(47.5/50)

Effect of Enzyme Catalase and Substrate 3.0% Hydrogen Peroxide Concentration on Reaction Rate

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**Introduction (8/8)**

 The main focus of this lab is enzymes, or biological molecules that speed up chemical reactions in living organisms *(Barron, 2014).* Enzymes lower the amount of energy needed to start a chemical reaction, known as activation energy, and is best defined by *Graph 1*. Without enzymes, the activation energy required would be much more than with enzymes, resulting in reactions too slow to support life. Enzymes act as biological catalysts, or substances that accelerate a process without being affected (*Hoefnagels, 2012*). Therefore, enzymes can proceed without themselves being consumed or changed. An enzyme functions by combining with a substrate, a reactive molecule, to form an enzyme-substrate complex *(Castro, 2014)*. This formation begins when a substrate binds to an enzyme’s active site, or space that readily accepts a substrate. The functions of enzymes have such a high degree of specificity because of the unique shape of the active site (Toone *et al.*1990). Once the substrate and the enzyme bind and the reaction is complete, the products are released, and the active site is empty and ready for the next substrate. This occurs repeatedly, allowing for many functions of life to be completed such as DNA replication, protein formation, and food digestion (*Hoefnagels, 2012*).

In optimal conditions, as stated before, enzymes are unaffected; however factors such as temperature, pH, and salt concentration can not only affect its reaction rate but also render an enzyme useless, which is known as denaturation *(Eed, 2012).* In this lab, the effects of enzyme and substrate concentration on the reaction rate are tested. The enzyme catalase, extracted from potatoes, reacts with hydrogen peroxide, the substrate of the reaction, to produce water and oxygen. If the enzyme concentration is increased, then the reaction rate will be decreased; and, similarly, if the substrate concentration is increased, the reaction rate will also be decreased. The reaction rate *r* is found by the equation: *r= 1/t,* when *t* is the time it takes for the reaction to be completed, which is indicated by the floatation of the disk in the substrate solution. This scale is used because it offers precise, comparable results and is more accurate than, for example, counting the bubbles produced in the reaction. The latter method is inconsistent and more susceptible for error than the reliable equation used for this experiment. As recorded in the results section, the larger the fraction, the quicker the results took place. In conclusion, hypothetically, the greater the concentration of enzymes and substrates, the less time it takes for the reaction to be completed.

**Graph 1: Activation Energy Diagram**



*(Graph 1 from Imgarcade.com)*

**Materials (2.5/2.5)**

 The materials used in this lab include a 100 mL-graduated cylinder, 2.1 cm filter paper disks, 50 mL beakers, small paper cups, a blender, water, ice, cheesecloth, and a potato. Five different paper cups consisted of enzyme catalase and water (*see table 1 for dilutions*). Then, five more solutions were made of the substrate 3.0% hydrogen peroxide and water (*see table 2 for dilutions*).

**Methods (2.0/2.5)**

The first step of this lab was extracting the enzyme catalase from the potato. This was done by peeling and cutting a fresh potato and weighing out 50 grams of tissue. The tissue, 50 mL of water, and a small amount of crushed ice were placed in a pre-chilled bender. For thirty seconds on high speed, the contents were mixed evenly, or homogenized. Next, carried out in an ice bath to keep the enzymes from denaturing, the potato extract was filtered, using cheesecloth. The filtrate was, then, poured into a graduated cylinder, and water was subsequently added until the solution reached one hundred milliliters. This solution was separated into 5 beakers emerged in ice in 10 mL increments from 0 to 40 mL, and water was added and mixed accordingly in measurements needed to obtain the desired amount of enzyme concentration. Then the substrate was prepared by pouring 40 mL of 3.0% hydrogen peroxide solution into paper cups. At this point the first independent variable enzyme catalase concentration was ready to be manipulated and tested. The filter paper disk was submerged in the prepared catalase solution for 5 seconds, removed and drained on a paper towel for 10 seconds, and then, once placed at the bottom of the substrate solution the time until the disk floated to the top of the solution was measured. This was repeated twice for each of the 5 different catalase concentration solutions. The second independent variable that was tested was the substrate concentration. To test this factor, five beakers were prepared by combining the five different measurements of the 3.0% hydrogen peroxide and the respective amount of water for each to make the desired concentration solution. The constant in this part of the experiment was the enzyme concentration of one hundred units/milliliters. Now, the disks will be submerged into the enzyme catalase solution for five seconds, drained on a paper towel for 10 seconds, and the time will, again, be measured and recorded from the time the disk is placed at the bottom until it floats to the top. These steps were repeated for each different substrate concentration solution *(Methods by Erol Altug, lab manual)*.

**Results**(13/15)

*Table 1*: Effect of Enzyme Catalase Concentration on Rate of Activity

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Enzyme concentration % | Enzyme Concentration(Units/mL) | Trial 1Time to Float Disc in seconds (*t*) | Trial 2Time to Float Disc in seconds (*t*) | Average | Rate= 1/*t* |
| 100 | 100 *(40 mL of enzyme solution, 0 mL of water)* | 1s | 1s | 1s | 1 |
| 75 | 75*(30 mL of enzyme solution, 10 mL of water)* | 5s | 5s | 5s | 1/5 |
| 50 | 50*(20 mL of enzyme solution, 20 mL of water)* | 15s | 10s | 12.5s | 2/25 |
| 25 | 25*(10 mL of enzyme solution, 30 mL of water)* | 25s | 30s | 27.5s | 2/55 |
| 0 | 0*(0 mL of enzyme solution, 40 mL of water)* | $$\infty $$ | $$\infty $$ | $$\infty $$ | 1/$\infty $ |

(Table from Erol Altug, lab manual; explain what this is briefly)

As represented by *Table 1,* an enzyme concentration of 100% has a rate of 1. An enzyme concentration of 75% has a rate of 1/5. An enzyme concentration of 50% has a rate 2/25. An enzyme concentration of 25% has a rate of 2/55. An enzyme concentration of 0% has a rate of 1/infinity (*Table 1 from Erol Altug, lab manual*).

*Table 2:* Effect of Substrate Concentration on Enzyme Activity

|  |  |  |
| --- | --- | --- |
| Substrate Concentration(From serial dilutions) | Trial 1Time to Float Disc in seconds (*t*) | Rate= 1/*t* |
| 0.0% H202*(0 mL of 3% H2O2, 40 mL of water)* | Doesn’t float | N/a |
| 0.75% H202*(10 mL of 3% H2O2, 30 mL of water)* | 20s | 1/20 |
| 1.5% H202*(20 mL of 3% H2O2, 20 mL of water)* | 8s | 1/8 |
| 2.25% H202*(30 mL of 3% H2O2, 10 mL of water)* | 6s | 1/6 |
| 3.0% H202*(40 mL of 3% H2O2, 0 mL of water)* | 3s | 1/3 |

(Table from Erol Altug, lab manual)

As represented by *Table 2,* a substrate concentration of 0.0% has a non-applicable rate. A substrate concentration of 0.75% has a rate of 1/20. A substrate concentration of 1.5% has a rate of 1/8. A substrate concentration of 2.25% has a rate of 1/6. A substrate concentration of 3.0% has a rate 1/3 (*Table 2 from Erol Altug, lab manual*).

**1. Basic Questions of the Experiment:**

**A. What is the enzyme of this reaction?**

*The enzyme of this reaction is catalase.*

**B. What is the substrate of this reaction?**

T*he substrate is 3.0% hydrogen peroxide.*

**C. What is the product of this reaction?**

 *This reaction results in water and oxygen.*

**D. What is the gas produced and how could you demonstrate that?**

*Oxygen is the gas produced, and it is demonstrated as the gas trapped in the fibers of the disk* *causing it to float (Erol Altug, lab manual).*

**2. How does enzyme activity vary with enzyme concentration?**

T*he enzyme activity increases as the enzyme concentration increases as supported by Table One.*

**3. How is the rate of enzyme activity affected by increasing the concentration to of the substrate?**

*The rate of the enzyme activity increases as the substrate concentration increases as shown in Table Two.*

**4. What do you think would happen if you increased the substrate concentration to 40.0% hydrogen peroxide?**

*If the substrate concentration of hydrogen peroxide were increased to 40.0%, the enzyme activity would most likely occur in less than three seconds.*

**5. How does changing the substrate concentration compare to changing the enzyme concentration in this experiment?**

*Changing the substrate concentration is similar to changing the enzyme concentration in this experiment because as both become more concentrated, the reaction rate is quicker.*

(Questions from Erol Altug, lab manual)

**Discussion and conclusion (15/15)**

These results support the initial statement that the higher the concentration of enzyme or substrate, the quicker the reaction took place. For example, when the concentration of enzyme catalase was 100%, it only took one second for the disk to float, which had a reaction rate of 1, where as it took the solution with only 25% concentration an average of 27.5 seconds, which had a reaction rate of 2/55. Also, the 3.0 % concentration of the substrate hydrogen peroxide took 3 seconds, which had a reaction rate of one-third; where as the concentration of 0.75% took 20 seconds, which had a reaction rate of one-twentieth. These rates are significant because the larger the rate *(1>2/55, 1/3>1/20),* the quicker the reaction took place. This scale serves as a precise measure to assess the time it took for the disk to float to the top indicating the completion of the chemical reaction. Supported by *Table 1* and *Table 2*, the lowest rate (1/ $\infty ) $is seen when the enzyme or substrate is least concentrated. In, contrast, the greatest rate (1) is revealed by a more concentrated solution. In addition, the reaction was not completed when the concentration of either the enzyme or substrate was zero percent. This occurred, because without the enzyme-substrate complex, the activation energy was too much to be fulfilled. To improve this experiment, more time should be allotted to complete at least two trials for each test allowing for more accurate results. Also, two or more people to keep time should be necessary to ensure true results. Overall, the experiment design of Tom Carroll, modified by Erol Altrug, clearly shows how the enzyme catalase and substrate 3.0% concentration affect the reaction rate. As stated by the hypothesis, if the concentration of either the enzyme or substrate is high, the reaction time will be shorter than that of a low concentration. These results show how much quicker essential reactions occur because of enzymes. In conclusion, survival is possible through enzymes and the chemical reactions they catalyze; additionally, enzyme and substrate concentration greatly affect the reaction rate.

**Error Analysis (2/2)**

The lack of time available to complete this lab may have affected the results, as a second trial for the effects of substrate concentration could not be performed. Therefore, the data could not be reaffirmed. Another possible error could have been in the timing of each trial. Each instance the timer began, it appeared somewhat inconsistent.

**Literature Cited Page**

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