Investigating Enzymes.

Aim;

My aim is to find out the effect of varying the concentration of the substrate (Hydrogen peroxide solution), will have on the Catalyse in side a piece of Liver when they react. I will use 5 different concentrations of the Hydrogen peroxide solution (H_2O_2).

Apparatus;

I will need the following to carry out my experiment into the Investigation. I will also need many of these items to improve the accuracy of this experiment;

Stop clock- To make sure I time each of the concentrations of the H_2O_2 correctly and accurately.

Piece of liver- The liver contains the Catalyse that I need to experiment with. I may need around 10 pieces of liver.

H_2O_2- The hydrogen peroxide will be the substrate in this experiment.

Measuring Cylinders- To measure the water and H_2O_2 accurately.

Boiling tubes- To hold the H_2O_2 and piece of liver in.

Delivery tube- to enable the O_2 to escape through when the reaction is taking place.

Water- To weaken the strength of the H_2O_2 and also to put in the other tube to watch the bubbles and count them.

Thermometer- So I can take the temperature of the Hydrogen peroxide, before I start my experiment.

Diagram;

Plan;

The first thing that needs to be done is to put on my safety glasses because there are chemicals in the room. I will then have to get my equipment out and set it up in the diagram but without the piece of the Liver in the tube already. I first have to measure out the correct amount of Hydrogen peroxide out in the measuring cylinder, for example 20cc. When I measure out the 20cc I will have to measure this as accurately as I can, I will do that by getting my eye, level with the 20cc mark on the cylinder and spill or add any H2O2 if necessary.

I will then pour the H2O2 into boiling tube A. I will also have to take the temperature of the H2O2, and record it. I will then measure 20cc in the measuring cylinder and then pour it into tube B. After that I will have carefully pick up the piece of liver a drop it in to tube A which contains the hydrogen peroxide. I will also have to start the stop clock simultaneously to when the liver reacts to the H2O2 accurately. I could also ask someone else to start the stop clock for the best possible results. Straight after that I have to fit the delivery tube securely onto the tubes, to make sure none of the gasses escape out of the tube. Once the stop clock reaches 45 seconds then I will start counting the bubbles.

I will only start counting the bubbles after 45 seconds because in my preliminary investigation, I found out that before that 45 second mark the bubbles come out to fast to count and there are too many as well. When the time gets to 45 seconds, I will start to count the bubbles then with also my partner keeping an eye on the time, until it reaches 1 minute and 15 seconds on the time, I will stop counting the bubbles and make a note of how many bubbles I have counted. I will count all the experiments with different concentrations when the clock reaches 45 seconds, to 1 minute 15 seconds, so all the experiments will have the bubbles counted for 30 seconds in total. Once the experiment has finished, then I will need to dispose of the piece of liver carefully and spill the used Hydrogen peroxide solution down the sink, and also change the water in tube B as well to make it a fair test. I will then have to do the experiment for each different concentration. The concentrations are shown in the table below.

Hydrogen peroxide H2O2	Substrate H2O2	Water (to weaken H2O2) H2O
5 Volume	0cc	0cc
4 Volume	16cc	4cc
3 Volume	12cc	8cc
2 Volume	8cc	12cc
1 Volume	4cc	16cc

Variables;

There many variables that I could change in this experiment and Keep the same. The main variables are;

- Instead of using a piece of Liver I could use a slice of potato because that contains Catalyse as well.
- I could test how the reaction rate of the hydrogen peroxide and the Catalyse changes if I vary the PH scale by adding a bit of acid or alkali.
- I could see if the reaction rate changes by varying the temperature of the Hydrogen peroxide.

The variable that I am going to use is changing the concentration of the Hydrogen peroxide to see if that has an effect on how much Oxygen is produced out of the reaction.

Fair test;

To make sure I have a fair test, I will have to follow several rules. I will have to make sure that the following are carried out appropriately;

- I use the same boiling tube sizes.
- I use the same delivery tube each time.
- I use the correct amount of water in boiling tube B.
- I use the correct amount of Hydrogen peroxide.
- I do repeat readings for each of the concentrations.

The most important one is that I allow the same time to count the bubbles for each of the concentrations, as this is crucial.

Safety;

For this experiment safety glasses must be worn at all times, as the hydrogen peroxide solution can be dangerous if it gets into your eyes and if it gets in contact with your skin, you need to rinse it off immediately. Your bags, coats and other personal possessions are placed out of the way securely or tucked neatly away under your desks. The Hydrogen peroxide is bleach, meaning that it will either stain or take the colour out of your clothes or bags. Our bags and coats also need to be tucked away because if the liver falls on any of them, it wouldn't be very pleasant to wear, it may even smell bad. I will have to take care and be aware of others around me who are working. After all, safety does come first.

Preliminary Investigation.

For my preliminary work I used a piece of Liver, which worked very well because it has plenty of catalyses to react with the Hydrogen peroxide. I will use Liver in my experiments. In my preliminary investigation, I set up my apparatus like I have shown in my diagram. I used 20cc of the $H2O2$ to see what happened in the highest concentration. I found out that when I added my piece

Liver, it reacted too much with the H_2O_2, I had to wait until the stop clock reached 45 seconds for the bubbles to have slowed down enough to count them. The bubbles were coming out fast because the piece of Liver had a lot of Catalyse, which reacted with the Hydrogen peroxide molecules by colliding with them. So I have learnt to wait 45 seconds from when I drop the piece of liver into the Hydrogen peroxide to count the bubbles. I then have to count the bubbles for 30 seconds until the stop clock reaches 1 minute 15 seconds.
My main worry is that at the end of the 30 second counting period that the reaction will slow down to much or finish, so there will no bubbles at all coming out. I have also found out that if I use the same piece of Liver for two experiments, in the second experiment, the Liver doesn't react well with the H_2O_2 because the Catalyse has been used up in the last experiments reaction. I have decided to use a new piece of Liver in every experiment.

Prediction;

My prediction is that when the catalyse inside the liver(the enzyme) reacts with the Hydrogen peroxide(the substrate) the reaction that will take place will be;

Hydrogen Catalyse Water +
Peroxide ………………… Oxygen.

The reaction will take place because the liver containing the Catalyse molecules, will collide with the Hydrogen peroxide molecules. That type of reaction is called a "Chance Reaction" because there is the chance that the H_2O_2 molecules might miss the Catalyse molecules or they might collide with them.
I think that the higher the concentration of the H_2O_2, the greater chance of a collision. That is because the higher the concentration, the more H_2O_2 molecules there are, so therefore the higher chance there is for a reaction. So this is the order in which the most bubbles will be counted, starting with the highest;
5 Volumes
4 Volumes
3 Volumes
2 Volumes
1 Volume
If you change the temperature of the Hydrogen peroxide then I think the reaction will be a lot slower and less, because the enzymes usually work at body temperature which is 27 C. The body temperature is the enzymes optimum condition that it can work in.

Results;

The following table shows my first set of results;
cc = Cubic centre metres
S = Seconds
C = Degrees Celsius

Amount of H_2O_2 in cc	Amount of H_2O in cc	Temp of H_2O_2 in C	Number of Bubbles	Time bubbles were counted (S)
20cc	0cc	23 C	77	30s
16cc	4cc	23 C	45	30s
12cc	8cc	23 C	38	30s
8cc	12cc	23 C	36	30s
4cc	16cc	23 C	22	30s

My repeat readings are;

Amount of H_2O_2 in cc	Amount of H_2O in cc	Temp of H_2O_2 in C	Number of Bubbles	Time bubbles were counted (S)
20cc	0cc	23 C	68	30s
16cc	4cc	23 C	41	30s
12cc	8cc	23 C	31	30s
8cc	12cc	23 C	28	30s
4cc	16cc	23 C	14	30s

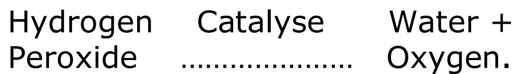
The average of my two results is in the table below;

Amount of H_2O_2 in cc	Amount of H_2O in cc	Temp of H_2O_2 in C	Number of Bubbles	Time bubbles were counted (S)
20cc	0cc	23 C	72.5	30s
16cc	4cc	23 C	43	30s
12cc	8cc	23 C	34.5	30s
8cc	12cc	23 C	32	30s
4cc	16cc	23 C	18	30s

I am now going to plot a graph using the information and Data I have collected through my experiments, which are shown in the tables above.

Conclusion;

When I changed the condition of the Hydrogen peroxide, by changing the temperature of it, the higher the concentration the more bubbles came out of tube B. This happened by a "Chance Collision". That means, when I dropped the piece of Liver containing the Catalyses, the H2O2 molecules could have missed the Catalyse molecules. The higher the concentration of Hydrogen peroxide, the more molecules there are and the better chance there is of a collision. A successful collision is when the molecules collide with each other and react.
The patterns in my graph with the averaged results plotted are that the higher the concentration of the Hydrogen peroxide, the higher the number of bubbles. So on my graph I have a vertical line going upwards.
The word equation that took place was;



The lock and key theory is that every enzyme has a different shape. They break up the reactant e.g. Catalyse by fitting around the Catalyse into the enzymes active site and breaks up the reactant, the enzyme can also be re-used. Enzymes are Proteins. Protein molecules are denatured by high temperatures and extreme PH environments. If a protein is denatured, then the active site into which the reactant fits in changes shape irreversibly so the reactant can't fit in there. That then means that the reactant can't be broken down.

Evaluation;

This experiment may not have been accurate because the pieces of Liver that I used in my experiment weren’t measured. Another very important point is that there wasn't a method of measuring the amount of catalyse in the Liver. That was the main problem in the experiment.
The size of all the bubbles weren't the same. At the beginning of the reaction when you drop the Liver in, the bubbles come out smaller and faster, I think that happens because they are pressured and pushed out so they break up into little pieces. I did repeat readings which confirmed my first readings. I could extend this experiment by seeing if O2 would displace the water in a beaker with a lid on.
I only got one anomalous result, this happened while using the 5 Vol of H2O2. I got this result possibly because I used a larger piece of Liver in this experiment then the others.