Enzyme Lab

Examination of how the concentration of substrate (H_2O_2) affects the rate of Enzyme Activity (Liver).

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Introduction

Enzymes are proteins produced by living things that operate as biochemical catalysts (speed up chemical reactions). These enzymes are formed from a sequence of amino acids that take on a specific three-dimensional structure. Along this structure, there is a spot called the active site, where the substrate "matches" the shape of the site. An analogy can be used to explain this in the way that a lock and key are specific and match each other. Here, the lock is the enzyme's active site while the key is the substrate.

Substrate is the medium or surface that an organism attaches to. This base or foundation is what the enzyme "works" on. The substrate binds with the enzyme's active site, and the enzyme catalyzes, leading to a chemical reaction.

Focused Problem

I will investigate how the concentration H_2O_2 (substrate) will affect the rate of the enzyme-catalyzed reaction (liver).

→ This will be observed by placing a disk of liver into a test tube, and adding 5ml of H_2O_2 into the test tube. The rate of enzyme-catalyzed reaction will be observed by measuring how much water is displaced from the 100ml graduated cylinder, which is filled with water (placed in a water basin). The amount of water displaced is a result of the enzyme-catalyzed reaction. By using different concentrations of the substrate (H_2O_2), we are able to find out just how much a change in the concentration of substrate affects the rate of the enzyme-catalyzed reaction. Therefore, by finding out what concentration produces what results, we can apply this to instances where we may want to alter the rate of enzyme-catalyzed reactions to aid us in a way that is in our convenience.

Hypothesis

The higher the concentration of substrate is, the faster (more water displaced) the rate of enzyme-catalyzed reaction will be. The lower the concentration of substrate is, the slower in the solution, the slower (less water displaced) the rate of enzyme-catalyzed reaction will be.

Independent Variable

The <u>concentration of substrate</u> used to react with the enzyme. The H_2O_2, which is the substrate in this experiment, reacts with the liver, which is the enzyme in this experiment. Different concentrations of H_2O_2 are created by adding calculated amounts of water to the 30% concentration of H_2O_2 to create "weaker" concentrations.

We created a formula to better understand this process of creating different concentrations of substrate. → $[(X * (A/100)) * (100/B)] - X = Z$

⇒ X = amount of H_2O_2 A = original substrate %

B = desired substrate % Z = amount of water added to the value X

Dependent Variable

The rate of enzyme-catalyzed reaction will be observed by measuring how much water is displaced from the 100ml graduated cylinder. This measurement will determine how the concentration of the substrate affects the rate of enzyme-catalyzed reaction.

Controlled Variables

Temperature

The temperature of the water was always held at a constant of room temperature (20 - 25°C). To make sure that the temperature would always stay the same, we placed our experiments at the same place where the same amount of sunlight was hitting. By keeping the experiment at the same place, we were able to keep the temperature at a near-constant level. By preventing a change in temperature, we made certain that an increase/decrease in temperature would not speed up/slow down the rate of enzyme-catalyzed reactions.

Time

The time in which we let our reaction react was a time span of 2 minutes. By keeping this constant time span, we were able to get accurate results of the amount of reaction in that certain time span. The time was very important in our experiment, as the reactions that occurred happened fairly quickly, and an inaccurate reading of time may have made big changes in our results. We kept the 2-minute rule by using a stopwatch.

Volume

The volume and amount water/ H_2O_2 used in our experiment was always held at a constant amount. The 100ml graduated cylinder placed upside down in the water basin contained a 100ml of water each time. In each test tube, 5ml of H_2O_2 was used.

Liver "Disks"

All our liver "disks" that we used we all the same, and constant. Using a hole-puncher, small circles were created from the paper. Each disk was covered on each surface with a fine layer of liver. The amount of liver we used in each experiment was very important, as a change in the amount of liver could change the reaction results drastically. By using these disks, we were able to keep the same surface area in which the enzyme was exposed to the substrate.

Scale

The same 100ml graduated cylinder was used in our experiment. This same 100ml graduated cylinder was used at all times to avoid any errors and complications that may have affected the results or the accuracy of our results in the experiment.

The same sized test tube in which we placed our enzyme and substrate in was always the same size. Once again, we did this to ensure that we got accurate results in our experiment, and we did this to avoid any inaccuracies that may change our results.

Apparatus

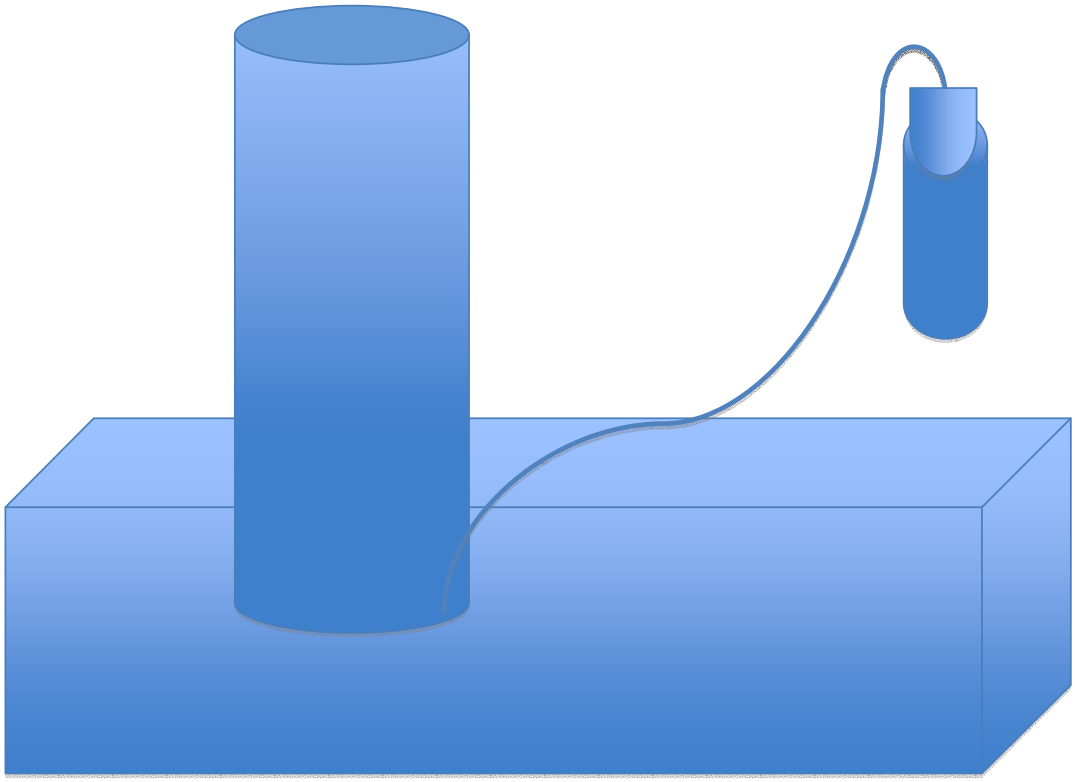
- 100ml Graduated Cylinder

- Water
- Hole-puncher
- Liver
- Mortar & Pestle
- Paper
- Sand
- H_2O_2 (30%)
- Forceps
- Petri Dish
- Test Tube
- Cork
- Tubing
- Water Basin
- Stopwatch

Procedure

1. Gather and prepare all the items from the list of apparatus'.
2. Punch out 5 disks from the paper using the hole-puncher.
3. Sprinkle a tiny portion of sand onto the liver, and grind it using the mortar and pestle.
4. Place each disk into the liver using the forceps and make sure that each surface of the disk is liver-covered. Place these disks into a petri dish as we make the substrate.
5. Fill a water basin up with water. Place the water filled 100ml graduated cylinder upside down into the water basin, letting no air enter, and no water escape.
6. Place one end of the tube that is connected to the cork under the 100ml graduated cylinder.
7. Prepare the desired concentration of substrate. The first concentration is 3% of H_2O_2. This is created using the formula, $[(X * (A/100)) * (100/B)] – X = Z$. This formula is explained previously in more detail under the "Independent Variable" section. For each set of results, different concentrations of substrate are needed (3%, 7%, 11%, 15%, and 19%).
8. Place one disk into a test tube.
9. Add 5ml of the substrate into the test tube, and at the same moment place the cork connected to the tubing over the test tube.
10. Time 2 minutes of the reaction using a stopwatch, and keep an eye on the displacement of water in the graduated cylinder.
11. As 2 minutes is reached, take off the cork from the test tube. The reaction has stopped.
12. Record how much water was displaced from the graduated cylinder.
13. Do this another 4 times for 3% of H_2O_2. After this set is done, repeat the whole procedure again, but this time changing 3% to 7%(+4% each set). Do each set of results 5 times to get an accurate reading of your results.

Setup



Raw Data

Table 1 – Water Displacement measurements in 100ml Graduated Cylinder from enzyme-catalyzed reaction using different concentrations of substrate (+4%) over a time period of 2 minutes

Substrate Concentration /±2 ml	3%	7%	11%	15%	19%
	Total displacement of water in graduated cy linder /ml ± 2ml				
Trial 1	46	52	58	56	58
Trial 2	44	50	54	62	58
Trial 3	46	52	58	56	62
Trial 4	40	50	62	60	54

Trial 5	46	46	58	58	62
Average	**44.4**	**50**	**58**	**58.4**	**58.8**

⇒Table 1 shows our collection of data, in which we recorded the total displacement of water in the graduated cylinder for each trial and concentration of our experiment (5x5). The average of these results is show in the last row.

Table 2 – Standard Deviations of Error Bars for Water Displacement measurements in 100ml Graduated Cylinder from enzyme-catalyzed reaction using different concentrations of substrate (+4%) over a time period of 2 minutes

Substrate Concentration /±2 ml	**3%**	**7%**	**11%**	**15%**	**19%**
Standard Deviation	**2.607680962**	**2.449489743**	**2.828427125**	**2.607680962**	**3.346640106**

⇒Table 2 shows us the standard deviation of our results. This is used to show some of the uncertainties/errors in our results that may occur.

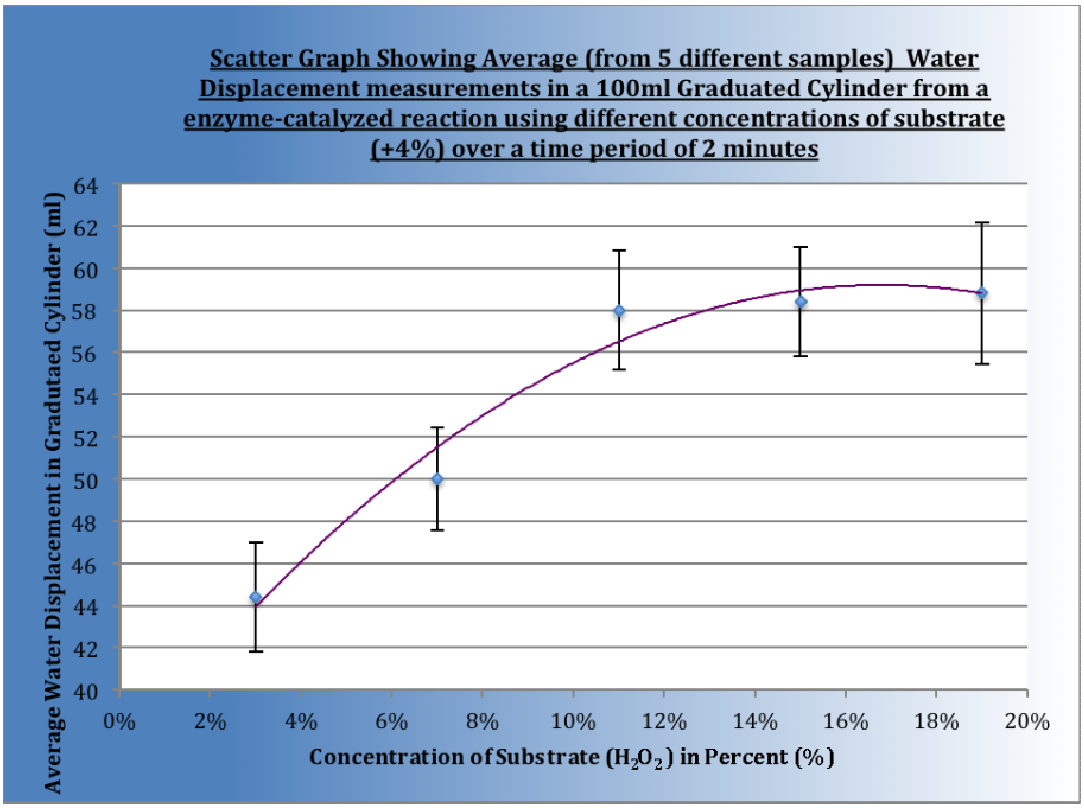
*Errors and Uncertainties

As stated in our tables, the amount of water displacement measured is accurate ±2 ml as the graduated cylinder we used had markings each 2 ml, making it hard to get the exact number at times. This is due to the fact that we used a 100ml graduated cylinder to match the quantity of our displacement results.

2 minutes was timed from the instant the cork was put on the test tube. Although we planned it so that we could put the cork on immediately when the reaction started, it is impossible to get the timing perfectly right every time. Therefore the 2 minutes we used to measure the rate of enzyme-catalyzed reaction is around ±0.5 seconds off.

By using the standard deviation figures (Table 2) gained from our test results in Table 1, we represent in uncertainties in our experiment that may have occurred.

Graph



The Scatter Graph above shows us the average water displacement in the Graduated Cylinder over different concentrations of the substrate. These figures are taken from Table 1. The error bars that are placed on each average are figures of standard deviation used from Table 2. The purple line is a polynomial line, which shows the general curve that this scatter graph is showing (similar to a line of best fit).

By looking at the graph above, it is clear that enzyme-catalyzed reaction rate (water displacement in graduated cylinder) increases as the concentration of the substrate increases. But it is also clear that at a certain point, around 15%, the line does not go any further up, but it maintains at the same level. The rising line has reached a plateau. This suggests that at a certain point, the rate of enzyme-catalyzed reaction reaches a maximum point, and this rate then is stable (no increase, no decrease).

Conclusion

From the data that was collected in Table 1, it is evident that as the concentration of the substrate increases, so does the rate of reaction. This general trend is deduced from Table 1, as the substrates with smaller concentrations have a smaller amount of water displaced in the graduated cylinder. As the concentration of the substrate increases, the larger the amount of water displaced in the graduated cylinder. This is due to increased molecular collisions. The higher the concentration of the substrate, the more molecules there are to collide, making it react faster. From this first table though, it is also evident that at a certain point (11%), the reaction rate does not

increase as much, if any at all. It is as if a limit is reached, and the figures have reached a plateau. This is due to the fact that all enzymes have a certain maximum rate at which they can work. All the active sites are taken, and no extra molecules will increase the reaction rate. This explains the reason as to why the water displacement levels hardly increase from 11% - 19% substrate concentration.

Table 2 shows the standard deviation of our figures collected in Table 1. This table reminds us that there will always be a certain level of uncertainty in our results collected from the experiment. The goal is to reduce these errors and make the experiment as accurate as possible.

The Graph shows the average water displacement for each concentration of substrate with error bars regarding the standard deviation of the figures. This polynomial line like a line of best fit passes through each error bar, meaning that our results were fairly accurate. Once again, like explained in Table 1, the graph shows us that reaction rate increases due to more collisions with higher concentrations of substrate. But a maximum is reached, where the reaction rate increases no further. Every enzyme molecule is working as fast as possible. No more substrate can increase the rate of reaction. This is shown by the straight line or plateau on the graph after a rapid increase.

My hypothesis was wrong, where I thought that reaction rate would keep increasing as substrate concentration increased. From the table and the graph, it is clearly shown that a certain "maximum" is reached, where reaction rate cannot increase anymore, even with extra added substrate. This maximum rate is reached, where every enzyme molecule is working at a 100%, and there are no active sites where reactions can take place.

Weaknesses/Limitations

One of the main weaknesses that may have made our results a little inaccurate was the amount of liver placed on each surface of the paper disks. Although we used these paper disks to "control" the amount of liver used for each experiment, some disks had a slight bit more liver on the surface than others. The stickiness of the liver was a factor to this unevenness of liver on each disk. As there may have been more liver in some trials than others, more reaction was able to take place, leading to some of the wide range of results. This also increases our standard deviation figures, meaning we were not able to be as precise as we wanted to be.

Another weakness was our method of closing the cork on the test tube over the reaction. As my partner dropped the substrate over the enzyme in the test tube, my job was to close the test tube to trap and record all the reaction that was taking place. As I am not a robot, some times I was not able to close the test tube off as quickly as other times, leading to an escape of some of the reaction. Obviously we were not able to record this "lost" reaction, therefore some of our results do not reflect the true amount of reaction taken place.

Improvements

To control the amount of liver used in the experiment, a good way may be to get frozen 1 cubic centimeter cubes of liver. This way, we are certain that one trial has no more liver than another. By being in this frozen state, we are then able to leave it for a

few minutes, and it would still be able to use. A 1 cubic centimeter cube would have a definite mass and volume, therefore making results much more accurate and trustworthy.

A better method to close the cork over the reaction in the test tube may be to have the cork on over the cubic centimeter of liver from the first place. Another opening at the top of the cork would lead to a syringe, where 5ml of H_2O_2 is placed. When the H_2O_2 is injected, we can start timing for our results without losing any of the reaction to the outside air. All the reaction would stay inside of the test tube. By doing this, we can be much more certain that no other "factors" are affecting the rate of reaction other than the concentration of the substrate. '