(34/50)

**Enzyme Catalyst and Reaction Lab**

Sarah Harber

Ziana Young, Aulbree Haskins, Shaila Bowles

**Introduction (4.5/5)**

In this experiment, enzymes (biological catalysts) were tested to see if the reaction would be sped up or slowed down (*i.e.* enzymatic rate). The catalase is found inside of every organism that is exposed to oxygen. All cells require catalase to initiate the breakdown in the cells. The substrate (substance being acted upon) is tested with different amounts of hydrogen peroxide and water. Catalase breaks down hydrogen peroxide into two waters and oxygen. “The active sites are chiral, and enzymes are now well accepted as catalysts” (Wong, Whitesides 1995). The activation site (the site that which the substrate bonds to) is held in place by bonds called hydrophobic (can not come into contact with water), hydrogen (where atoms of hydrogen come together), and ionic bonds (one of the strongest and hardest bond of ions to break). These bonds allow the reaction to occur, “ this means identifying the catalytic residues and their roles and establishing the structure of any reaction intermeddiates and transition site” (Mulholland, 2008) because if none of these bonds existed the activation energy would not be needed as there would be nothing to breakdown. The activation site is specific to one reaction as its shape is unique; however, the active site can become denatured (unable to be used by its original because its shape has changed to fit another) by some factors including pH (scale that measures the amount of hydronium (H+) ions in a solution), salt concentration, temperature, and small molecules. The effects of pH in a solution can cause the reaction to slow down as it does not have the optimal circumstances, and in extreme cases can cause the active site to be changed. Salt concentration can also inflict the enzyme as if the concentration is to the atoms will began to stick together and not form the correct ions to complete the reaction. Temperature is a giant participant in changing the normality of the reaction. The higher the temperature the faster the reaction occurs, however if the temperature goes above the optimal level, it can cause the reaction to become disruptive which makes the reaction come to a halt. Also, if the temperature is dropped too low it can cause the reaction to slow and eventually stop as it would not have enough kinetic energy to continue the reaction. Small molecules also effect the activation time, including an activator (increases rate of reaction) and an inhibitor (decreases the reaction rate). The activator unfolds the enzyme and an inhibitor reduce the stability of the enzyme structure. The reaction rate can further be changed by enzyme concentration/ substrate concentration. “Enzyme, synthetic catalysts, and catalytic antibodies can be all used to perform asymmetric reactions” (Jacobsen, Finnley 1994).

**Hypothesis (3/3)**

If the concentration is higher, then the reaction will occur faster; likewise if the concentration is low, then the reaction will happen slower.

**Materials (2.5/2.5)**

The materials needed for this experiment include potato catalase (previously made by instructor, 100 mL graduated cylinder, filter paper discs, small disposable cups, forceps, hydrogen peroxide, water, and ice. The potato catalase was mixed with varying amounts

of water (See Table 1). Also, a mixture of varying amounts of water and hydrogen peroxide

were used (See Table 2).

**Table 1: Catalase Concentration**

|  |  |  |  |
| --- | --- | --- | --- |
| Enzyme Concentration % | mL of original catalase | mL of cold water | Units per mL |
| 100.00% | 40 | 0 | 100 |
| 75.00% | 30 | 10 | 75 |
| 50.00% | 20 | 20 | 50 |
| 25.00% | 10 | 30 | 25 |
| 0.00% | 0 | 40 | 0 |

Descriptive text here…

**Table 2: Hydrogen Peroxide Dilutions**

|  |  |  |  |
| --- | --- | --- | --- |
| Beaker | Hydrogen Peroxide Dilution | ML of 3% H2O2 | mL of water |
| 0.00% | 0.00% | 0 | 40 |
| 0.75% | 0.75% | 10 | 30 |
| 1.50% | 1.50% | 20 | 20 |
| 2.25% | 2.25% | 30 | 10 |
| 3.00% | 3.00% | 40 | 0 |

Descriptive text…

**Methods (2.5/2.5)**

First step was to gather all materials. Next, I used the prepared potato catalase to begin the procedure of combing different amounts of water and catalase into separate, labeled, 100 mL beakers (refer to Table 1). Next, I set the set each dilution up and used forceps to hold the paper disc in the hydrogen peroxide for five seconds using a stopwatch. After the five secondswas completed, I took the disc out and let it rest of a paper towel for ten seconds, not touching it. Then, the disc was placed at the bottom of the hydrogen peroxide solution, and a stopwatch was used to count the time it took for the disc to rise to the top. All solutions were tested twice. The results were recorded (see Table 3). The next experiment was tested using the dilutions of hydrogen peroxide and water (refer to Table 2). The same experiment was completed using the hydrogen peroxide dilutions were tested twice and the results were recorded.

**Results (9/15)**

**You need to write your results in paragraph format 1st. Ex. 100% enzyme concentration was recorded with an average rate of x. 75% with average concentration rate of y, etc.**

**Table 3: Results of Effect of Enzyme Concentration on Rate of Activity**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Enzyme concentration % | Enzyme concentration (units/mL) | Trial 1  (time in sec. for disc to float) | Trial 2  (time in sec. for disc to float) | Average | Rate |
| 100 | 100 | 3 | 3.2 | 3.1 | 0.32 |
| 75 | 75 | 4.8 | 4 | 4.4 | 0.23 |
| 50 | 50 | 5.8 | 3.9 | 4.85 | 0.21 |
| 25 | 25 | 7.2 | 5.4 | 6.3 | 0.16 |
| 0 | 0 | infinite | infinite | infinite | 0 |

**Descriptive text**

**Table 4: Results of Effect of Substrate Concentration on Enzyme Activity**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Substrate Concentration | Trial 1  (time for disc in sec.) | Trial 2  (time for disc in sec.) | Average | Rate |  |
| 0.0% H2O2 | infinite | infinite | infinite | 0 |  |
| 0.75% H2O2 | 7.6 | 2.73 | 5.17 | 0.19 |  |
| 1.5%H2O2 | 5.8 | 3.2 | 4.5 | 0.22 |  |
| 2.25% H2O2 | 3.5 | 1.5 | 2.5 | 0.4 |  |
| 3.0% H2O2 | 3.8 | 3 | 3.4 | 0.29 |  |

**Descriptive text**

**1.Basic questions of the experiment**

**a. What is the enzyme of this reaction?***Catalase*

**b. What is the substrate of this reaction?** *Hydrogen Peroxide*

**c. What is the product of this reaction?** *Water and Oxygen*

**d. What is the gas produced and how could you demonstrate that?** *Oxygen is released and can be demonstrated by the floating of the disc as the oxygen is left during the reaction.*

**2.How does enzyme activity vary with enzyme concentration?** *As the enzyme decreases, the activity rate goes up.*

**3.How is the rate of the enzyme activity affected by increasing the concentration of the substrate?** *When the concentration of the substrate increases, the reaction takes longer.*

**4.What do you think would happen if you increased the substrate concentration to 40.0% hydrogen peroxide?** *The reaction would not occur.*

**5.How does the changing the substrate concentration compare to changing the enzyme concentration in this experiment?** *When the substrate concentration was decreased, the discs took longer to float; however, when the enzyme concentration was increased, the disc floated to the top faster.*

**Conclusion (7.5/15)**

The conclusion has supported the hypothesis half way by happening faster at higher concentrations in the hydrogen peroxide but not in the potato catalase (refer to Tables 1 and 2). If the potato catalase was at 100% concentration the enzyme began to denature which is shown by its 3.1 seconds to the top. Also, at 0% concentration of the potato catalase, the enzyme becomes denatured and will never float to the top. The greatest rate seen was with the concentration of 25% potato catalase, the disc floated to the top in 0.16 seconds. (Refer to Table 1) The enzyme was denatured by the pH of the catalase. The other reaction, which supported the hypothesis, was shown to have faster times as the concentration was higher. When the concentration was at 3.0% hydrogen peroxide, the disc floated fastest (Refer to Table 2). The disc was pushed up by the oxygen that was being released as the reaction occurred. The significance of the results is that depending on the solution tested results in whether the reaction is sped up or slowed down in higher concentration. Further testing should be done to aid in the results of this experiment as one experiment cannot be proven correct until others are tested.

**Error Analysis (2/2)**

During this experiment, many factors could have led to the incorrect data. The hydrogen peroxide could have stopped functioning correctly on the second trial in both the substrate and catalase experiments. This could have caused the paper to float irregularly. Also, the experiments could have also been effected by the use of tap water instead of distilled water. Distilled water has been filtered out all the contaminants, while tap water can still have some minerals that have not been filtered out. This could have caused the water to have more force against the disc to float.

**Grammar (1/3) 6-8 errors)**

**Citations**

* **Chemistry & Biology Volume 1 & 2, Eric N. Jacobsen and Nathaniel S. Finney, 1994**
* **Enzymes in Synthetic Organic Chemistry, C. H. Wong and G. M. Whitesides, 1995**
* **Computational Enzymology: Modeling the Mechanisms of Biological Catalsyts, Adrian J. Mulholland, 2008**