

Determining the rate of action of an enzyme essay sample



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Aim:

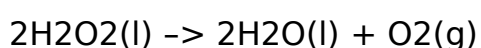
The purpose of this investigation is to experimentally determine the effect a change in substrate concentration, Hydrogen Peroxide, (H₂O₂) will have on the rate of action (measured in time taken to surface due to buoyancy of O₂ gas) of an enzyme (Catalase).

Background:

The human body functions due to the action of enzymes which speed up chemical reactions within the body by lowering the activation energy of a reaction.

Hydrogen Peroxide is produced in large amounts within the human body, most notably within the liver. Hydrogen Peroxide is a by product of various reactions within the body. It is a toxic chemical and in high concentrations is poisonous to the body systems. Its prolonged presence would ultimately destroy the body's cells by inhibiting metabolic reactions. As a result, the body must find a way of ridding itself of this detrimental Hydrogen Peroxide. It does this through the implementation of the enzyme Catalase.

Catalase, like all enzymes adheres to the induced fit relationship between Hydrogen Peroxide and itself. Accordingly it acts only on H₂O₂ initiating a reaction, via lowering the activation energy, that results in the breakdown of this harmful substance into harmless substances which can be either used or excreted by the body. The Hydrogen Peroxide breaks down to form water and oxygen. This reaction can be seen below:



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The Catalase molecules are extremely effective as one molecule of the enzymes can interact with six million molecules of hydrogen peroxide in one minute.

Hypothesis

It is hypothesised that the rate of action of the Catalase will increase proportionally with the increase of substrate availability; however this increase will slow until it finally plateaus at a point known as the optimum reaction rate. At this point, useful substrate concentration is at its maximum. This is achieved when the amount of useful enzyme present cannot react with any more substrate and hence the graph will cease to increase, but rather stay at the same level if the substrate is continually renewed

This is based on the fact that though an enzyme, being a catalyst, is not used up in a chemical reaction it can only bind with a maximum number of substrates, and convert these into H₂O and O₂.

The concentration of substrate will determine the speed of the reaction as it controls how many Hydrogen peroxide molecules are available to be converted by the enzymes and hence the production of O₂. This gas is ultimately the telling measure of the rate of reaction as it controls the buoyancy of the paper sheet and hence how quickly it surfaces after sinking.

Variables

Variable

Method

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Error

Controlled:

The amount of enzyme used

A sheet doused in liver juice will be used for each test

Excess liver bits

The amount of distilled water used to distil the solution

20mL of Distilled water will be used to distil the solution

±0.5mL

Size of sheet of filter paper

The filter paper sheets will be 1cm x 1cm

±0.5mm

Manipulated:

The concentration of substrate present in the reaction

Concentrations of 3, 4, 6 and 8% will be used

0.5%

Respondent:

The time taken for the filter paper sheets to surface

Timing the sheets from when they reach the bottom of the beaker until the surface

0.5sec

Extraneous:

The temperature of the reaction environment

N/A

May increase reaction speed

The humidity of the reaction environment

N/A

N/A

The pressure of the reaction environment

N/A

N/A

The possible effects of soiled equipment on pH

N/A

Denature enzymes and decrease number of Catalase molecules available to convert H₂O₂

The exact amount of catalase in the Liver juice

N/A

N/A

Denaturation of catalase due to natural causes and age

N/A

N/A

Materials

Amount

Equipment

Error

25mL Measuring Cylinder

0.5mL

Mortar and Pestle

Impurities on cleaning

15g

Liver(Fresh)

Temperature may denature catalase

10mL

Hydrogen Peroxide (H₂O₂) 3%

0.5%

10mL

Hydrogen Peroxide (H₂O₂) 4%

0.5%

10mL

Hydrogen Peroxide (H₂O₂) 6%

0.5%

10mL

Hydrogen Peroxide (H₂O₂) 8%

0.5%

200mL

Distilled Water

Impurities on distillation

Stop Watch

0.005

Tweezers

Improper cleaning- contamination

50mL Beaker

0. 5mL

10

Filter Paper (1cm x 1cm)

0. 05cm

Beaker Marking Pen

Pipettes

0. 05mL

Scissors

Ruler

0. 05cm

Method

1. Collect materials.
2. Crush Liver in the mortar and pestle until juice is present.
3. Using the marker, indicate on one (1) test tube and one (1) beaker the concentration of 3% Hydrogen Peroxide.

4. Fill the test tube with 25mL of Distilled water.
5. Fill the beaker with 10mL of 3 % Hydrogen Peroxide.
6. Using the pipette take a sample of the 3 % Hydrogen Peroxide and drop five (5) drops into the test tube.
7. Lightly swirl the test tube to combine the two solutions.
8. Using the tweezers, pick up one of the filter paper squares and immerse it in the crushed liver in the mortar and pestle.
9. Using the second pair of tweezers remove any solid forms of liver from the filter paper.
10. Place the catalase doused filter paper into the test tube.
11. On contact with the solution, begin the stop watch. The paper will slowly sink to the bottom, O₂ bubbles will begin to form and lift the paper back to the surface
12. Stop the timer when the paper reaches the top of the test tube.
13. Record this time.
14. Repeat steps 3- 13, replacing the 3% Hydrogen Peroxide with each of the other concentrations.

Data Collection

Results:

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Figure 1 shows the time taken for each of the filter sheets to surface in seconds

Concentration(C) (%)

0.5%

3%

4%

6%

8%

Time(T)

(seconds)

0.5

Trial One

35

22

16

14

Trial Two

30

18

12

10

Trial Three

29

18

13

11

Total (Average)

31

19

14

12

Qualitative Results:

* On contact with the solution, small O₂ bubbles began to form on the surface of the Liver.

- * These small bubbles collected around the sheet of filter paper
- * The filter paper slowly began to rise from the bottom of the beaker
- * No other physical characteristics observed throughout reaction

Graphs:

Graph 1 shows the indirectly proportional relationship between time and concentration. Graphically represents 1 over the values in figure 1 .

Relationship: C is proportional to 1/T

Graph 2 shows the extended graph of the function which accurately models the data in graph 1 converted to a rate.

Data Processing:

Data Processing

Sample Calculation Average:

Mean(x) = $\sum(x) \text{ (min)} / (\text{Number of } x)$

Where

Mean= the average value of the selected data

X= data values

\sum = the total sum of the data values

Number of x= the number of values in the x data series

$$\text{e. g. Mean} = (35 + 30 + 29) / 3$$

$$= 31 \text{ seconds}$$

Sample Calculation Rate of Reaction

$$\text{Rate} = 1 / \text{Time}$$

$$\text{Rate (molecules of buoyancy/sec)} = 1 / \text{Time(sec)}$$

Calculation [Whole Average]

$$\text{Rate} = 1 / 31$$

$$= 0.032258 \text{ molecules/sec}$$

Figure 2 shows one divided by the time taken to surface so as to achieve a model of comparative rates

Concentration(C) (%)

0.5%

3%

4%

6%

8%

1/Time(T)

(seconds)

0.000005

Trial One

0.028571

0.045455

0.0625

0.071429

Trial Two

0.033333

0.055556

0.083333

0.1

Trial Three

0.034483

0.055556

0.076923

0.090909

Total (Average)

0. 032258

0. 052632

0. 071429

0. 083333

Conclusion

Statement

The rate of reaction between the Catalase and the hydrogen peroxide increase proportionally to the concentration of substrate. This continued until 8% was reached, at this point the rate was maintained. The greater the concentration of substrate the greater number of molecules was available to be converted into water and oxygen gas. Therefore the rate of reaction increased in the order of 3%, 4%, 6% and the fastest was 8%.

Explanation

The purpose of this investigation was achieved in the rate of action of the Catalase increased proportionally with the increase of substrate until the optimum reaction point was reached at which point the rate began to plateau. To account for error due to measuring falsities and equipment interaction the graph of concentration vs. rate was drawn. The rate of action was extrapolated using a log based trendline to compensate for the curving

function. This extrapolation proved that a concentration of 8% H₂O₂ solute will reach the optimum rate of action of Catalase.

Graph 1 shows that in all cases the concentration increase resulted in a quicker surfacing time for the filter sheets doused in liver juices. If it is assumed that all sheets have equal numbers of Catalase molecules then it can be concluded that to reach the maximum rate of reaction a concentration of 8% of H₂O₂ molecules must be present in the system.

Evaluation:

Various assumptions were made when conducting this experiment, which are obvious when considering the large variation in results for the three trials. Clearly, since these results were significantly different extraneous variable alone cannot account for the entire discrepancies between values. There was a methodical error in making assumptions about concentration accuracies. It was false to presume that the concentrations marked on the bottles were entirely correct, as well that they had not been tampered with or through other courses been altered. The assumptions were rough approximations based on little evidence; hence they quite possibly are the reason for the slightly skewed results.

Also, when conducting the experiment considerations were not made as to the consistent quality of the liver/ Catalase. The condition of the liver was not monitored throughout the process which suggests that the enzymes may have been effect by temperature, humidity, and other outside variables. This could results in the denaturisation of the enzymes which would later be used

in the experiment and would skew the results, altering the experiments controlled variable and ultimately providing false results.

In the future, to account for these errors, a mass spectrometer should be employed so test the exact concentrations of Hydrogen Peroxide, as well as the quality of the distilled water. More drops of substrate could have been added to provide a clearer result. Rather than a mortar and pestle being used to crush the liver, a blender would be more appropriate to ensure a better consistency and eliminate the effects of a changing enzyme concentration. Finally, the results should be duplicated in order to improve the quality of the mean results and to increase the R² value of the graph.