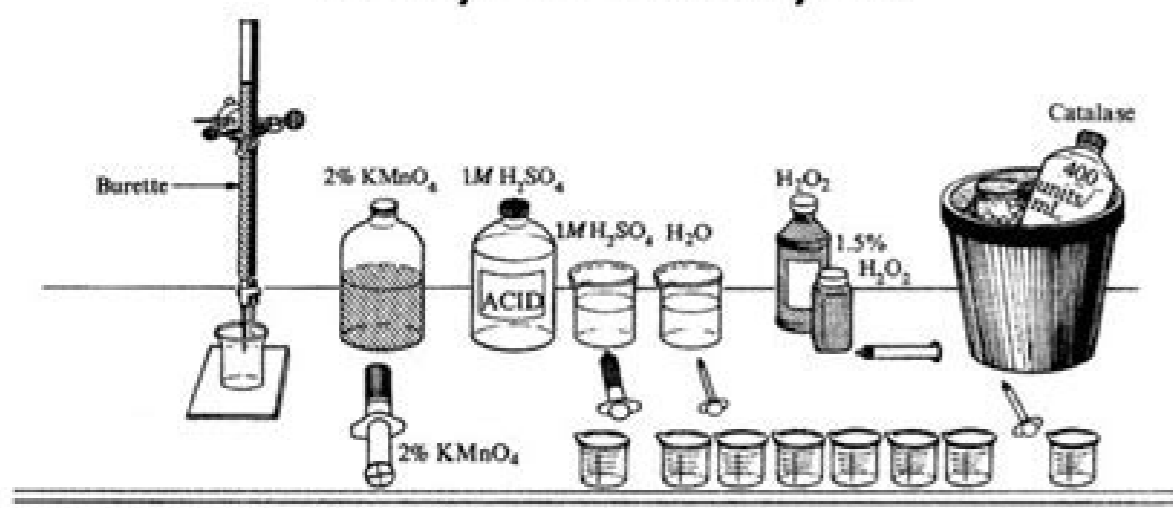


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# LAB 2 Enzyme Catalysis



NAME: Michael Hahn Lab Day: Monday  
 XXIII: Section: 048 Week: 1  
 Date: Monday 17<sup>th</sup> September 2012 Experiment: 6

### Experiment 6: Activation energy for an enzyme-catalyzed and acid-catalyzed hydrolysis

**Theory:**  
 Enzymes lower the ability to bring about vast increases in the rates of reactions. In most cases, the rates of enzyme-catalyzed reactions are faster than those of uncatalyzed reactions by factors of  $10^6$  to  $10^{12}$ . Enzymes show remarkable specificity for their substrates and the formation of specific products. According to Emil Fischer theory, enzymes have the ability to distinguish between  $\alpha$  and  $\beta$  - glycosidic linkages that led him to formulate the lock and key hypothesis for enzyme specificity.

According to the lock and key hypothesis, the specificity of an enzyme and its substrate comes from their geometrically complementary shapes. In an enzyme-catalyzed reaction, the enzyme and the substrate combine to form an enzyme-substrate complex. Formation of the enzyme-substrate complex often induces a conformational change in the enzyme called an induced fit that allows it to bind the substrate more effectively.

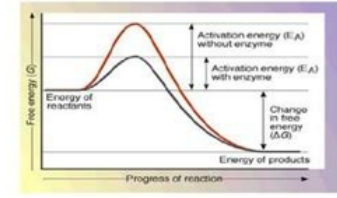
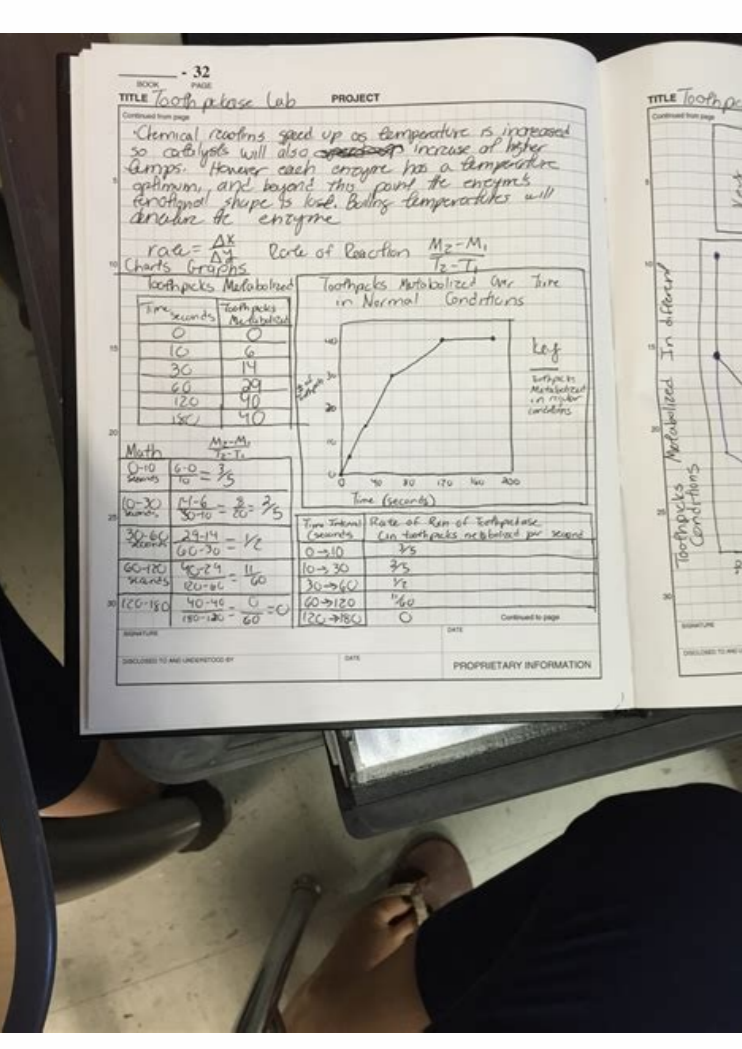


Diagram above showing activation energy, in the amount of energy needed to get the reaction to the transition state, in which bonds are broken and new bonds are formed. Enzymes are protein that catalyze chemical reactions. Enzymes use less activation energy and so speed up the reaction (Enzymes lower the activation energy needs to start up a reaction).



Preston Fernandez Optimal pH of Liver and Potato Catalase Reactivity HL BIO P.8

### Sample Calculations

#### Calculating Average Uncertainty

$$\frac{\text{Range}}{2} = \text{Average Uncertainty}$$

Manipulating Averages into Percentages (The concentration of enzyme is different for potato and chicken liver, by converting the averages into percentages it will be easier and more realistic to compare and contrast the catalase in potatoes to the catalase in enzymes)

$$\frac{\text{Average}}{\text{Highest Average of Chicken Liver Catalase}} \times 100 \quad \frac{\text{Average}}{\text{Highest Average of Potato Catalase}} \times 100$$

Above are the formulas used to calculate the averages into percentages.

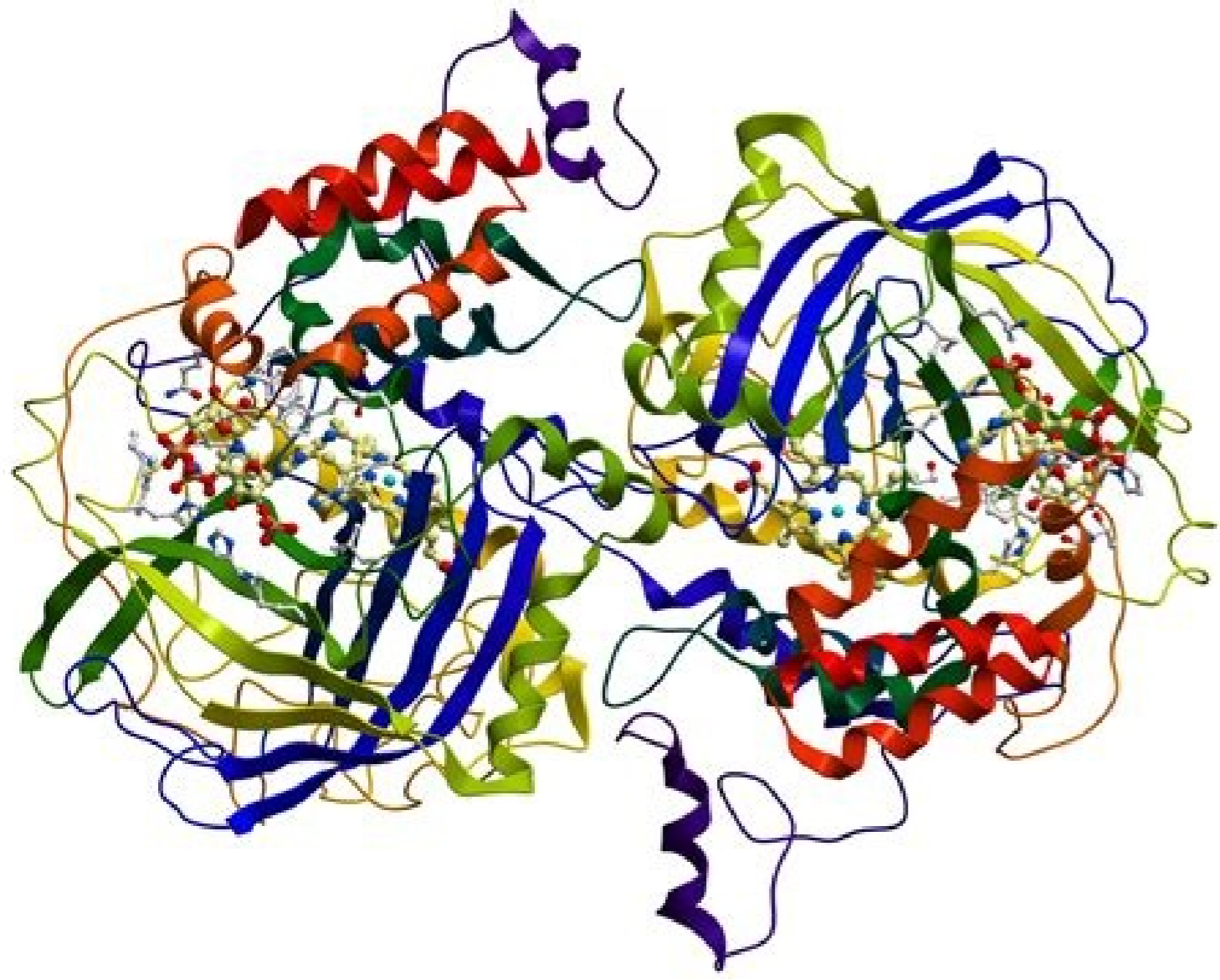
The Calculations Shown below is the manipulation of averages to percentages for chicken liver.

$$\frac{6.540}{6.540} \times 100 = 100\% \quad \frac{1.536}{6.540} \times 100 = 23\% \quad \frac{5.954}{6.540} \times 100 = 91\%$$

### Processed Data Table

Optimal pH of Liver Catalase and Potato Catalase Enzyme Reactivity				
pH	% Optimal pH of Liver Catalase Enzyme Reactivity		% Optimal pH of Potato Catalase Enzyme Reactivity	
	Avg. kPa/s	% Optimal	Avg. kPa/s	% Optimal
5	1.536	23	.03548	86
7	6.540	100	.04123	100
9	5.954	91	.03451	84

The table above is processed data that shows the % optimal the catalases are at different pHs



Ap biology lab 02 enzyme catalysis answers. Lab two enzyme catalysis answers. Ap biology lab 2 enzyme catalysis lab answers. Enzyme catalysis lab questions answers. Ward's ap biology lab 2 enzyme catalysis answers. Enzyme catalysis pre lab answers. Carolina enzyme catalysis lab answers. Pearson lab 2 enzyme catalysis answers.

**Lab 2 Enzyme Catalysis**  
**Introduction:** Enzymes are proteins produced by living cells. They are biochemical catalysts meaning they lower the activation energy needed for a biochemical reaction to occur. Because of enzyme activity, cells can carry out complex chemical activities at relatively low temperatures. The substrate is the substance acted upon in an enzyme-catalyzed reaction, and it can bind reversibly to the active site of the enzyme. The active site is the portion of the enzyme that interacts with the substrate so that any substrate that blocks or changes the shape of the active site affects the activity of the enzyme. The result of this temporary union is a reduction in the amount of energy required to activate the reaction of the substrate molecule so that products are formed. The following equation demonstrates this process:  $E + S \rightarrow ES \rightarrow E + P$  Enzymes follow the law of mass reaction. Therefore, the enzyme is not changed in the reaction and can be recycled to break down additional substrate molecules. Several factors can affect the action of an enzyme: salt concentration, pH of the environment, temperature, activations and inhibitors. If salt concentration is close to zero, the changed amino acid side chains of the enzyme molecules will attract one another. The enzyme will then denature and form an inactive precipitate. Denaturation occurs when excess heat destroys the tertiary structure of proteins. This usually occurs at 40 to 50° Celsius. If salt concentration is high, the normal interaction of charged groups will be blocked. An intermediate salt concentration is normally the optimum for enzyme activity. The salt concentration of blood and cytoplasm are good examples of intermediate concentrations. The pH scale is a logarithmic scale that measures the acidity or H<sup>+</sup> concentration in a solution and runs from 0 to 14, with 0 being highest in acidity and 14 lowest. Amino acid side chains contain groups such as -COOH that readily gain or lose H<sup>+</sup> ions. As the pH is lowered an enzyme will tend to gain H<sup>+</sup> ions, disrupting the enzyme's shape. If the pH is raised, the enzyme will lose H<sup>+</sup> ions and eventually lose its active shape. Reactions usually perform optimally in neutral environments. Chemical reactions generally speed up as the temperature is raised. More of the reacting molecules have enough kinetic energy to undergo the reaction as the temperature increases. However, if the temperature goes above the temperature optimum, the conformation of the enzyme molecules is disrupted. An activator is a coenzyme that increases the rate of the reaction and can regulate how fast the enzyme acts. It also makes the active site a better fit for the substrate. An inhibitor has the same power of activator regulation but decrease the reaction rate. An inhibitor also reduces the number of S-S bridges and reacts with the side chains near activation sites, blocking them. The enzyme used in this lab is catalase. It has four polypeptide chains that are each composed of more than 500 amino acids. One catalase function is to prevent the accumulation of toxic levels of hydrogen peroxide formed as a by-product of metabolic processes. Many oxidation reactions that occur in cells involve catalase. The following is the primary reaction catalyzed by catalase, the decomposition of hydrogen peroxide to form water and oxygen:  $2 H_2O_2 \rightarrow 2 H_2O + O_2$  (gas) Without catalase this reaction occurs spontaneously but very slowly. Catalase speeds up the reaction notably. The direction of an enzyme-catalyzed reaction is directly dependent on the concentration of enzyme, substrate, and product. For example, lots of substrate with a little product makes more product. Another example is lots of product with a little enzyme forms more substrate. Much can be learned about enzymes by studying the kinetics of enzyme-catalyzed reaction. 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. **Hypothesis:** Enzyme catalase, when working under optimum conditions, noticeably increases the rate of hydrogen peroxide decomposition. **Materials:** Exercise 2A The materials needed for exercise 2A of the lab are: 30 mL of 1.5% (0.44 M) H<sub>2</sub>O<sub>2</sub>, a 50- mL glass beaker, 6 mL of freshly made catalase solution, a test tube, boiling water bath, 1 cm<sup>3</sup> of liver, a knife for maceration, paper towels, safety goggles, lab apron, pencil, eraser, and paper to record results. Exercise 2B The materials needed for exercise 2B are: 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub>, two clean glass beakers, 1 mL of H<sub>2</sub>O, 10 mL of H<sub>2</sub>SO<sub>4</sub>, a white sheet of paper, a 5 mL syringe, approximately 5 mL of KMnO<sub>4</sub>, paper, pencil, eraser, safety goggles, and lab aprons. Exercise 2C The materials needed for exercise 2C of the lab are: 20 mL of 1.5% H<sub>2</sub>O<sub>2</sub>, two glass beakers, 1 mL of H<sub>2</sub>O, 10 mL of H<sub>2</sub>SO<sub>4</sub>, a white sheet of paper, a 5 mL syringe, approximately 5 mL of KMnO<sub>4</sub>, paper, pencil, eraser, safety goggles, and lab aprons. Exercise 2D For this part of the experiment, the materials needed are 12 cups labeled 10, 30, 60, 120, 180, and 360 on two each, six cups labeled acid, 60 mL of 1.5% H<sub>2</sub>O<sub>2</sub>, a clean 50- mL beaker, 6 mL of catalase extract, two 5- mL syringes, KMnO<sub>4</sub>, a timer, paper, pencil, black marker, eraser, safety goggles, and lab aprons. **Methods:** Exercise 2A Transfer 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub> into a 50- mL glass beaker and add 1 mL of freshly made catalase solution. Remember to keep the catalase solution on ice at all times. Record the results. Then transfer 5 mL of purified catalase extract to a test tube and place it in a boiling water bath for five minutes. Transfer 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub> into a 50- mL beaker and add 1 mL of the cooled, boiled catalase solution. Again record the results. To demonstrate the presence of catalase in living tissue, cut 1 cm of liver, macerate it, and transfer it into a 50- mL glass beaker containing 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub>. Record these results. Exercise 2B Put 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub> into a clean glass beaker. Add 1 mL of H<sub>2</sub>O. Add 10 mL of H<sub>2</sub>SO<sub>4</sub> (1.0 M) using extreme caution. Mix this solution well. Remove a 5 mL sample and place it into another beaker. Assay for the amount of H<sub>2</sub>O<sub>2</sub> as follows. Place the beaker containing the sample over white paper. Use a 5- mL syringe to add KMnO<sub>4</sub> a drop at a time to the solution until a persistent pink or brown color is obtained. Remember to gently swirl the solution after adding each drop. Record all results. Check with another group before proceeding to see that results are similar. Exercise 2C To determine the rate of spontaneous conversion of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> in an uncatalyzed reaction, put about 20 mL of 1.5% H<sub>2</sub>O<sub>2</sub> in a beaker. Store it uncovered at room temperature for approximately 24 hours. Repeat the steps from Exercise 2B, using the uncatalyzed H<sub>2</sub>O<sub>2</sub>, to determine the proportional amount H<sub>2</sub>O<sub>2</sub> of remaining after 24 hours. Record the results. Exercise 2D If a day or more has passed since Exercise B was performed, it is necessary to reestablish the baseline. Repeat the assay and record the results. Compare with other groups to check that results are similar. To determine the course of an enzymatic reaction, how much substrate is disappearing over time must be measured. First, set up the cups with the times and the word acid up. Add 10 mL of H<sub>2</sub>SO<sub>4</sub> to each of the cups marked acid. Then put 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub> into the cup marked 10 sec. Add 1 mL of catalase extract to this cup. Swirl gently for 10 seconds. (Calculate time using the timer for accuracy.) At 10 seconds, add the contents of one of the acid filled cups. Remove 5 mL and place in the second cup marked 10 sec. Assay the 5- mL sample by adding KMnO<sub>4</sub> a drop at a time until the solution obtains a pink or brown color. Repeat the above steps except allow the reactions to proceed for 30, 60, 120, 180, and 360 seconds, respectively. Use the times' corresponding, marked cups. Record all results and observations. **Results:** Table 1: Test of Catalysis Activity Experiment. **Observations:** Hydrogen Peroxide + Fresh Catalase Bubbling in solution with the release of oxygen. Hydrogen Peroxide + Boiled Catalase No reaction occurred. Hydrogen Peroxide + Liver Much bubbling in solution with the release of O<sub>2</sub>. **Table 2: Establishing a Baseline #1** Baseline Calculations (syringe contains KMnO<sub>4</sub>) Readings Final Reading of Syringe 1.2 mL Initial Reading of Syringe 5.0 mL Baseline 3.8 **Table 3: Uncatalyzed H<sub>2</sub>O<sub>2</sub> Decomposition (Syringes Contain KMnO<sub>4</sub>)** Results Final Reading of Syringe 1.3 mL Initial Reading of Syringe 5.0 mL Amount of H<sub>2</sub>O<sub>2</sub> Spontaneously Decomposed 3.7 mL Percent of H<sub>2</sub>O<sub>2</sub> Spontaneously Decomposed in 24 Hours 94.3% **Table 4: Establishing a Baseline #2** Baseline Calculations (syringe contains KMnO<sub>4</sub>) Readings Final Reading of Syringe 1.5 mL Initial Reading of Syringe 5.0 mL Baseline 3.5 **Table 5: Time-Course Determination Potassium Permanganate (mL)** Time in Seconds 10 30 60 120 180 360 Baseline 3.53 53.53 53.53 53.53 53.53 Final Reading 1.31 61.82 02.42 7 Initial Reading 5.05 05.05 05.05 05.05 Amount of KMnO<sub>4</sub> Consumed 3.73 43.23 02.62 3 Amount of H<sub>2</sub>O<sub>2</sub> Used 0.20 10.30 50.91 2 **Effect of Time on the Amount of H<sub>2</sub>O<sub>2</sub> Remaining after an Enzyme Catalyzed Reaction** Exercise 2A: 1.a. What is the enzyme in this reaction? The enzyme in this reaction is the catalase solution. 1.b. What is the substrate in this reaction? The substrate is hydrogen peroxide. 1.c. What are the products in this reaction? The products are water and oxygen gas. 1.d. How could you show that the gas evolved is oxygen? Referring to the equation 2H<sub>2</sub>O<sub>2</sub> + Catalase solution → H<sub>2</sub>O + O<sub>2</sub>, the only gas released is oxygen. 2. How does the reaction compare to the one using the unboiled catalase? Explain the reason for this difference. With the boiled catalase, there was no sign of bubbling because the catalase was denatured by the heat and caused no reaction. 3.a. What do you observe? I observe quite a bit of gas being released from the solution. 3.b. What do you think would happen if the liver were boiled before being added to the hydrogen peroxide? I think that no signs of a reaction occurring would be shown. The catalase that occurs naturally within the liver would have been denatured. 4. From the formula described earlier recall that rate = G y/G x . Determine the initial rate of the reaction and the rates between each of the time points. Record the rates in the table below. **Time Intervals (seconds)** Initial 0-10 10-30 30-60 60-120 120-180 180-360 **Rates** 37/100-3/200-1/150-1/300-1/150-1/6005. When is the rate the highest? Explain why. The rate is the highest in the first ten seconds because the rate decreases as the concentration of the catalase decreases over time. 6. When is the rate the lowest? For what reason is the rate low? The rate is lowest during the last time period of 360 seconds because the most time has passed. The catalase concentration has been reduced and the product amount has increased, blocking the enzymes from reacting with the hydrogen peroxide. 7. Explain the inhibiting effect of sulfuric acid on the function of the catalysis. Relate this to enzyme structure and chemistry. The sulfuric acid's high concentration of H<sup>+</sup> ions gives the acid a low pH. Because enzymes can only function in the pH range of six to eight, the addition of an acidic solution denatures the enzyme, stopping the reaction. 8. Predict the effect of lowering the temperature would have on the rate of the enzyme activity. Explain your prediction. Enzymes generally only work at the between the temperatures of forty and fifty degrees Celsius. Lowering the temperature would slow the reaction until the enzyme is denatured and no longer able to react. 9. Design a controlled experiment to test the effect of varying pH, temperature, or enzyme concentration. **Part One (the effects of a strong acid on enzyme activity):** Add 10 mL of 1.5- % hydrogen peroxide to a 50- mL beaker, and add 1 mL of catalase solution. Mix well and then add 1 mL of (0.5 M) HCl to the beaker. Observe the reaction and record the results. **Part Two (the effects of a neutral solution on enzyme activity):** Add 10 mL of 1.5- % hydrogen peroxide to a 50- mL beaker, and add 1 mL of catalase solution. Mix well and then add 1 mL of (0.5 M) NaOH to the beaker. Observe the reaction and record the results. **Error Analysis:** Several errors could have occurred throughout the experiment. Miscalculations involving numbers and amounts of solutions would have a severe effect upon the results. Mathematical errors may also have occurred. When the catalase arrived, it had melted. Because it is to remain on ice at all times, this may have caused errors. The age of the hydrogen peroxide effected results. For example, when calculating the percent of hydrogen peroxide spontaneously decomposed after 24 hours, new hydrogen peroxide yielded a much higher percentage than the aged hydrogen peroxide. Errors occur in every experiment and that is why it is necessary to repeat an experiment several times for the most accurate results. **Discussion and Conclusion:** Catalase, or enzymes, drastically increases the rate of hydrogen peroxide decomposition. This lab shows how catalase added to hydrogen peroxide leads to the release of oxygen, boiled catalase is denatured, and the presence of catalase in living things can lead to the breaking down of hydrogen peroxide in the body. In the lab it was shown that the natural decomposition hydrogen peroxide is slower than decomposition taking place with the addition of enzymes. If hydrogen peroxide was required to decompose naturally, life could not survive. The addition of catalase increases this decomposition rate allowing life to continue.

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