Determining the rate of action of an enzyme

Skills to be assessed: Design, Data Collection & Processing and Conclusion & Evaluation

Aim:

The purpose of this investigation is to experimentally determine the effect a change in substrate concentration, Hydrogen Peroxide, (H_2O_2) will have on the rate of action (measured in time taken to surface due to buoyancy of O_2 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 H_2O_2 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:

$$2H_2O_{2(l)} \rightarrow 2H_2O_{(l)} + O_{2(g)}$$

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 wit the increase of substrate availability; however this increase will slow until it finally plateaus at a point know 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 H_2O and O_2.

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 O_2. 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**	**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 H_2O_2
The exact amount of catalase in the Liver juice	N/A	N/A
Denaturisation of catalase due to natural causes and age	N/A	N/A

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Materials

Amount	Equipment	Error
5	25mL Measuring Cylinder	±0.5mL
1	Mortar and Pestle	Impurities on cleaning
15g	Liver(Fresh)	Temperature may denature catalase
10mL	Hydrogen Peroxide (H_2O_2) 3%	±0.5%
10mL	Hydrogen Peroxide (H_2O_2) 4%	±0.5%
10mL	Hydrogen Peroxide (H_2O_2) 6%	±0.5%
10mL	Hydrogen Peroxide (H_2O_2) 8%	±0.5%
200mL	Distilled Water	Impurities on distillation
1	Stop Watch	±0.005
2	Tweezers	Improper cleaning- contamination
4	50mL Beaker	±0.5mL
10	Filter Paper (1cm x 1cm)	±0.05cm
1	Beaker Marking Pen	
4	Pipettes	±0.05mL
1	Scissors	
1	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, O_2 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:

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

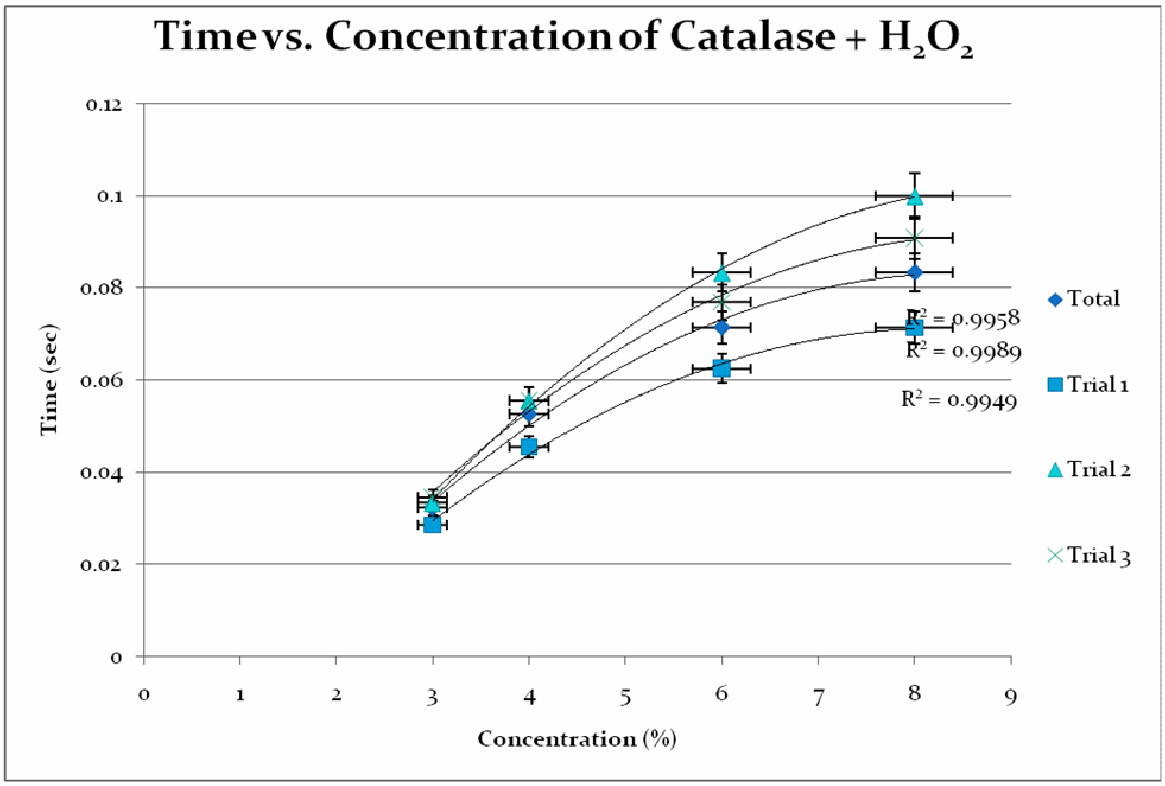
Qualitative Results:

- On contact with the solution, small O_2 bubbles began to form on the surface of the Liver.
- These small bubbles collected around the sheet of filter paper
- The filter paper slowly begun to rise from the bottom of the beaker
- No other physical characteristics observed throughout reaction

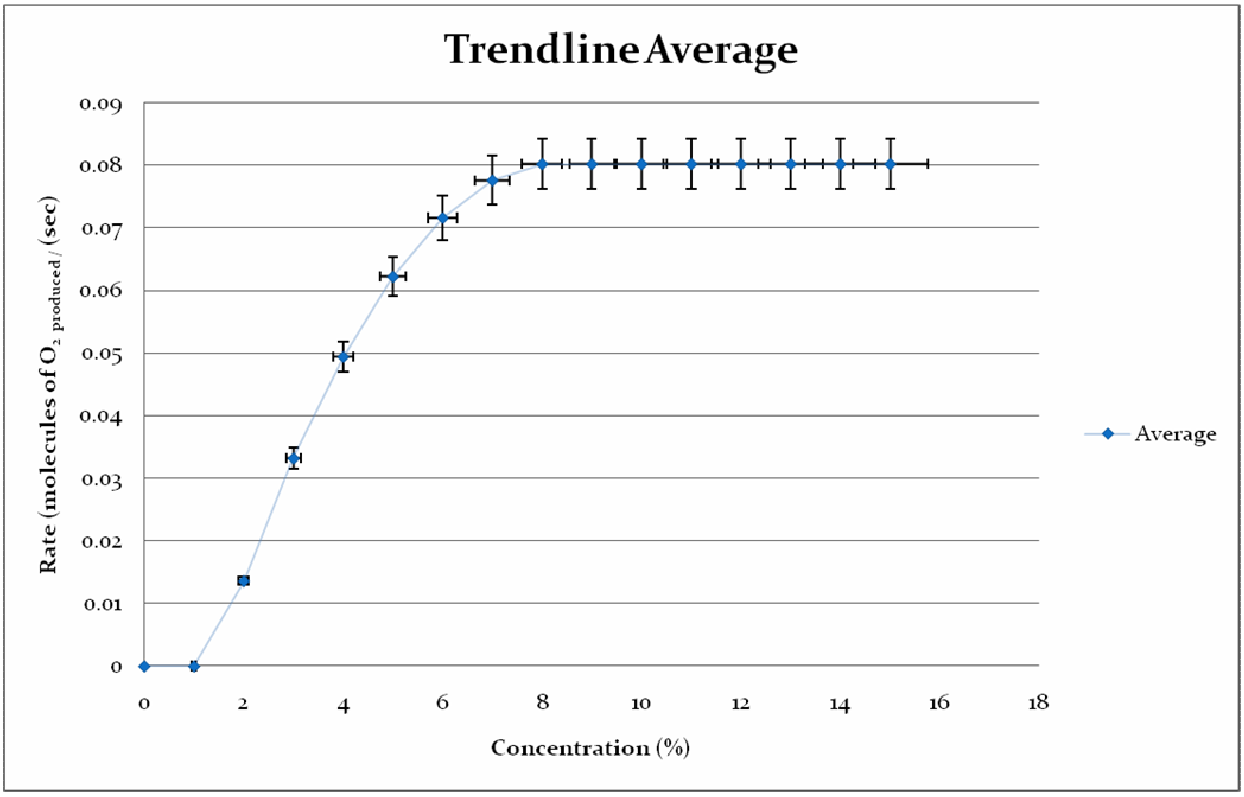
Graphs:

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) = □(x) (min) / (Number of x)
Where
Mean= the average value of the selected data
X= data values
□= the total sum of the data values
Number of x= the number of values in the x data series

e.g. Mean = (35 + 30 + 29) / 3
= 31seconds

Sample Calculation Rate of Reaction

Rate = 1 / Time

Rate (molecules of buoyancy/sec) = 1) / Time(sec)

Calculation [Whole Average]

Rate = 1/ 31

= 0.032258 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) ±0.0000005	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 or 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% H_2O_2 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 H_2O_2 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 R^2 value of the graph.