Effect of temperature on lipase

Theory The higher the substrate concentration the more quickly product is produced (rate of reaction increases) until enzyme saturation is reached at which time more substrate has no further effect. Enzymes such as Catalase are protein molecules which are found in living cells. They are used to speed up specific reactions in the cells. They are all very specific as each enzyme just performs one particular reaction.

Catalase is an enzyme found in food such as potato and liver. It is used for removing Hydrogen Peroxide from the cells. Hydrogen Peroxide is the poisonous by-product of metabolism. Catalase speeds up the decomposition of Hydrogen Peroxide into water and oxygen as shown in the equations below. It is able to speed up the decomposition of Hydrogen Peroxide because the shape of its active site matches the shape of the Hydrogen Peroxide molecule. This type of reaction where a molecule is broken down into smaller pieces is called an anabolic reaction.

Hypothesis The prediction would be such that as the substrate concentration increases, the rate of reaction will go up at a directly proportional rate until the solution becomes saturated with the substrate hydrogen peroxide. When this saturation point is reached, then adding extra substrate will make no difference. The rate steadily increases when more substrate is added because more of the active sites of the enzyme are being used which results in more reactions so the required amount of oxygen is made more quickly.

Once the amount of substrate molecules added exceeds the number of active sites available then the rate of reaction will no longer go up. This is because the maximum number of reactions are being done at once so any extra substrate molecules have to wait until some of the active sites become

available. Variables Independent Variable(s) Concentration of substrate Dependent Variable(s) Rate of enzyme activity Control Variable(s) Temperature pH Pressure Apparatus S. No Item Qty. Size, Capacity, Amount 1 Graduated cylinder 1 500mL, 500cm3 2 Metal Stand 1 - 3 Catalase from Chicken Liver 1 gm each 4 Hydrogen Peroxide (2%-14%) 1 each 10 mL each 5 Test Tubes 7 - 6 Test Tube Rack 1 - 7 Distilled water - 1 L 8 Stop watch 1 - 9 Pipette 1 10 mL 10 Tub 1 2000mL 11 Cork with hole for transferring tube 7 - 12 Transferring tube 7 - 13 Rubber tube 7 100cm Procedure 1. Add 1gm of chicken liver to one test tube. Add 10mL of hydrogen peroxide solution at a concentration of 2% to the other test tube. Use a pipette to measure out the volumes. It is very important to accurately measure the amounts of Hydrogen Peroxide and chicken liver to ensure a fair test. 2.

Pour the hydrogen peroxide solution into the test tube containing the chicken liver and immediately put the cork with a transferring tube plugged into it connecting it to a rubber tube leading to a filled inverted graduated cylinder to measure the amount of gas in mL (cm3) formed. 3. Bubbles should start to rise up the tube and the water level in the graduated cylinder should move down. 4. Record the water level after every 30 seconds for a total period of 5 minutes. 5. Do the same for 4%, 6%, 8%, 10%, 12% and 14% and record the readings for them individually.

When the concentration of Hydrogen Peroxide is increased, the rate of reaction increases at a directly proportional rate until the concentration of Hydrogen Peroxide reaches about 10%. If you double the concentration of Hydrogen Peroxide then the rate of reaction doubles as well. When the concentration is doubled from 8-16% the rate goes up from 1. 65-2. 97 Cm3

Oxygen produced per second, which is an increase of 1. 8 times. I would expect the rate to increase two times if the Hydrogen Peroxide concentration is increased two times because there are twice as many substrate molecules which can join onto the enzymes active sites.

The reason that the number is less than two times could be put down to the fact that at 10% the Enzyme's active sites may already be close to being saturated with Hydrogen Peroxide. There may also be some experimental error which causes the inaccuracies. After 10% the increase in the rate of reaction slows down. This is shown by the gradient of the graph going down. At this point virtually all the active sites are occupied so the active sites are said to be saturated with Hydrogen Peroxide. Increasing the Hydrogen Peroxide Concentration after the point of saturation has been reached will not cause the rate of reaction to go up any more.

All the active sites are being used so any extra Hydrogen Peroxide molecules will have to wait until an active site becomes available. The theoretical maximum rate of reaction is when all the sites are being used but in reality this theoretical maximum is never reached due to the fact that not all the active sites are being used all the time. The substrate molecules need time to join onto the enzyme and to leave it so the maximum rate achieved is always slightly below the theoretical maximum. The time taken to fit into and leave the active site is the limiting factor in the rate of reaction.

Limitations a) There is a slight delay between pouring the Hydrogen Peroxide into the catalase, putting the bung on and starting the stopwatch. This will slightly affect all the results but as I carried out all the three steps in the same way for all the experiments it should not make any difference to the

overall result. b) It is also impossible to precisely measure out the amounts of Hydrogen Peroxide and catalase each time. As the scale on the pipettes shows the volume to the nearest mm3 the volume of the solutions that I used should be correct to the nearest mm3.

The volume of gas in the test tube to start with is slightly affected by the amount which the bung is pushed down each time, if the bung is pushed down further then the volume in the tube will be less so the 30cm3 of gas is reached faster. c) Due to the fairly slow speed of our reactions it is only possible to measure the time of the reaction to the nearest 0. 1 second even though the stopwatch shows the measurements to the nearest 0. 01 second. d) Human errors such as inappropriate readings, time difference in readings, stopping flow of air by accidentally compressing rubber tube... could also have been made