Enzyme catalysed decomposition of hydrogen peroxide

Objective

The purpose of my experiment is to determine the effect of different concentration of copper Hydrogen Peroxide on the activity of catalase and the production of oxygen.

Background Knowledge

Catalase is an enzyme which is globular protein - the secondary protein structure is folded into a spherical or globular shape. Hydrogen bonds, ionic bonds, disulphide bridges and hydrophobic interactions (between groups of amino acids) maintain the specific three dimensional shape of the enzyme. This specific 3D shape is very essential for the functioning of enzymes. The part of the enzyme which binds with the substrate is called the active site. The shape of the active site differs from one enzyme to another. This makes the enzyme react only with a specific substrate, which fits the active site. Enzymes also lower the activation energy and provide an alternate (lower energy) pathway for the reaction to proceed. Thus the rate of reaction speeds up (catalyst).

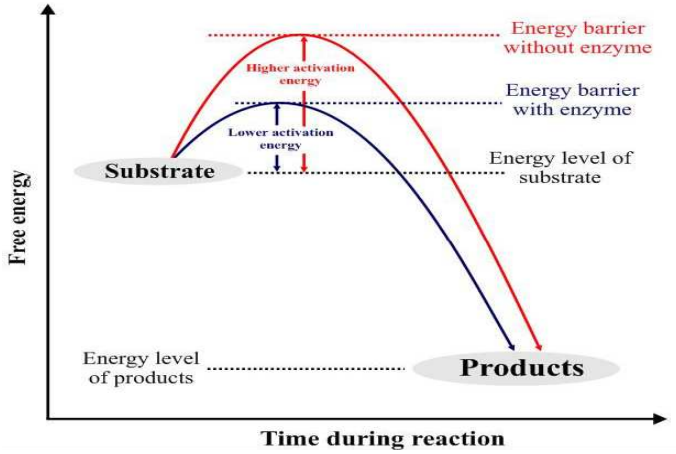

Figure 1

The enzyme active site binds to hydrogen peroxide (substrate) and decomposes it to oxygen and water.

$$\text{Hydrogen Peroxide} \xrightarrow{\text{Catalase (Yeast)}} \text{Water + Oxygen}$$

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Figure 2

There are many factors which affect the activity of enzymes; the concentration inhibitor is one of the factors which have a massive effect the activity of enzyme.

Enzyme catalysed decomposition of hydrogen peroxide

An enzyme inhibitor is a substance that slows down the rate at which an enzyme - catalysed reaction takes place. Many enzyme inhibitors work by binding with the enzyme, with the result that the enzyme can no longer bind with its substrate
Some inhibitors have shapes rather like the enzyme's normal substrate molecule, allowing them to bind at the active site of t he enzyme. They are called ***active site-directed inhibitors.***

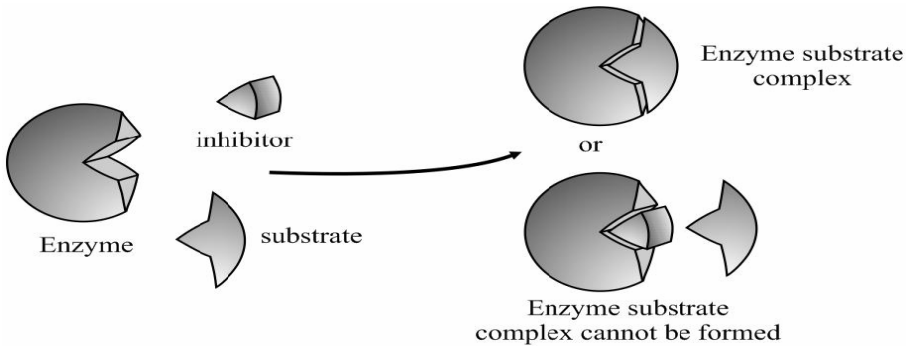

Figure 3

If there is an inhibitor molecule in the active site, the substrate cann ot bind there. Some active site-directed inhibitors bind permanently to the active site, so that they permanently inactivate the enzyme. While the inhibitor is out of the active site, it is possible for a substrate molecule to slot in, so the inhibitor wil l not completely stop the reaction, just slow it down. The inhibitor and the substrate are competing for the active site, so these inhibitors are sometimes called competitive inhibitors.
Other inhibitors bind to parts of the enzyme other than the active s ite. They are called ***non-active site-directed inhibitors****. They are sometimes called non****-competitive inhibitors****.*

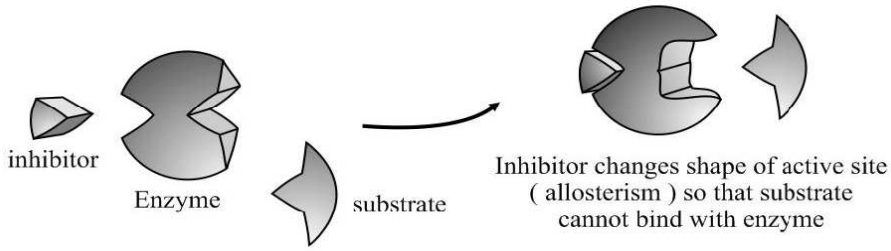

Figure 4

Non-competitive inhibitors are substances that forms strong covalent bonds with an enzyme and consequently may not be displaced by the addition of excess substrate. Therefore, non-competitive inhibition is irreversible. A non-competitive inhibitor may be bonded at, near, or r emote from the active site. In any case, the basic structure of the enzyme is modified to the degree that it ceases to work. See the graphic on the left. If the inhibition is at a place remote from the active site, this is called ***Allosteric inhibition****. Allosteric means "other site" or "other structure". The interaction of an inhibitor at an Allosteric site changes the structure of the enzyme so that the active site is also changed.*

Enzyme catalysed decomposition of hydrogen peroxide

Hypothesis:

Preliminary experiment

Before I began my actual investigation I had conducted 5 different preliminary experiment to decide on what apparatus I would need, what quantities of enzyme and substrate should be used in the investigation , and what the best method of getting a fair test would be. There was an option of using a glass syringe to collect the gas given off or to use a measuring cylinder in a bucket of water.

Experiment 1

Aim: *is to find what the effect of substrate concentration will have on the activity of catalase*

Plan: *I will use 5 different concentrations of Hydrogen peroxide (the substrate).* ***(5 vol, 10 vol, 15 vol, 20 vol, 25 vol)***

Variables:

I will;

Keep the temperature constant at 40 ^{o}C by using a water bath.

Keep the pH constant at pH 7 using a buffer

Keep the volume and concentration of catalase (yeast) constant and time

Result:

Volume of oxygen produced (Cm^3)					
	H_2O_2 solution (vol)				
Time (S)	***5***	***10***	***15***	***20***	***25***
10	*2.0*	*4.5*	*7.5*	*15.5*	*14.0*
20	*3.5*	*8.5*	*12.0*	*22.5*	*20.5*
30	*4.0*	*9.5*	*13.5*	*27.0*	*26.0*
40	*4.0*	*10.0*	*13.5*	*30.5*	*28.5*
50	*4.0*	*10.0*	*13.5*	*31.0*	*29.0*
60	*4.0*	*10.0*	*13.5*	*31.0*	*29.0*

Experiment 2

Aim: *is to find what the effect of enzyme concentration will have on the rate of reaction.*

Plan: *I will use 5 different concentrations of catalase (yeast).* ***(2.5%, 5%, 7.5%, 10% and 12.5%)***

Variables:

I will;

Keep the temperature constant at 40 ^{o}C using a water bath.

Keep the pH constant at pH 7 using a buffer

Keep the volume and concentration of Hydrogen peroxide constant and the time.

Result*:*

Volume of oxygen produced (Cm^3)					
	Yeast solution (%)				
Time (S)	***2.5***	***5***	***7.5***	***10***	***12.5***
10	*1.5*	*3.5*	*8.0*	*10.5*	*8.5*
20	*3.0*	*6.5*	*14.5*	*17.0*	*15.0*
30	*3.5*	*7.0*	*18.0*	*20.5*	*18.0*
40	*3.5*	*7.0*	*18.0*	*20.5*	*18.0*
50	*3.5*	*7.0*	*18.0*	*20.5*	*18.0*

60	*3.5*	*7.0*	*18.0*	*20.5*	*18.0*

Experiment 3
Aim: *is to find out the effect of temperature on the rate of reaction.*
Plan: *Temperatures used ranged from 20-60 degrees celsius in increments of 10*
Variables:
I will;

Keep the volume and concentration of catalase (yeast) constant
Keep the pH constant at pH 7 using a b uffer
Keep the volume and concentration of Hydrogen peroxide constant and the time.

Result:

Volume of oxygen produced (Cm^3)					
	Temperature (^oC)				
Time (S)	***20***	***30***	***40***	***50***	***60***
10	*1.5*	*3.5*	*15.0*	*6.5*	*1.5*
20	*2.5*	*6.0*	*26.5*	*9.0*	*2.0*
30	*2.5*	*6.5*	*32.0*	*10.0*	*2.0*
40	*2.5*	*6.5*	*34.5*	*10.5*	*2.0*
50	*2.5*	*6.5*	*34.5*	*10.5*	*2.0*
60	*2.5*	*6.5*	*34.5*	*10.5*	*2.0*

Experiment 4
Aim: *is to find out the effect of pH on the rate of reaction.*
Plan: *pH used ranged from 1 , 3, 5, 7, & 9 in increments of 2*
Variables:
I will;

Keep the volume and concentration of catalase (yeast) constant
Keep the temperature constant at 40 ^oC by using a water bath.
Keep the volume and concentration of Hydrogen peroxide constant and the time

Result:

Volume of oxygen produced (Cm^3)					
	Buffer solution (pH)				
Time (S)	***1***	***3***	***5***	***7***	***9***
10	*1.0*	*2.5*	*6.0*	*14.5*	*7.5*
20	*1.5*	*3.5*	*8.5*	*19.5*	*9.0*
30	*1.5*	*3.5*	*9.0*	*24.0*	*9.5*
40	*1.5*	*3.5*	*9.0*	*26.5*	*9.5*
50	*1.5*	*3.5*	*9.0*	*27.0*	*9.5*
60	*1.5*	*3.5*	*9.0*	*27.0*	*9.5*

Enzyme catalysed decomposition of hydrogen peroxide

Experiment 5
Aim: *is to find out the effect of inhibito rs on the rate of reaction.*
Plan: *copper sulphate used ranged from 0.0m -0.2m in increments of 0.02*
Variables:
I will;

Keep the volume of catalase (yeast) constant
Keep the temperature constant at 40 ^{o}C by using a water bath.
Keep the volume of Hydrogen peroxide constant

Result:

Conclusion
Catalase is made of a central heme and four polypeptide chains. Its active site binds to hydrogen peroxide and decomposes it to oxygen and water. My data suggests that enzyme activity depends on several modifiable variables. Factors that increase binding of H_2O_2 to catalase, such as increasing substrate and enzyme amounts, and increasing temperature, will increase the rate of reaction. After all active sites are occupied, adding more hydrogen peroxide will not affect the rate of reaction. Factors that cause catalase denaturation, such as high temperatures, extreme pH, and non-competitive inhibitors, can change its 3-dimensional structure, rendering it less active.

Fair tasting
In this investigation, the variables that affect the activity of the enzyme, catalase, were considered and controlled so that they would not disrupt the success of the experiment.

Controlled variable
1. ***pH :****In this experiment, the pH will be kept constant using pH 7 buffer solutions, this is because change in pH affects the ionic and hydrogen bonding in an enzyme and so alters it shape. Each enzyme has an optimum pH at which its active site best fits the substrate. Variation either side of pH results in denaturation of the enzyme and a slower rate of reaction.*
2. ***Temperature:*** *In this experiment, the temperature will be kept constant at 40 $^{\circ}$c (optimum temperature) using a water bath. This is because as temperature increases up to the optimum, the rate of enzyme activity also increases. In other word the substrate molecules gain more kinetic energy and the collisions between active sites of enzymes and substrate molecule become more frequent. The rate of enzymes substrate complex formation increases so enzymes activity speeds up. Beyond the optimum temperature, the rate of enzyme activity decreases because the high temperature causes the enzyme molecule to lose its specific 3D shape (due to breaking of Hydrogen bonds and ionic bonds). The active sites cannot bind with substrate, so enzyme substrate complexes cannot form.*
3. ***Volume of yeast solution (catalase):*** *The volume of yeast solution (catalase) will also be kept constant at 10ml using a 10 cm ^{3} measuring cylinder to ensure the accuracy.*
4. ***Concentration of yeast solution (catalase) enzyme :****In this experiment, the Concentration of yeast solution (catalase) will be kept constant at 5% this is because as the enzyme concentration increases (at constant substrate concentration) the rate of reaction increases until it reaches a maximum rate (V*

max). This is because there will be more number of free active sites, at any given time. So the rate of enzyme substrate complex formation increases. Thus rate of reaction increases. The rate doesn't increase beyond the V max because the substrate concentration becomes a limiting factor. Even though there will be many free active sites, there will not be enough substrate molecules to bind with them. So, rate of enzyme substrate complex formation remains constant at V max. But, increasing substrate concentration would further increase the rate of reaction.

5. ***Volume of hydrogen peroxide (substrate):*** *The volume of hydrogen peroxide (substrate) will also be kept constant at 10ml using a 10 cm^3 syringe to ensure the accuracy.*
6. ***Concentration of hydrogen peroxide (substrate):*** *In this experiment, the Concentration of hydrogen peroxide (substrate) will be kept constant at 20Vol this is because as the substrate concentration increases (at constant enzyme concentration) the rate of reaction increases until it reaches a maximum rate (V max). This is because there will be less number of free active sites and more number of substrate molecules to bind with the enzyme active site , at any given time. So the rate of enzyme substrate complex formation increases. Thus rate of reaction increases. The rate doesn't increase beyond the V max because the enzyme concentration becomes a limiting factor. Even though there will be many substrate molecules, there will not be enough enzyme molecules to bind with them. So, rate of enzyme substrate complex formation remains constant at V max. But, increasing enzyme concentration would further increase the rate of reaction.*

7. ***Time:*** *The time will be recorded every 10 seconds interval for 60 seconds using digital stop watch.*

8. ***Volume of copper sulphate (inhibitor):*** *The volume of copper sulphate (inhibitor) will also be kept constant at 10ml using a 10 cm^3 syringe to ensure the accuracy.*

Independent variables

- ***Concentration of copper sulphate (inhibitor):*** *This is the variable that will change and affects the volume of oxygen that is produced. I will add different volumes of distilled water to the copper sulphate solution to make different concentrations copper sulphate solutions. The concentrations to be used are as followed; 0.00 $moldm^{-3}$, 0.02 $moldm^{-3}$, 0.04 $moldm^{-3}$, 0.06 $moldm^{-3}$, 0.08 $moldm^{-3}$, 0.1 $moldm^{-3}$, 0.2 $moldm^{-3}$.*

Dependent variable

- ***Volume of oxygen:*** *In this investigation, the dependent variable will be the volume of oxygen produced. This volume oxygen produced will depend on the Concentration and volume of copper sulphate (inhibitor) and the rate of activity of the catalase's ability, to covert the hydrogen peroxide to water and oxygen. The volume of oxygen produced will be measured using a gas syringe every 10 seconds for 60 seconds. Since I was using 100cm^3 of gas syringe, to ensure accurate results the volume of the gas will be measured to 1d.p.*

Enzyme catalysed decomposition of hydrogen peroxide

Apparatus

Apparatus nomenclature	*Use*	*Reason for choice*
1x Bung	Used to seal the conical flask. It encloses the conical flask from the outside without displacing the inner volume and prevents any oxygen from being released out of the conical flask.	To prevent any oxygen from being released out of the conical flask.
1x 100 cm^3 Gas syringe	Used to draw a volume of a gas from the conical flask and measures the volume of oxygen given off in a reaction. Attached directly to the conical flask by a rubber tube, for collecting and measuring the exact amount of Oxygen given off directly from the beaker during the experiment this will be used to measure the volume of oxygen produced. It allows the volume to be read accurately to 1d.p. Collecting the volume of gas this way is more accurate and reliable than counting the number of bubbles produced in a boiling tube, which would be the alternate method.	It has an accuracy of 0.1 – The Gas syringe has been chosen to be used to measure the oxygen given off in the reaction rather than other pieces of equipment which perform the same job. This choice was made because the gas syringe is a lot easily to operate than other option and you can clearly see the amounts of gas given off. This allows you to record the results quickly. Other options like counting the bubbles of gas are less reliable as you cannot be sure of the number of bubbles as they are too quick.
1x Goggles	They are worn throughout the course of the experiment.	To prevent harmful substances such as hydrogen peroxide and copper sulphate from contact with eyes.
Copper sulphate	It is used as one of the reactants in our experiment. Various concentrations of it will be reacted with the other solutions.	This solution contains metal ions that work as inhibitors on the catalysis reaction. Our aim is to investigate in detail the exact effect of these ions
Water bath	It will continuously heat	I insert the test tubes and

	water at a specified temperature, in my case 35° C.	beakers containing the reactants that I want to acclimatise into the water bath and leave them in there for a period of 3 minutes. After such time the temperature of the solutions (reactants) will be 35 °C
Conical flask	It contains solutions that are used during the course of the expThis is the reactions described above takes place. It contains the substrate, Hydrogen peroxide, the source of catalase, yeast, water, to vary the concentrations and finally the copper sulphate, the variable in concern. Attached directly to the gas syringe for measurement, this is part of the fair testeriment	I used mine to keep the buffer solution in a safe place.
Pipette	To measure small amounts of solutions.	For small amounts of solutions it is more practical and easy to use a pipette instead of a syringe
Distilled water	To be introduced into the copper sulphate solutions to create different concentrations.	I aim to investigate how different concentrations of copper sulphate solution affect the rate of the catalysis. I used distilled water because???
Buffer solution (pH 7)	It is put in a test tube and acclimatised before mixing it with the potato tubes and the other solutions	It is used to maintain the pH of the reaction constant at 7 throughout, which is the optimum pH for the reaction involving catalase.
Hydrogen peroxide solution 20%	It is put in a test tube and acclimatised before making it react with catalase. The concentration of the Hydrogen Peroxide was 20%	It functions as the substrate in the catalysis reaction. I decided to study catalase, and since enzymes are specific, Hydrogen Peroxide is the only substrate that can fit into catalase's active sites.

Enzyme catalysed decomposition of hydrogen peroxide

Stopwatch	To measure the length of time of the reaction, and also to measure the length of time of the acclimatisation process undergone by the 3 test tubes containing the reactants, and the beaker which functions as the environment in which the reaction is happening. The accuracy of the stopwatch was to half a second. For measuring the total time for the experiment (120seconds) and the time intervals in which the amount of oxygen produced would be measured. (20 seconds).	The test tubes containing the reactants are inserted into the water bath set at 35 C, so that the solutions temperature rises to the one in the water bath, the same is carried out with the beaker. This process has to be undergone for a specific length of time, measured by the stopwatch.
Graduated syringe x2 **10 ml**	One is for injecting the Hydrogen Peroxide into the test tube at the start of the reaction; the other one is used to mix right amounts of other solutions.	To make sure that the hydrogen peroxide doesn't make contact with the other reactants before the right apparatus is set up for the counting of the gas produced. The other syringe is used to measure for example, the amount of distilled water to inject into the copper sulphate solutions. Syringes are used to measure the quantities of yeast and hydrogen peroxide. This is because they can measure the quantities more accurately than pipettes can. Using a syringe allows you to measure out the different concentrations very accurately compared with using a pipette. This improves the reliability of the investigation if the

		quantities are measured more accurately
Test tube rack	To contain the test tubes	I used it to maintain the test tubes in place, in an orderly manner.
Clamp x2	One is used to hold up the measuring cylinder and the other one to prevent the syringe from tipping over. Made up of the head, boss, stand and clamp. Generally for support i.e. for holding and stabilizing the gas syringe in one position during the experiment	To avoid spillings that would interfere with the good exit of my experiment.
Rubber pipe	The rubber connection is used for the transfer of the oxygen produced straight forwardly from the reaction into the gas syringe for measurement and recording, without any medium of barrier	

Results

0.00M of $CuSO_4$ (Distilled Water)			
	Volume of oxygen produced (Cm^3)		
Time (S)	***First trial***	***Second trial***	***Average***
10	*27.5*	*28.0*	*27.8*
20	*44.0*	*43.5*	*43.8*
30	*54.0*	*54.5*	*54.3*
40	*61.5*	*61.0*	*61.3*
50	*65.0*	*66.0*	*65.5*
60	*67.0*	*67.5*	*67.3*

0.02M of $CuSO_4$			
	Volume of oxygen produced (Cm^3)		
Time (S)	***First trial***	***Second trial***	***Average***
10	*26.5*	*25.0*	*25.8*
20	*42.0*	*41.0*	*41.5*
30	*52.5*	*53.0*	*52.8*
40	*58.5*	*56.5*	*57.5*

50	*62.5*	*61.0*	*61.8*
60	*64.0*	*63.0*	*63.5*

0.04M of $CuSO_4$			
	Volume of oxygen produced (Cm^3)		
Time (S)	***First trial***	***Second trial***	***Average***
10	*24.0*	*24.5*	*24.3*
20	*37.0*	*38.0*	*37.5*
30	*46.0*	*45.5*	*45.8*
40	*52.5*	*53.0*	*52.8*
50	*56.5*	*56.0*	*56.3*
60	*59.0*	*58.5*	*58.8*

0.06M of $CuSO_4$			
	Volume of oxygen produced (Cm^3)		
Time (S)	***First trial***	***Second trial***	***Average***
10	*11.0*	*10.0*	*10.5*
20	*23.5*	*22.0*	*22.8*
30	*36.0*	*35.5*	*35.8*
40	*44.0*	*42.5*	*43.3*
50	*46.5*	*47.5*	*47.0*
60	*50.5*	*52.0*	*51.3*

0.08M of $CuSO_4$			
	Volume of oxygen produced (Cm^3)		
Time (S)	***First trial***	***Second trial***	***Average***
10	*10.5*	*9.5*	*10.0*
20	*23.0*	*23.5*	*23.3*
30	*35.5*	*35.0*	*35.3*
40	*40.5*	*37.0*	*38.8*
50	*42.0*	*41.0*	*41.5*
60	*43.5*	*43.5*	*43.5*

Enzyme catalysed decomposition of hydrogen peroxide

0.1M of $CuSO_4$			
	Volume of oxygen produced (Cm^3)		
Time (S)	*First trial*	*Second trial*	*Average*
10	*8.0*	*9.5*	*8.8*
20	*22.5*	*21.5*	*22.0*
30	*34.5*	*34.0*	*34.3*
40	*36.0*	*39.0*	*37.5*
50	*39.0*	*37.0*	*38.0*
60	*39.5*	*37.5*	*38.5*

0.2 M of $CuSO_4$			
	Volume of oxygen produced (Cm^3)		
Time (S)	*First trial*	*Second trial*	*Average*
10	*5.5*	*6.5*	*6.0*
20	*11.0*	*13.5*	*12.3*
30	*17.0*	*15.5*	*16.3*
40	*20.5*	*22.0*	*21.3*
50	*23.0*	*23.5*	*23.3*
60	*24.0*	*23.0*	*23.5*

	Average Volume of oxygen produced (Cm^3)						
Time (s)	**0.00M**	**0.02M**	**0.04M**	**0.06M**	**0.08M**	**0.1M**	**0.2M**
10	27.8	25.8	24.3	10.5	10.0	8.8	6.0
20	43.8	41.5	37.5	22.8	23.3	22.0	12.3
30	54.3	52.8	45.8	35.8	35.3	34.3	16.3
40	61.3	57.5	52.8	43.3	38.8	37.5	21.3
50	65.5	61.8	56.3	47.0	41.5	38.0	23.3
60	67.3	63.5	58.8	51.3	43.5	38.5	23.5

Concentration of $CuSO_4$ (Mol/dm^3)	**Average rates (Cm^3/s)**
0.00	2.8
0.02	2.63
0.04	2.63
0.06	1.2
0.08	1.2
0.10	1.18
0.20	0.63

Analysing
Graph 1

Enzyme catalysed decomposition of hydrogen peroxide

Notice on this graph the production oxygen increased by 43.8cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 61.3 cm^3 and after 60 seconds the amount of oxygen produced was 67.3 cm^3.
The production of oxygen increased proportionally the first 10 seconds, slight increase between 10-40 seconds and after 40 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 60 seconds

Graph 2

As you can see on this graph the production oxygen increased by 41.5 cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 57.5 cm ^{3} and after 60 seconds the amount of oxygen produced was 63.5 cm ^{3}.
The amount of oxygen produced increased proportionally the first 10 secon ds, slight increase between 10-30 seconds and after 30 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 60 seconds

Graph 3

On this graph the production oxygen increased by 37.5 cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 52.8 cm^3 and after 60 seconds the amount of oxygen produced was 58.8 cm ^{3}.
Again the amount of oxygen produced increased proportionally the first 10 seconds, slight increase between 10-30 seconds and after 30 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 60 seconds

Graph 4

As you can see on this graph the production oxygen increased by 22.8 cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 43.3 cm ^{3} and after 60 seconds the amount of oxygen produced was 51.3 cm ^{3}.
The amount of oxygen produced increased proportionally the first 30 seconds this time, slight increase between 30-40 seconds and after 40 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 60 seconds

Graph 5

On this graph the production oxygen increased by 23.3 cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 38.8 cm ^{3} and after 60 seconds the amount of oxygen produced was 43.5 cm^3.
Again the amount of oxygen produced increased proportionally the first 30 seconds, slight increase between 30-50 seconds and after 50 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 60 seconds

Graph 6

You can see on this graph the production oxygen increased by 20 cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 37.5 cm ^{3} and after 60 seconds the amount of oxygen produced was 38 .3 cm^3.
Again the amount of oxygen produced increased proportionally the first 30 seconds, slight increase between 30-40 seconds and after 40 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 6 0 seconds

Graph 7

As you can see on this graph the production oxygen increased by 12.3 cm ^{3} during the first 20 seconds, after 40 seconds the amount of oxygen produced was 21.3 cm ^{3} and after 60 seconds the amount of oxygen produced was 23.5 cm ^{3}.
Again the amount of oxygen produced increased proportionally the first 40 seconds, slight increase between 40-50 seconds and after 50 seconds the amount of oxygen produced starts to levels off. Overall the production oxygen increased through out 60 seconds

Explanation

*On **graph 1** the production of oxygen increased proportionally the first 10 seconds sand slight increase between 10-40 seconds and this is because there was equal amount enzyme and substrate number of free active sites at any given time. So the rate of enzyme substrate complex formation increases until it reaches the maximum rate (V_{max}). After 40 seconds the amount of oxygen produced starts to levels off this is because both enzyme and substrate concentration becomes a limiting factor so the rate of reaction remains constant at (V_{max}).*

*On **graph 2 and 3** the production of oxygen increased proportionally the first 10 seconds sand slight increase between 10-30 seconds this is because there was equal amount enzyme and substrate molecules sites during the first 30 seconds and less time for the inhibitor (0.02 moldm^{-3} of copper sulphate was used) to work because it was less concentrated than the substrate molecules. So the rate of enzyme substrate complex formation increases until it reaches the maximum rate (V_{max}). After 30 seconds the amount of oxygen produced starts to levels off this is because enzyme concentration becomes a limiting factor as most of enzyme molecules are permanently denatured by copper ions. Even though there are plenty of substrate molecules, there are no enough free active sites to bind with the substrate molecules, at any given time. So enzyme substrate complex formations remain constant at (V_{max}).*

*On **graph 7** the production of oxygen increased proportionally the first 20 seconds sand slight increase between 20-25 seconds this is because there was equal amount enzyme and substrate molecules sites during the first 25 seconds and less time for the inhibitor (0.2 moldm^{-3} of copper sulphate was used) to work. So the rate of enzyme substrate complex formation increases until it reaches the maximum rate (V_{max}). After 25 seconds the amount of oxygen produced starts to levels off this is because enzyme concentration becomes a limiting factor as most of enzyme molecules are permanently denatured by copper ions. Even though there are plenty of substrate molecules, there are no enough free active sites to bind with the substrate molecules, at any given time. So enzyme substrate complex formations remain constant at (V_{max}).*

Anomalous result

*On **graph 4, 5 and 6** there are some **anomalous** results. (Explained under evaluation section)*

Enzyme catalysed decomposition of hydrogen peroxide

Initial Rate

In this investigation an initial rate graph has to be plotted to show the difference in initial rate as concentration increases. It is used to work out the initial rate at the beginning of the reaction so I can compare the initial rates of all the concentrations to see if they change.

Initial rate is worked out by calculating the slope closes to the tangent to the curve, as close to 0 as possible. This means I will measure from just before the graph starts to curve.

Sample calculation

e.g. On graph 1 starts to curve on about 20 second. This means I will take 20seconds. I then look on the graph to see the amount of gas produced in this time. It says 56 cm^3 of oxygen. This mean 56 cm^3 are produced per 20sec.
I then do a calculation using the following formula :-

$$\text{Initial rate} = \frac{\text{Volume}}{\text{Time}} \qquad \frac{56\ cm^3}{20} = 2.8\ cm^3/sec$$

A table to show the average rate of the volume of oxygen given off as the copper sulphate concentration changes

Concentration of $CuSO_4$ (Mol/dm^3)	***Average rates (Cm^3/s)***
0.00	*2.8*
0.02	*2.63*
0.04	*2.63*
0.06	*1.2*
0.08	*1.2*
0.10	*1.18*
0.20	*0.63*

Graph 9

Notice on this graph, the average rate of reaction was 2.8cm^3/s during the controlled experiment (0.0 mol/dm^3 of copper sulphate), the average rate of reaction reduced to 2.63 cm^3/s when 0.02 mol/dm^3 of copper sulphate was used, again the average rate of reaction remains 2.63cm^3/s when 0.04 mol/dm^3 of copper sulphate was used.

When 0.06 mol/dm^3 of copper sulphate was used the average rate of reaction was 1.2cm^3/s. Again the average rate of reaction remains 1.2cm^3/s when 0.08 mol/dm^3 of copper sulphate was used. The average rate of reaction reduced to 1.18cm^3/s when 0.1 mol/dm^3 of copper sulphate was used. When 0.2 mol/dm^3 of copper sulphate was used the average rate of reaction was 0.63cm^3/s. The average rate of reaction halved compared to 1.18cm^3/s (when 0.1 mol/dm^3 of copper sulphate was used). Overall as the concentration of copper sulphate increase the average rate of reaction decreases and the plotted results on the graph produce a strong negative correlation.

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Trends

As you noticed from all the graphs there are several patterns and trends which can be seen clearly.

- *The initial rate of reaction (production of oxygen) decreased as the concentration of copper sulphate (**see graph 9**).*

- *The initial rate reaction was 0.63cm^3/s when 0.2 mol/dm^3 of copper sulphate is used but the initial rate of reaction doubled (it was 1.18cm^3/s) when 0.1 mol/dm^3 of copper sulphate is used. As you can see from the result, when the concentration of copper sulphate decreased by 0.1 mol/dm^3 the initial rate of reaction doubles.*

- *Graph 1&6 has a similar trend, when the concentration of copper sulphate decreased by 0.1mol/dm^3 the initial rate of reaction doubles.*

- *All, the three graphs (Graph 1, 2& 3) shows, the production of oxygen increased proportionally during the 10 seconds of each experiment. This is because both the 2 concentration of copper sulphate were week and their effect on the activity of catalase was negligible during the first 10 seconds of each experiment.*

- *On both (graph 2 and 3) the graph starts to c urve at 30 seconds. This is because the rate of enzyme substrate complex formation can only increase up until it reaches the maximum rate (V_{max}). After 30 seconds the amount of oxygen produced starts to levels off this is because enzyme concentration becomes a limiting factor as most of enzyme molecules are permanently denatured by copper ions.*

Conclusion

My prediction was," the greater the concentration of copper sulphate the lower the production of oxygen and less, the activity of catalase". The overall result supports my prediction .

Evaluation

Sources of error:

There are several sources of error in this experiment. Since the investigation takes a very long time to complete, the experiment had to be continued over several days. As a result, different H_2O_2 and catalase (yeast) solutions were used on each day. Some of the solutions may have been older than the original solutions used which may have had an effect on their reactivity. Also, different concentrations of solutions may have been used. For example, when the original yeast solution ran out, a new solution had to be made and probably resulted in a slightly different concentration.

Limitation of experimental techniques and uncertainties associated with the equipment:

First I will calculate the uncertainties associated with the measurements that I have taken. I will choose the lowest value where I have a choice to illustrate the 'worst case scenario'

Measuring cylinder:

The uncertainty associated with the measuring cylinder reading is 0.5cm^3

The % uncertainty in a measuring cylinder reading of 50cm^3 =

$$\frac{0.5}{50} \times 100 = 1.0\%$$

Percentage error = 1.0%

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Thermometer:

The thermometer I used in this experiment is accurate to 0.5 ^{o}C

The uncertainty associated with the thermometer reading is 0.5 ^{o}C

The % uncertainty in a thermometer reading 40 ^{o}C

$$= \frac{0.5}{40} \times 100 = 1.25\%$$

Percentage error = 1.25%

Gas syringe:

The gas syringe I used in this experiment is accurate to 0.5 cm^3

The uncertainty associated with the gas syringe reading is 0.5cm^3

The % uncertainty in a gas syringe reading of 100cm^3

$$\frac{0.5}{100} \times 100 = 0.5\%$$

Percentage error = 1.25%

I have listed some of the limitations of the experimental techniques in the following table

Aspect of procedure	***Limitation***	***Effect on the overall result***

Reading on Graduated syringe	*The syringe was graduated to the nearest 0.5ml*	*The overall effect is very Insignificant because it was unlikely to be a possible error when measuring 10 ml of Hydrogen Peroxide, yeast solutions or the other solutions (copper sulphate & buffer solution) using 10ml Graduated syringe*
Making recordings at identical intervals(every 10 seconds)	*The difficulty was that it was difficult to read off values exactly on time when the reaction was still taking place.*	
Reading on gas syringe	*The syringe was graduated to the nearest 0.5ml*	

Improvement:
If I was given a chance to conduct another similar exp eriment, I would change some of the apparatus, improve the procedures and techniques that were during the experiment.
My experiment could be improved in numerous ways.

Further investigation

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This experiment can be extended to investigate the type of inhibitor that was used through out the experiment i.e. (Competitive or Non - competitive inhibitor)