

# Study of catalase and human amylase



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An enzyme is a catalyst that speeds up the rate of reactions by lowering the activation energy. Each enzyme works better under optimal conditions, which favor the most active shape for the enzyme molecule. Enzyme and substrate concentration, temperature, and pH are environmental factors important by producing the most reaction rate. Also, the different of factors are used to examine the effects of catalase and human amylase. Gas pressure Sensor, Vernier Gas Pressure interface, and Logger Pro are used to collect the pressure of oxygen. At 300C and pH 7 of catalase, the concentration of substrates and enzyme increase and the rates of reaction also rise. However, substrate concentration goes up at 400C and pH 7 of human amylase.

#### Introduction:

Enzymes are catalytic proteins, which control a chemical reaction by increasing the rate of a reaction without being consumed in the process (Enzyme 2007). The catalytic speeds up the chemical reaction by lowering the activation energy, which needed to break the chemical bonds between reactants to combine with other substances. In reactions, the substances at the beginning of the process are called substrate and enzyme can covert substrate molecules to product molecules. At lowering the activation energy barrier, the enzyme has specific substrates to absorb enough energy to reach the transition state (Campbell 2008).

Enzymes are very specific in environmental factors, which affect the reaction rate. The enzyme only works on the substrate that fits the active site and no other (Campbell 2008). The enzyme binds to the substrate called the active

site, which is made up amino acids (Enzyme 2007). The substrates enter active site, and the enzyme changes shape such that its active site enfolds the substrate and catalyze the reaction more easily. Moreover, the more substrate molecules are available, the more often they access the active sites (Campbell 2008). Enzyme works better under optimal conditions including enzyme concentration, substrate concentration, temperature, and pH because they favor the most active shape for the enzyme molecule. Nevertheless, the enzyme will denature and become less efficient the rate of reaction when the conditions get extreme alteration (Campbell 2008).

Each enzyme has a different specific temperature, which affects on the rate of reaction. Most human enzymes have optimal temperature of about 35-40°C. Substrates collide with active site, the kinetic energy of the molecules converts to increase chemical potential energy (Campbell 2008). Therefore, the temperature increases to reach the activation energy and the rate of an enzymatic reaction increase. In addition, if the chemical potential energy rises greater, some of the weak bonds of 3-D shape of the active protein are broken (Enzyme 2007). It will denature of the protein and inactivate the protein. Thus, the rate of reaction decrease when there are too much heat. Just as each enzyme has an optimal temperature, pH level also has an optimal of the range pH 6-8. The pH affects the structure of enzymes by altering basic amino acids or ionization of acidic (Campbell 2008). Changes of pH affect to 3-D shape of the protein, and enzymes become denatured.

Substrate concentration and enzyme concentration affect the rate of reaction of enzymes. When substrate concentration increases, it means that more substrate is added; the reaction rate increases because of using more

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the active site of the enzyme (enzyme 2007). However, when the active site of the substrate is reached at further point, enzymes are saturated to limit reaction rates. Thus, when the substrate concentration is constant, the rate of reaction of is constant. Nevertheless, when the substrates remain constant, the enzyme concentration increases and the rate of reaction also increases until certain limiting concentration.

In the experiment, by different environment factors catalase is used to the actual experiment and human amylase is used to the simulation experiment. Catalase is enzyme present in all living cells. It decomposes hydrogen peroxide into oxygen and water and protects cells( ). Amylase a digestive enzyme made primarily by the pancreas and salivary glands. The primary function of the enzyme amylase is to break down starches in food so that they can be used by the body to trigger specific chemical reactions (Amylase tests. 2006). In addition, both the salivary and pancreatic amylases are  $\alpha$ -amylases in human physiology;  $\alpha$ -amylase is an enzyme that hydrolyses alpha-bonds of large alpha-linked polysaccharides such as starch and glycogen (Amylase tests. 2006).  $\alpha$ -amylase is predicted to work best in the human body temperature of 37 °C and pH from 5.6 to 6.9. If body heat exceeds 37°C by too much cells become impaired or permanently damaged, at lower temperature metabolism decreases without permanent damage until ice crystals form in the cells. Also, if pH is extremely high or low, the activity will decrease for most enzymes. Catalase is predicted in the temperature of 37.50C , the pH under of 8, and the enzyme and substrate concentration is high.

Methods:

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In this experiment, we tested catalase activity by using a yeast solution to determine the effects of enzyme concentration, substrate concentration, temperature, and pH. Moreover, we measured the rate of chemical reaction by producing the pressure of oxygen and breaking down of hydrogen peroxide.

First, we connected the Gas pressure Sensor to the Vernier Gas Pressure interface . Then we connected the Vernier Gas Pressure interface to the laptop. From the Biology with Computers folder, we opened the file “ 06 Enzyme (Pressure)” from Logger Pro program. Then we used a clean large test tube and placed the enzyme solution at the very bottom of the test tube. In addition, we used an Erlenmeyer flask to keep the test tube from moving during the experiment. Also, we used the rubber stopper to insert and create a tight seal onto the test tube, and the stopper valve was in the closed position. We drew up the substrate solution (3% H<sub>2</sub>O<sub>2</sub>+ H<sub>2</sub>O) into the syringe and connected the syringe to the rubber stopper assembly. Later, we opened the valve of the syringe and injected the peroxide solution into the test tube and immediately closed the valve and clicked the collect button on the Logger Pro. While we waited three minutes to collect data, we didn't swirl or move the test tube. When data collection had finished, we removed the rubber stopper assembly and discarded the contents of the test tube. Then, we selected experiment and stored latest run in the Logger Pro software. We clicked on the graph where the data values began to increase, dragged the mouse point to the point where the graph began to look non-linear, and clicked the Linear Fit button to a linear regression. For all the experiments, we used the same processes.

For enzyme concentration, we used 15 mL of water and 15 mL of 3% hydrogen peroxide. We placed one drop of yeast at the bottom of the test tube, and we drew up 6 mL of substrate solution. Following the above procedures, we performed the experiment and determined the rate as the slope of the curves we generated during the experiment. We repeated the experiment using different enzyme concentrations of two, three, four, and five drops and calculated the slopes as mentioned before. Therefore, we recorded the data in table one.

Moreover, for substrate concentration we added 1 mL of water with 5 mL of 3% hydrogen peroxide. We placed three drops of enzyme solution at the very bottom of the test tube, and we drew up the 6 mL of substrate solution into the syringe from the beaker. In the same methods above, we performed the experiment and determined the rate as a slope of the curves during the experiment. We repeated the experiment using different substrate concentrations of 2, 3, 4, and 5 mL of water and 4, 3, 2, and 1 mL of 3% hydrogen peroxide. Consequently, we recorded the data in table two.

Similarly, we used 3 mL of water with 3 mL of 3% hydrogen peroxide for testing the effect of temperature on the enzyme. We added three drops of yeast at the bottom of the test tube and sealed it with the rubber stopper assembly. Then we placed the test tube in the flask half full with ice water and waited for three minutes. Also, at the same we placed syringe in ice for three minutes. We recorded the temperature from the thermometer placed in the ice. After the three minute period, we removed the syringe from the ice and connected it to the rubber stopper assembly, and we followed the general procedures to determine the rate of reaction. We repeated the

experiment using different temperature of room temperature, 30°C water bath, 40°C water bath, 50°C water bath, and 60°C water bath and calculated the slopes as mentioned before. Thus, we determined the data in table three.

Finally, we added 3 mL of the pH 3 solution and 3 mL of 3% hydrogen peroxide. We placed three drops of yeast solution at the very bottom of the test tube, and we drew up the solutions into the syringe. In the same methods above, we performed the experiment and determined the rate as a slope of the curves during the experiment. We repeated the experiment using different pH solutions of pH 5, pH 7, pH 9, and pH 11. As the result, we recorded the data in table four.

#### Results:

Figure 1. Relationship between the rate of reaction and temperature for the human amylase. The data is collected from a simulated experiment by using the program Enzyme Investigation. In this experiment, the rate of reaction of human amylase are based on the constant of substrate concentration of 0.01 mole/L, enzyme concentration of  $1.0 \times 10^{-6}$  mole/L, and pH of 7. Human amylase's optimal temperature is 40°C.

Figure 2. Relationship between the rate of reaction and the effect of pH for the human amylase. The data is collected from a simulated experiment by using the computer software Enzyme Investigations. While temperature at 25°C, substrate concentration of 0.01 mole/L, and enzyme concentration of  $1.0 \times 10^{-6}$  mole/L remain constant, pH changes different level from 1 to 14.

Figure 3. Relationship between the rate of reaction and the effect of substrate concentration for human amylase. The data is collected from a simulated experiment by using the computer software Enzyme Investigations. In this experiment, temperature at 40°C, enzyme concentration of  $1.0 \times 10^{-6}$  mole/L, and pH of 7 remain constant.

Figure 4. Relationship between the rate of reaction and the effect of temperature for the enzyme catalase by using the computer software Logger Pro and determined on a three minutes period each trial. Catalase's optimal temperature is 30°C. In this experiment, the rate of reaction is based on the constant of substrate concentration of 0.5 mL and yeast of 3 drops.

Figure 5. Relationship between the rate of reaction and the effect of pH for enzyme catalase. The data is collected from the actual experiment by using the computer software Logger Pro and determined the experiment on a three minutes period each trial. In this experiment, 3% H<sub>2</sub>O<sub>2</sub> of 3 mL and yeast of 3 drops remain constant, but level of pH varies from 3 to 11.

Figure 6. Relationship between the rate of reaction and the effect of enzyme concentration for enzyme catalase. The data is collected from the actual experiment by using the computer software Logger Pro and determined on a three minutes period per trial. In this experiment, 15 mL of H<sub>2</sub>O and 15 mL of 3% H<sub>2</sub>O<sub>2</sub> remain constant, but more drops for enzyme solutions (yeast) are added.

Figure 7. Relationship between the rate of reaction and the effect of substrate concentration for the enzyme catalase. The data is collected from the actual experiment by using computer software Logger Pro and

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determined on a three minutes period. The effects are based on constant of enzyme solutions of 3 drops.

Discussion: