Determining the rate of action of an enzyme

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

Design

Aim:

The purpose of this investigation is to experimentally determine the effect a change in liver surface area will have on the rate of action (measured in oxygen production) of the enzyme (Catalase) on the substrate Hydrogen Peroxide (H_2O_2).

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 with the increase of surface area.

This is based on the surface area to volume ratio; by having a higher surface area to volume ratio it will in turn allow more substrate to react with the available enzyme's active sites until a plateau is reached in the reaction rates where the maximum number of active sites are being bound to by substrates.

The condition of the liver will determine the amount of surface area exposed to the substrate and hence be the definitive factor in this investigation. Accordingly, the rate of action of the Catalase will be greatest when the liver is ground, it will be followed by the sliced liver and then the whole liver. This is because the liver with the greatest surface area will have the most hydrogen peroxide molecules converted into oxygen bubbles in the time period. Reaction rate will then be determined by calculating the number of bubbles produced in the defined time.

Variables

Variable	Method	Error
Controlled:		
The amount of enzyme used	4g of Liver will be used in each test	±0.5g
The amount of distilled water used to dilate the solution	20mL of Distilled water will be used to dilate the solution	±0.5mL
The amount of dishwashing liquid	10 Drops of PineOCleen will be used in each test tube	±0.5drops
The amount of substrate present in the reaction	5mL of Hydrogen peroxide will be used in each test tube	±0.5mL
Manipulated:		
The surface area of the liver	The liver will be produced in three different states; ground, chopped and whole cubes	Human error
Respondent:		
The rate of action of the Catalase	The height of the detergent bubbles in the column will be measured at 30second intervals in a measuring cylinder	0.5mL
Extraneous:		
The temperature of the reaction environment	N/A	N/A
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	N/A
The exact amount of Catalase in the Liver juice	N/A	N/A
Denaturisation of Catalase due to natural causes	N/A	N/A

Materials

Amount	Equipment	Error
40g	Fresh Liver	Denaturisation of Enzymes
9	25mL Measuring Cylinder	±0.5mL
3	100mL Measuring Cylinder	±0.5mL
1	Scalpel	Residue
1	Scales	±0.005g
10mL	Detergent	±0.5mL
200mL	Distilled Water	Impurities
30mL	3% H_2O_2	±0.5%
1	100mL Beaker	±0.5mL
9	Test Tube	Residue
1	Test Tube Rack	N/A
9	Pipettes	±0.5mL
1	Marker	N/A
1	Stop Watch	0.5sec
1	Tweezers	N/A
3	Petri Dish	Residue

Method

1. Collect materials.
2. Weigh three (3) approximately cube shaped pieces of fresh liver to be the same weight of 4.00g
3. Prepare the liver into its three different conditions
    a. Ground- Crush Liver in the mortar and pestle until juice is present.
    b. Sliced- Using a scalpel
    c. Whole
4. Using the marker, indicate on one (1) test tube the ground liver
5. Fill the test tube with 20mL of Distilled water.
6. Add 5mL of 3 % Hydrogen Peroxide to the test tube
7. Add 10 drops of the dishwashing liquid to the test tube using the pipette
8. Lightly swirl the test tube to combine the two solutions.
9. Place the ground liver into the test tube and begin timing on the stop watch
10. At thirty second intervals record the height of the bubble column, continue this for two minutes or until bubbles cease to grow
11. Record all results in table
12. Repeat steps 4- 11, replacing the liver with the sliced, then whole liver
13. Repeat steps 2-11 three separate times for trials

Data Collection

Results:

Raw Data

Figure 1: Total volume of measuring cylinder throughout reaction of whole liver in Catalase-hydrogen peroxide solution over 120sec.

Trial	Weight (g)± 0.005g	Total Volume of Solution (+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
1	4.01	42	50	52	55
2	3.97	45	50	54	56
3	4.00	50	55	57	58

Note: start at approximately 25mL solution

Figure 2: Total volume of measuring cylinder throughout reaction of sliced liver in Catalase-hydrogen peroxide solution over 120sec.

Trial	Weight (g)± 0.005g	Total Volume of Solution(+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
1	4.39	40	50	55	55
2	4.39	50	55	60	60
3	4.33	50	55	60	60

Note: start at approximately 25mL solution

Figure 3: Total volume of measuring cylinder throughout reaction of ground liver in Catalase-hydrogen peroxide solution over 120sec.

Trial	Weight (g)± 0.005g	Total Volume of Solution(+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
1	3.89	50	57	70	71
2	3.82	51	63	70	72
3	4.27	48	55	75	75

Note: start at approximately 25mL solution

Qualitative Observations:

- On contact with the solution, small O_2 bubbles began to form on the surface of the Liver.
- These bubbles quickly floated to the top where they began forming larger bubbles with the assistance of the Detergent.
- The detergent wall of bubbles began to climb the side of the test tube and eventually filled up in the center.
- The bubbles were murky grey, and had a thicker than normal texture.
- As time progressed the liver seemed visibly softer, though the size remained the same
- No other physical characteristics observed throughout reaction

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 = (17 + 20 + 25) / 3
= 63/3
= 21mL

Sample Calculation Rate of Reaction

Speed = Distance / Time

Speed (mL/sec) = Δ Amount of Total Volume of Solution (mL) / Δ Time(sec)

Calculation [Whole Average]

Rate = (56 – 25)/ 120

= 31 / 120

= 0.26mL/sec

Sample Calculation Experimental Total Volume

Total Volume O_2 Detergent bubbles= Total Volume solution – Original Volume solution

Total= Total Solution – 25

Total= 50 – 25

= 25mL

Volume Detergent Bubbles

Figure 4: Total volume of bubbles throughout reaction of whole liver in Catalase-hydrogen peroxide solution over 120sec.

Trial	Weight (g)± 0.005g	Total Volume of Solution (+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
1	4.01	17	25	27	30
2	3.97	20	25	29	31
3	4.00	25	30	32	33

Figure 6: Total volume of bubbles throughout reaction of sliced liver in Catalase-hydrogen peroxide solution over 120sec.

Trial	Weight (g)± 0.005g	Total Volume of Solution(+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
1	4.39	15	25	30	30
2	4.39	25	30	35	35
3	4.33	25	30	35	35

Figure 6: Total volume of bubbles throughout reaction of ground liver in Catalase-hydrogen peroxide solution over 120sec.

Trial	Weight (g)± 0.005g	Total Volume of Solution(+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
1	3.89	25	32	45	46
2	3.82	26	38	45	47
3	4.27	23	30	50	50

Figure 7:Average total volume of bubbles throughout reaction liver in Catalase-hydrogen peroxide solution over 120sec.

Average Results

Condition	Weight (g)± 0.005g	Total Volume of Solution(+25mL) 30sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 60sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 90sec (mL) ± 0.5mL	Total Volume of Solution(+25mL) 120sec (mL) ± 0.5mL
Whole	3.99	21	27	29	31
Sliced	4.37	22	28	33	33
Crushed	3.99	25	33	47	48

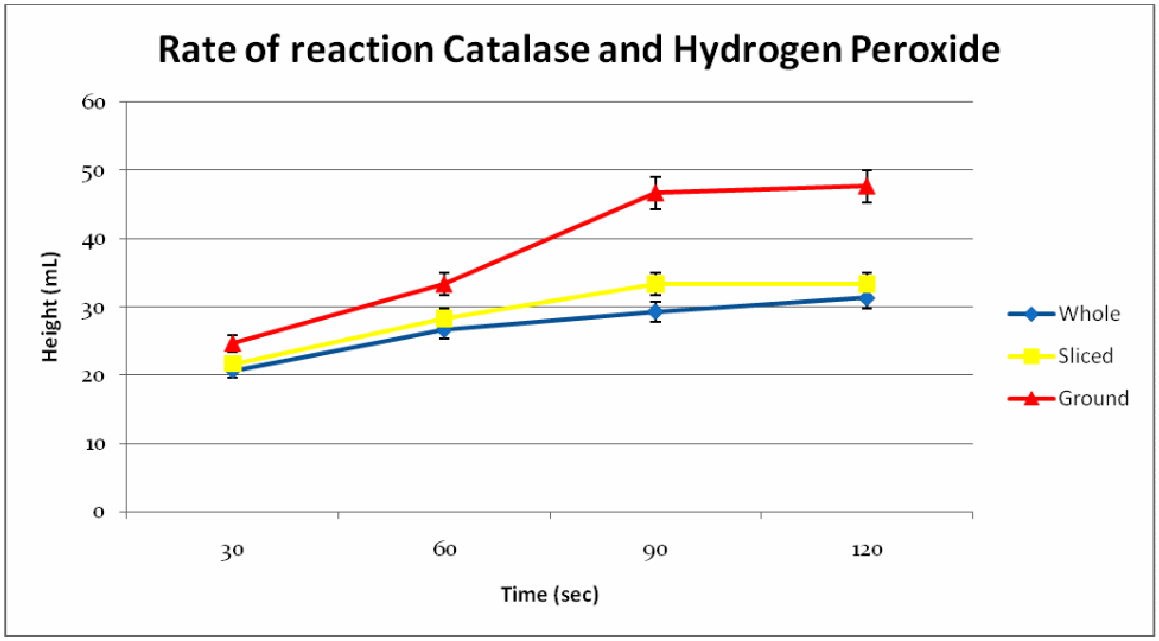
Figure 8:Average rate of reaction in Catalase-hydrogen peroxide solution over 120sec.

Average Rate

Condition	Weight (g)± 0.005g	Rate of Reaction (mL/sec) ±0.5mL
Whole	3.99	0.26
Sliced	4.37	0.28
Crushed	3.99	0.40

Graphs:

Figure 9: Average total volume of bubbles throughout reaction liver in Catalase-hydrogen peroxide solution over 120sec.



Conclusion

Statement:

The rate of the liver and hydrogen peroxide reaction increased proportionally to the surface area. The greater the surface area the more active sites were exposed to the substrate and the faster oxygen was produced. Therefore the ground liver was the fastest reaction, then the sliced liver and the slowest to react was the whole liver.

Explanation:

The purpose of this investigation was achieved, as it was determined that the surface area of the liver did affect the action of the enzyme. The hypothesis is accepted as the data in Graph 1 shows that the ground liver had the fastest rate of reaction. This can be seen by its steeper gradient and higher maximum. This was followed by the sliced liver which had a shallower gradient and a lower maximum. Last was the whole liver which produced a similar gradient to the sliced, though slightly less steep, nevertheless it was still reacting after 120seconds. The gradient is, in figure 9 a measure of Oxygen output of the system over time. Accordingly, it is representative of the rate of oxygen production per second.

Both the ground and the sliced liver seem to plateau after 90 seconds of reaction time which would suggest that this is the point at which all of the substrate molecules have been acted upon by the Catalase. This should not occur at the same time as the ground supposedly reacts much faster than the whole. The plateau does not account for the fact that the maximum number of active sites on the enzymes are being used up by substrates, because the volume of gas being produced should sill increase despite the rate staying the same, this is not the case for this experiment. The reason for this error becomes evident when considering the results of the sliced liver which indicate that the volume decreases over 30 sec. It is evident that the bubbles have burst and are skewing the datas collected. To account for error due to measuring falsities and equipment interaction the graph of time vs. bubble amount was drawn and a curve of best fit chosen to model the data most effectively. This curve was a polynomial function and when extrapolated could be used to give accurate approximation of the relationship between time and oxygen production.

Evaluation

Weaknesses/Limitations	Effect of Assumptions	Improvements
Though no outliers were particularly identified some of the data was very close to being out of the normal range.	The skewed data may have affected the accuracy of the averages taken and ultimately the experiment action of Catalase.	Repeating the experiment more than the already tried three (3) times would help to gain a smaller normal range and eventually eliminate obvious outliers
A methodical error identified was the tendency of both the sliced and crushed liver to become stuck on the side of the test tube when placed inside and hence not being completely submerged in the substrate.	This would have affected the amount of Catalase available to convert the substrate to O_2 molecules changing the controlled variable and effectively altering the results.	Place the liver into the test tube and leave to settle (tap) before placing the premeasured 25mL of solution on top of the liver.
The assumption that the weights of the liver were approximately the same.	The changing amount of liver would again change the amount of Catalase present to convert the substrate hydrogen peroxide.	Use more precise tools when sectioning off the liver.
The temperature of the environment was not monitored throughout the process and may have therefore been different for each experimental run.	This temperature may have denatured the enzymes within the liver, decreasing the amount of enzymes able to do effective work	Keep the liver in a fridge until use to maintain a constant temperature
The results gap between the different conditions of liver was not significant enough to expose patterns	Small errors may have a noticeable effect on the trends exhibited by the data	Add more substrate to gain a more separated result.
The allowed time for the reaction was not enough to completely react the Catalase with the Hydrogen Peroxide in all cases. This is not required but would allow for in depth analysis of the data	By not allowing the reactions to completely finish the experimental rate becomes an assumption for the samples which do not completely react within the 120 second time period	Continue the experiment for 360 seconds. This will allow for the very slowest of reactions to take place and completely react with each other
The oxygen bubbles produced escaped from the test tube and/or burst and did not therefore allow for the total oxygen production to be measured	The oxygen production was the main measurement used to determine the rate of reaction. If the total oxygen production was altered then the entire results may be affected	To avoid the loss of oxygen gas it would be more effective to measure the gas produced via the movement of a syringe so as to limit gas loss and give accurate quantitative results