Biology Coursework –
Investigating a factor that affects enzyme activity.

Read Baurtally 11W
21/10/02

Planning

Aim
To investigate a factor which will affect the activity of catalase, whilst keeping all variables constant.

Possible Independent Variables
Here are a number of possible independent variables that could be changed in the experiment:

Independent variable	Continuous/Discontinuous	Easy to measure?
Volume of substrate used	Continuous	Yes
Type of enzyme	Discontinuous	Yes
Overall mass of piece of meat	Continuous	No
Type of substrate used	Discontinuous	Yes
Temperature of substrate	Continuous	Yes
pH of substrate	Continuous	**No**
Concentration of substrate used	Continuous	Yes

The independent variable I have chosen, or the one to be changed throughout the experiment will be 'the concentration of substrate used', which will range from 0.25M to 1.25M with increments of 0.25M. With reference to the table above, it has been chosen, as it is continuous (i.e. it has a numerical value of some sort and this can be altered) so the results can facilitate a graph.

Dependent Variable
Rate at which the bubbles of oxygen rise, which will be calculated by observing how many bubbles of oxygen rise to the surface of a measuring cylinder (by means of downward displacement) in one minute. This will be measured in bubbles per ten seconds.

Control variables:

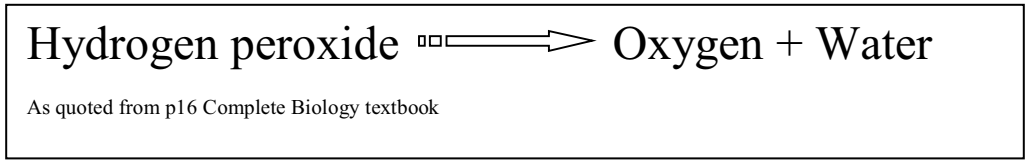
- Volume of substrate used: 100ml
- Temperature: taken place at room temperature 21 degrees centigrade
- Type of substrate used: Hydrogen peroxide
- Mass of meat used: 5g

- Amount of water in the test tube in which the oxygen bubbles downward displaces in the water. This is so the time taken for each individual bubble to effectively rise to the bottom of the test tube will take the same amount of time.
- Number of oxygen bubbles to rise: 30

Prediction

I predict that the higher the concentration of the hydrogen peroxide substrate, the faster thirty bubbles of oxygen will rise. I also think that the relationship will be directly proportional: if I double the concentration of the hydrogen peroxide the rate at which the bubbles are rising will also double. This should facilitate a graph not dissimilar to this:

Scientific Theory



As quoted from p16 Complete Biology textbook

The catalase in the meat samples being used catalyses the breakdown of hydrogen peroxide.

With the enzyme catalase present, the activation energy of the reaction is lowered. The molecules of substrate fit into active sites of enzymes. The substrate now reacts to form a molecule of product to form an enzyme substrate complex. After this the enzyme molecule leaves the active site and the enzyme is now free to react with more substrate.

By increasing the concentration of the solution, we are increasing the number of substrate molecules present in a controlled sample of hydrogen peroxide. In doing this we increase the probability that a substrate molecule of hydrogen peroxide will come in contact with the active site of the catalase enzyme. Therefore the rate of reaction to take place should be higher in higher concentrations.

Diagram of apparatus used

Procedure

1. Fill up a conical flask with 100ml of chosen concentration of hydrogen peroxide.
2. Close it airtight with a bung by attaching a delivery tube to the top of the conical flask.
3. Place the tube into a measuring cylinder already full of water.
4. Make sure that the measuring cylinder is upside down, and the top of it is submerged in a tub of water.
5. Remove the bung from the conical flask momentarily and put the portion of meat into it promptly sealing the flask with the bung again.
6. Using a stopwatch, time how long it takes for thirty bubbles to rise to the bottom of the measuring cylinder through from the delivery tube.
7. Repeat this process with the other concentrations of hydrogen peroxide.

Safety

- Safety goggles must be worn at all times as hydrogen peroxide is a highly corrosive and dangerous liquid, and if it comes into contact with the eye it can result in an irritation and at worst blindness.
- A lab coat should also be worn as it protects the skin against the hydrogen peroxide.
- Hands must be washed after all experiments just in case they have come in contact with the hydrogen peroxide. In addition to this skin may have come into contact with raw meat and get contaminated, so it is necessary to wash your hands.

Preliminary work

For the preliminary work we set up a similar experiment to this one. We kept the concentration of the hydrogen peroxide fixed at 1M (the same was for the weight of the sources). We were looking to find a suitable source for the catalase enzyme. We were able to test for it from the following sources: potatoes, beef and liver. The results were recorded to two decimal places.
Here are the results:

Source of Catalase	**Bubbles of oxygen risen in 1 minute**	**Rate of oxygen bubbles rising per ten seconds**
Beef	48	8
Potato	28	4.67
Liver	N/A	N/A

As a result we decided to use beef as our source for catalase. This was because the liver produced too much oxygen and almost blew the top of the bung off the conical flask, making the test potentially dangerous. The potato experiment took perhaps too long, as we were looking to repeat the experiment two more times in order to obtain an average of results as we were under a time limitation.

Observation

Tabulate results

Experiment 1

Concentration of hydrogen peroxide (M)	No. of bubbles of oxygen risen in 1 minute	Rate of bubbles rising per 10 seconds
0.25	19	3.17
0.50	39	6.50
0.75	57	9.50
1.00	77	12.83
1.25	98	16.33

Experiment 2

Concentration of hydrogen peroxide (M)	No. of bubbles of oxygen risen in 1 minute	Rate of bubbles rising per 10 seconds
0.25	22	3.67
0.50	41	6.83
0.75	55	9.17
1.00	80	13.33
1.25	102	17.00

Experiment 3

Concentration of hydrogen peroxide (M)	No. of bubbles of oxygen risen in 1 minute	Rate of bubbles rising per 10 seconds
0.25	20	3.33
0.50	58	9.67
0.75	61	10.17
1.00	80	13.33
1.25	99	16.50

Average of Experiments 1-3

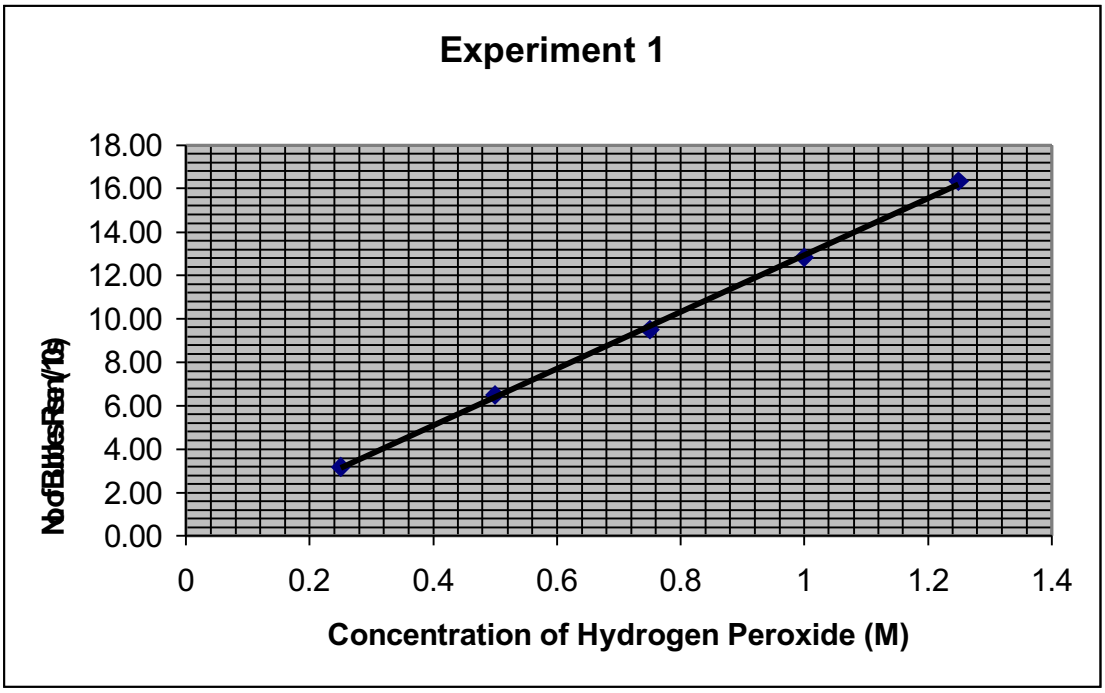
Concentration of hydrogen peroxide (M)	No. of bubbles of oxygen risen in 1 minute	Rate of bubbles rising per 10 seconds
0.25	20	3.39
0.50	40	6.67
0.75	57	9.61
1.00	79	13.17
1.25	99	16.61

*Denotes anomalous result

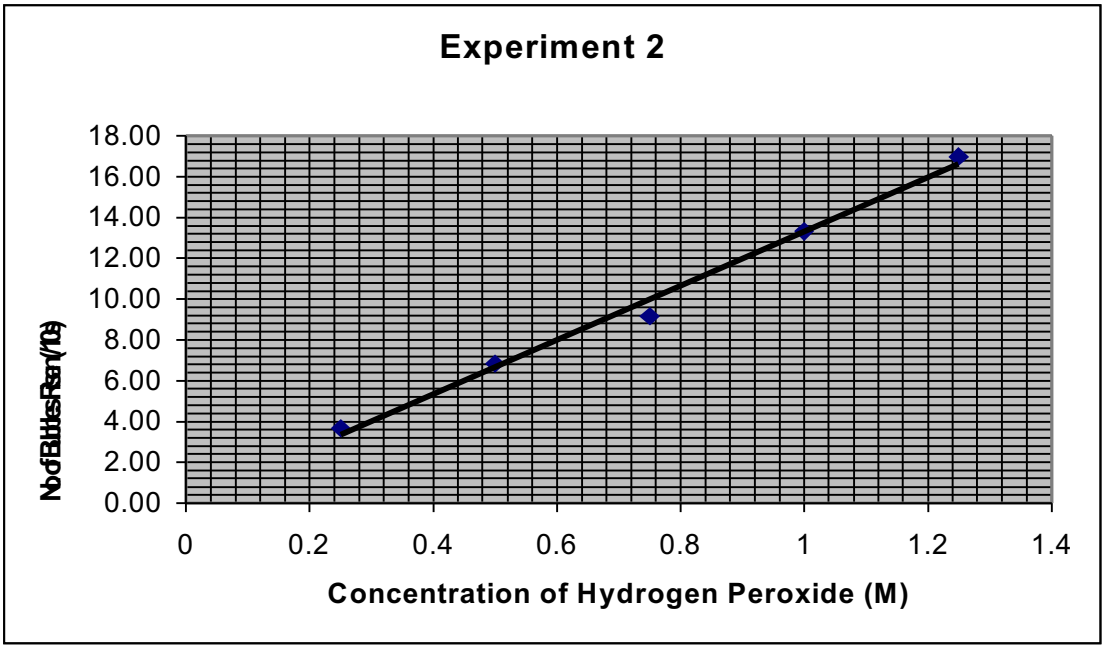
Analysis

Graphs

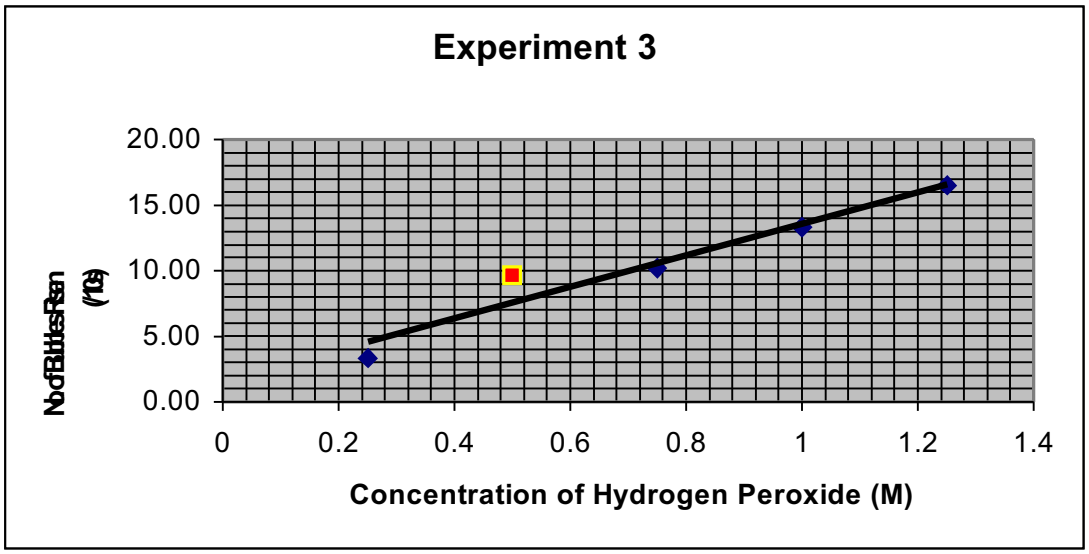
Experiment 1



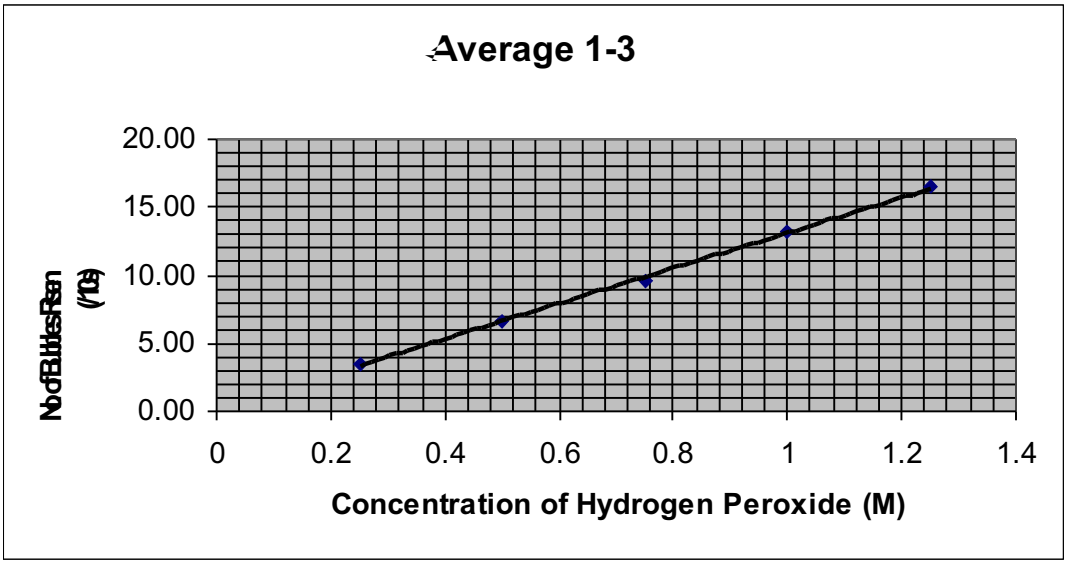
Experiment 2



Experiment 3



Average of experiments 1-3



Analysis

Graphical and Scientific Analysis

An average graph was formed to give us a better idea of what to expect in general from these experiments, and to be sure that the occurrences of one experiment was not a 'one-off'. However, experiment three contained an anomalous result. An anomalous result is a result that is unexpected, or which does not follow the set trend, and in this case it was an irregular rate of oxygen production. An average was obtained for this particular result from only experiment one and two, which has proven sufficient as the line of best fit follows through this plotted point.

The positive correlation of the graph as well as the line of best fit show that the relationship is directly proportional, as if the concentration is increased, the rate of oxygen production increases accordingly. For instance, quoting the average graph made, if 3.39 bubbles per ten seconds are rising from 0.25M of hydrogen peroxide, doubling the strength of the hydrogen peroxide to 0.5M upped the rate to 6.67 per ten seconds. Therefore 3.39 x 2 = 6.78 which is almost exactly 6.67 /10s proving that the relationship is directly proportional. My initial prediction made was therefore correct, and the graph made resembles the graphs in my final analysis. The only slight exception to this is the anomalous result found in experiment three, which will be discussed in the evaluation. This also supports my scientific reasoning, where I stated that the rate of oxygen production will increase as there will be more of a chance of the substrate and enzyme's active site coming into contact as the concentration of hydrogen peroxide is increased. This is represented by the positive correlation of the results.

Evaluation

Overall the experiment was fairly successful as the average graph does show proportional results as predicted initially. The only downside was that there were a limited number of concentrations of hydrogen peroxide. If there were increments of 0.5M between the available concentrations, the line of best fit would have had passed through many more of the points. To overcome this problem next time we would have to set out more time to perform this task. The anomalous result achieved in experiment three might have happened as for this particular experiment the conical flask was washed with warmer water. This would have then warmed up the hydrogen peroxide up slightly. The rate of reaction was therefore faster with this slight increase in temperature, as at low temperatures the substances reacting did not posses as much kinetic energy as with the heat. This in turn increased the rate of collisions increasing the rate of reaction.

Proposals for Further Investigations

In addition to this experiment, higher concentrations of hydrogen peroxide could be used, as it is possible that the rate of oxygen produced will not continuously increase. I would like to see whether there is a maximum ratio of substrate to enzyme achieved, where no higher concentrations give faster rates of reactions. We could say that this would prove my theory that the enzymes would be effectively saturated with the substrate. A manometer could be used to work out exactly how much oxygen (cm^3) is produced instead of the number of bubbles produced to work out an accurate rate of oxygen production (cm^3/s).