 seconds.

Time	Acid - height of bubbles (mm)	Neutral - height of bubbles (mm)	Basic - height of bubbles (mm)
15 s			
30 s			
60 s			
75 s			
Time Reaction finished			

Discussion:

1) What are enzymes?

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2) What are their roles in chemical reactions?

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3) Describe what happened to test tube 2 when hydrogen peroxide was added to the liver. What gas was produced? What liquid was left behind?

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4) Are enzymes reusable? _____

5) What effect does temperature have on catalase enzyme reaction rates?

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6) What effect does pH have on catalase enzyme reaction rates?

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Part C - Design an Experiment

Lactase enzyme supplement is a product designed to help people who cannot digest milk sugar (lactose) because they are missing the enzyme lactase. Many people are lactose-intolerant, a condition that is mainly genetic. Lactase breaks down lactose into two subunits: glucose and galactose.

To test for the presence of monosaccharides and reducing disaccharide sugars in food, the food sample is dissolved in water, and a small amount of Benedict's reagent is added. The solution should progress in the colors of blue (with no glucose present), green, yellow, orange, red, and then brick red when there is a large amount of glucose present. (Google benedict's test to see the way this looks.)

Design an experiment where you would determine how quickly lactase enzyme works to break down milk sugar at different temperatures. Be specific in your description, use drawings if necessary.

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