

Examining the Effect of Substrate Concentration on the Rate of Enzyme Activity

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-Introduction

The role played by enzymes is essential to perpetuate life. Enzymes are globular proteins made up of long chains of amino acids. These specific proteins act as catalysts to accelerate chemical reactions, due to the fact that most chemical reactions in biological cells occur too slowly. However, they do not only act as catalysts. When the human body has an abundance of enzymes, it can protect itself and repair the damage from countless diseases. Thus, without enzymes, we obtain diseases earlier in life; we also age at a faster rate, and acquire physical impairments and mental retardation as our bodies' decadence. As a result, a human being or an animal cannot live without enzymatic function. Enzymes are of great importance to our wellbeing, and are essential to our survival.

Examining the different factors that affect the rate of enzymatic activity is important and necessary. This is due to the fact that the rate at which enzymes work is vital to the homeostasis of a living organism. Homeostasis is crucial to a living organism; if the rate enzymatic activity were to happen too quickly, or too slowly, the internal balance of the organism would be distorted, which would result in harmful symptoms.

-Aim

The aim of this investigation is to examine whether there is a distinguishable trend between the rate of enzymatic activity and varying concentrations of substrates. Simply,

How will altering the substrate concentration affect the rate at which enzymes catalyze reactions?

This investigation will consist of a set-up that will allow us to accurately manipulate the substrate concentration of hydrogen peroxide, and adding the different concentrations to a controlled amount of pork liver that consists of catalase, a naturally occurring enzyme. In this case, hydrogen peroxide is the substrate. The rate at which this substrate of the enzyme is broken down will be calculated by the measuring the temperature by placing a thermometer near the core of the reaction. The highest temperature at each of the different substrate concentrations will be recorded and used. This particular situation will aid us into discovering a possible trend between the rate of enzymatic activity and substrate concentration.

We predict that as we increase the concentration of substrates added to the enzyme, the rate of enzymatic activity will increase. Thus, we anticipate that the maximum temperatures will increase as we increase the substrate concentration levels. We believe this because as substrate concentration increases, the more reactions will take place at once, meaning, the amount of substrates attaching to the active sites at a particular moment will be proportionally greater as substrate concentration increases.

-The Independent Variable

The independent variable in this investigation is the substrate concentration. We will manipulate the substrate concentration mixing controlled amounts of hydrogen peroxide solutions to create different concentrations.

-The Dependent Variable

The dependent variable in this investigation is enzyme activity. This will be measured by calculating the maximum temperature of each of the reactions when different substrate concentrations are added to a controlled amount of pork liver.

-The Controlled Variable(s)

There were numerous factors that had to be kept constant. The first one was the temperature. If left varied, the temperature of the atmosphere may have distorted the data. This particular problem was remedied by conducting this experiment in the same room at the same temperature.

Without even starting the experiment, we knew that the type of substrate used had to be constant throughout. In this case, we used hydrogen peroxide, a naturally occurring substrate, throughout the whole experiment. This is because this particular substrate was the substrate for the enzyme catalase in the liver. The use of different substrates may have produced different results.

The pH levels of the water used to create the solutions were to be kept constant. We made sure that the water we used was pH 7 by using pH indicators to clarify the level. Furthermore, we use distilled water, thereby increasing the possibilities of having matching pH levels in all of our solutions.

The apparatus used for each setup was kept constant. Using different graduated cylinders and/or thermometers at different times during the experiment may have corrupted the data.

The amount of enzyme used for each setup had to be as equal as possible. This was absolutely crucial, because if different amounts of enzymes were used throughout the experiment, the data might have been skewed by the variance in catalase for each time the experiment was conducted. To remedy the problem, we manually punched out paper discs with a hole-puncher, dipped them into a grounded liver, and used them as measurable units of enzyme concentration. Eventually, we allotted three discs per setup (each reaction).

Another factor we had to keep absolutely constant was the type of liver used. For example, cow liver and chicken liver would have both reacted and broken down the hydrogen peroxide, albeit at different rates. Hence, we decided to use chicken liver throughout the whole experiment.

The amount of substrate mixture catalyzed by the enzymes was kept constant as well in order for concentration rather than amount to be the factor that manipulated the data. We decided that three drops from a specific dropper (controlled) of solution was to be used for each setup.

-Apparatus

1. Paper
2. Pork liver
3. Pestle/mortar

4. Tweezers
5. 30% concentration hydrogen peroxide
6. Distilled water
7. Thermometer
8. Dropper
9. Graduated Cylinders (300ml, 10ml, 50ml)
10. Glass tubes
11. A particular stand for the glass tubes
12. Hole-puncher
13. Beakers
14. Syringe

-Manipulation of the Independent Variable

We will prepare five substrate solutions of varying substrate concentration.

-Diagram

-Method ¹

¹ All numbers in this investigation are calculated to three significant figures. Also, to make sure we collected accurate data, we decided to pursue the five-by-five method, meaning that we would test each setup a minimum of five times.

Before the experiment was conducted, we had to prepare five substrate solutions of varying substrate concentration. Because we were only able to attain a 30% hydrogen peroxide solution, we had to add certain amounts of distilled water to reach a certain hydrogen peroxide/distilled water ratio that would have allowed us to manipulate the percent of hydrogen peroxide levels in each of the solutions created. Simply, we were to add certain amounts of water (mL) to a controlled amount of 10mL of hydrogen peroxide solution with a 30% concentration. Using our mathematical knowledge, we came up with the following table:

Table 1: Mixtures of substrate Solutions

Concentration of Hydrogen Peroxide in mixture (%)	Amount of distilled water required to be added to 10mL of Hydrogen Peroxide solution with a 30% concentration (mL) ± 0.5 mL	Total Amount of the solution (mL) ± 0.5 mL
30	0	10
25	2	12
20	6	18
15	18	36
10	72	108
5	540	648

With the information in the table about ready and mathematically accurate, we were ready to start our experiment.

1. Place pork liver into a mortar.
2. Grand the pork liver against the mortar with a pestle until a paste is created.
3. Punch out as many paper discs as needed using a hole-puncher and paper.
4. Using a pair of tweezers, place three paper discs in the liver paste -- front and back.
5. Obtain a 100mL graduated cylinder, and place 10mL of 30% hydrogen peroxide into the graduated cylinder.
6. Obtain the amount of distilled water required to make a 30% hydrogen peroxide solution by placing a correct amount of distilled water into a graduated cylinder.
7. Obtain a beaker, and mix the steps 5 and 6 into the same beaker to create a solution.
8. Place the three discs covered with pork liver paste into a small glass tube with a supporting stand to keep the glass test tube vertical.
9. Place the tip of an electronic thermometer to the bottom of the test tube, where the three discs are located.

10. Place a dropper into the solution created in step 7 and suck an amount of the solution that will allow three drops of that solution to be ejected out of that dropper and onto the three discs inside the glass test tube.
11. Pour three drops of the solution, using the dropper, onto the three discs at the bottom of the test tube.
12. Watch the temperature that's written on the thermometer, and record the highest temperature reached before the reaction starts to cool down.
13. Repeat steps 4 to 12 four more times.
14. Repeat step 13 with four other batches of substrate solution.

-Results

Table 1: The maximum temperature (± 0.1 °C) reached after three drops of a solution containing 30% hydrogen peroxide concentration was added to three paper discs saturated with pork liver paste

Trial <i>n</i>	Maximum Temperature after 3 drops of a solution containing 30% hydrogen peroxide substrate concentration / ± 0.1
Trial One	57.4
Trial Two	60.5
Trial Three	58.6
Trial Four	57.3
Trial Five	54.7

Table 2: The maximum temperature (± 0.1 °C) reached after three drops of a solution containing 25% hydrogen peroxide concentration was added to three paper discs saturated with pork liver paste

Trial n	Maximum Temperature after 3 drops of a solution containing 25% hydrogen peroxide substrate concentration / ± 0.1
Trial One	57.1
Trial Two	56.7
Trial Three	57.4
Trial Four	56.9
Trial Five	57.6

Table 3: The maximum temperature (± 0.1 °C) reached after three drops of a solution containing 20% hydrogen peroxide concentration was added to three paper discs saturated with pork liver paste

Trial n	Maximum Temperature after 3 drops of a solution containing 20% hydrogen peroxide substrate concentration / ± 0.1
Trial One	53.9
Trial Two	52.4
Trial Three	53.6
Trial Four	51.2
Trial Five	52.2

Table 4: The maximum temperature (± 0.1 °C) reached after three drops of a solution containing 15% hydrogen peroxide concentration was added to three paper discs saturated with pork liver paste

Trial n	Maximum Temperature after 3 drops of a solution containing 15% hydrogen peroxide substrate concentration / ± 0.1
Trial One	41.7
Trial Two	40.7
Trial Three	41.5
Trial Four	42.5
Trial Five	45.3

Table 5: The maximum temperature (± 0.1 °C) reached after three drops of a solution containing 10% hydrogen peroxide concentration was added to three paper discs saturated with pork liver paste

Trial n	Maximum Temperature after 3 drops of a solution containing 10% hydrogen peroxide substrate concentration / ± 0.1
Trial One	31.5
Trial Two	30.6
Trial Three	32.5
Trial Four	33.2
Trial Five	31.9

Table 6: The maximum temperature (± 0.1 °C) reached after three drops of a solution containing 5% hydrogen peroxide concentration was added to three paper discs saturated with pork liver paste

Trial <i>n</i>	Maximum Temperature after 3 drops of a solution containing 5% hydrogen peroxide substrate concentration / ± 0.1
Trial One	27.0
Trial Two	26.7
Trial Three	27.9
Trial Four	28.1
Trial Five	28.9

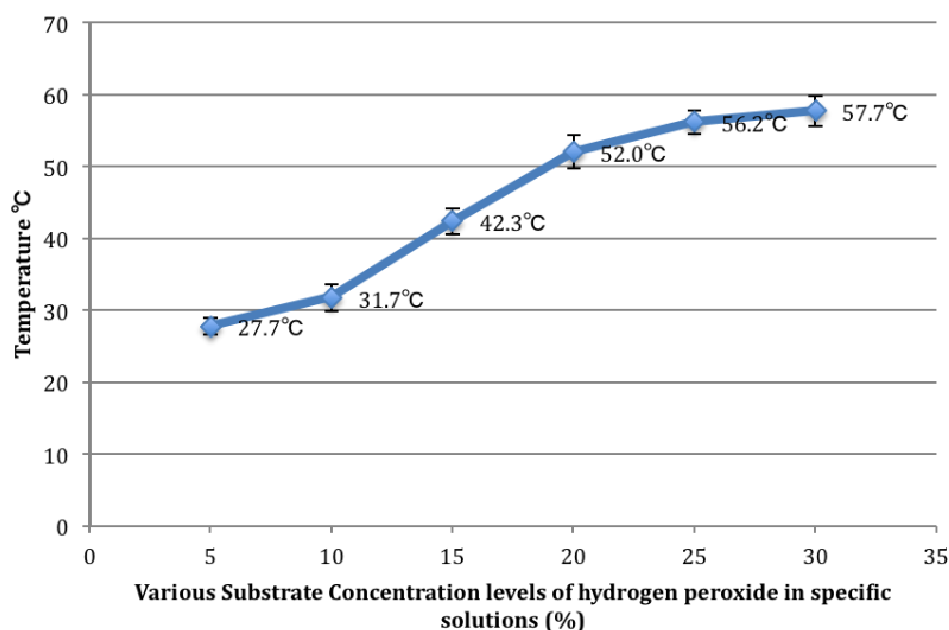
Next, the average of the maximum temperatures reached at each trial was calculated for each setup (different substrate concentration levels).

Table 7: The average maximum temperature (± 0.1 °C) reached after 3 drops of a solution containing various hydrogen peroxide substrate concentration levels were added to three paper discs that were saturated with pork liver paste

Concentration of Hydrogen Peroxide in mixture (%)	The Average Maximum temperature reached after 3 drops of a solution containing <i>n</i> % hydrogen peroxide substrate concentration / ± 0.1
30	57.7
25	56.2
20	52.0
15	42.3
10	31.7
5	27.7

The data above was recorded while the experiment was carried out. Once we collected sufficient and relevant data, we were able to calculate the average temperature reached (table 7), and afterwards were able to reflect the data onto a graph.

Line Graph displaying the average maximum temperature ($\pm 0.1^\circ\text{C}$) reached at each of the various substrate concentration levels (%) and its corresponding error bars showing the standard deviation of the data



The line graph above shows the average maximum temperature reached at each of the various substrate concentration levels tested. The six points were connected with specific linear lines to clearly illustrate the shape of our results. Evidently, the higher the concentration level increased the maximum temperature of the reaction. However, as seen on the graph, error bars showing the standard deviation of the data are evident. Before we constructed this graph, we calculated the standard deviation of each of the five trials for each setup.

The following equation allowed us to calculate the standard deviation for each of the other substrate concentrations.

$$s = \sqrt{\frac{\sum X^2 - \frac{(\sum X)^2}{n}}{n - 1}}$$

Using the standard deviation equation written previously, we came up with the following table.

Table 8: The average maximum temperature reached (± 0.1) at each substrate concentration level (%) and corresponding standard deviations that are accurate to three significant figures

Substrate Concentration (%)	Average maximum temperature reached ± 0.1	Standard Deviation (accurate to three significant figures)
30	57.7	2.11
25	56.2	1.67
20	52.0	2.32
15	42.3	1.78
10	31.7	1.87
5	27.7	1.19

-Conclusion

The contents of Table 7 were derived from the raw data found in tables one through six. Prior to experiment, we decided to pursue the five-by-five method, meaning that we would try each setup five times. This is because we wanted to collect sufficient and relatively accurate data. Upon placing an electronic thermometer near the core of each reaction, the maximum temperature of each reaction was collected.

This particular investigation was conducted to study if there was a recognizable change in the rate of enzyme reactions when the level of substrate concentration was manipulated. Again, we chose to tackle this problem by calculating the maximum temperature reached during each reaction. Because we chose to test six different substrate concentration levels, we ended up with six tables, each with five trials. The average was calculated, and was recorded in Table 7. Table 7 was then reflected onto a line graph.

By examining the line graph, there was a sharp increase in temperature between the substrate concentrations 10% and 15%. The temperature continued to rise at a relatively steady rate until the substrate concentration level at 20%. At this point, the rate of enzyme activity started to decrease; it still rose, however. From 25% substrate concentration, the temperature certainly decreased even more to the point where it almost completely flattens out and takes a near plateau shape. Hence, we can deduce that:

As substrate concentration increases, the rate of enzyme activity increases until it levels out at approximately 25%.

This particular trend can be explained logically. As substrate concentration increases, the amount of hydrogen peroxide molecules available to be combined with the enzyme increases. That is why the overall effect is an increase in the rate of activity (hence, an increase in the maximum temperature). However, there is a key factor that must be considered. Since the amount of enzyme is being kept constant throughout the whole investigation, at one point, all of the active sites on the enzymes will be occupied by substrates (lock and key). Thus, at one point, increasing the amount of substrate will not affect the rate of enzyme activity. This explains the slowing of the increase in reaction rate. When all active sites are occupied, the rate of enzyme activity decreases, thus the temperature decreases, and the rate consequently flattens out.

In conclusion, our hypothesis was partially correct. We hypothesized that as substrate concentration increased, the rate of enzyme activity would increase. As we deduced from our results, we understood that the rate did indeed increase as we increased the substrate concentration. However, we overlooked the fact that the rate would eventually level out due to occupied active sites. Overall, the investigation was a success.

-Weaknesses/Limitations

This investigation was indeed a success and our “question” was clearly answered. However, there were many evident weaknesses, and opportunities for improvement.

One first obstacle we encountered was probably the hardest to try and overcome. The insertion of enzymes proved to be fickle and inconsistent. After getting the enzymes onto respective discs, inserting them into the glass test tubes was quite the frustrating taste. Occasionally, the discs would stick onto the side of the tubes, which gave us no choice but to try and slide the discs down with additional materials. This left smears of liver on the side of the test tubes; as a result, it may have changed the amount of enzyme on each disc drastically, or moderately. A method to help improve this problem would be to use a beaker instead of a skinn y and small glass test tube.

Another big obstacle we encountered was the collecting of the enzyme. After grinding the animal liver in a mortar to make it into a paste, we dipped paper discs to extract a controlled amount of enzyme onto the discs. However, because of the level of inefficiency in this method, the three discs were almost always carrying different amounts of liver paste. This could have been remedied by using a much more effective way of taking liver paste out of the mortar.

In the process of mixing batches of hydrogen peroxide to produce solutions containing difference substrate concentrations, drawing the solution with a syringe and injecting the fluid into another beaker may have left some of the solution behind on the sides of tubing or the syringe. Hence, the amount of substrate concentration needed for each setup may not have been exact. A method of improvement would be to find a simpler way to transfer the liquid.

We came across a particular systematic error in our investigation: the thermometer. As written, the electronic thermometer used measured accurately, there was always that $\pm 0.1^{\circ}\text{C}$ possibility of error. To remedy this issue, we could have used more expensive equipment. Measuring the temperature was a vital part of our investigation; therefore, if we had used a thermometer that was a lot more accurate than the one we used, it may have produced more precise results.

The final weakness of our investigation was inevitable human error. Our experiment required liquids to be measured accurately using beakers and graduated cylinders. The only way for us to know whether a particular volume was correct or not was to confirm it ourselves by using our mere sense of sight. If our data was skewed in any way because of this possible weakness, then we could have used our eyes more efficiently. For example, if our eyes were level to the beakers, the amounts added could have been a lot more accurate.