Optimum temperature for catalase in potato

Hydrogen peroxide is a common by-product produced during metabolism in living organisms. On accumulation, hydrogen peroxide can have various implications on living cells such as skin disorders (Schallreuter & Rokos 2006). Decomposition of hydrogen peroxide gives out harmless water and oxygen, as shown by the equation 2H2O2 (aq) Ã¯Æ’ 2H2O (l) + O2 (g).

The rate of decomposition of hydrogen peroxide is low and it can be increased by an enzyme called Catalase. An enzyme is essentially a biological catalyst that can increase the rate of reaction but remains chemically unchanged at the end of the reaction (Pang 1997, p. 63). Catalase readily speeds up the breakdown of hydrogen peroxide at a rate of millions of hydrogen peroxide molecules per second (Goodsell 2004). It is particularly important in liver cells and kidney cells for removal of any toxins present in the blood stream to maintain health (Alberts et al. 2002).

By varying the temperatures using water baths and measuring the time taken for first bubbling and when bubbling remains constant, the rate of breakdown of hydrogen peroxide can be calculated by the reciprocals of the measured time. The temperature at which the reaction rate is the greatest is referred to as optimum temperature (Pang 1997, p. 70). That is the say, enzyme catalase catalyses the breakdown of hydrogen peroxide the most effectively at this temperature.

Aims

In this experiment, the influence of temperature on the activity catalase is examined. We aim to find out how its activity changes over a range of

temperatures, in order to establish the optimum temperature of this enzyme catalysed reaction.

Methods

Equipment

Water bath (10°C, 35°C, 45°C, 60°C)

Ice

Beakers x2 (for the ice bath)

Thermometer (to ensure that the ice bath is <10°C

Test tubes x10

Non-permanent marker

Timer

Hydrogen Peroxide x200mL

Cork Borer

Scalpels

Watch glasses

Aluminium Foil

Ruler

Potato

Pipettor

Safety

Hydrogen Peroxide (H2O2) is corrosive and hence safety glasses must be worn to prevent eye contact.

The decomposition of Hydrogen Peroxide would produce pure oxygen so combustible materials must be kept well away.

Experimental procedures

Set up the following apparatus according to the following conditions:

Test Tube

Temperature

A

< 10°C

B

35°C

C

45°C

D

60°C

E

Room temperature

Label the test tubes to be used, according to the above table.

Cover the stock H2O2 with aluminium foil to prevent decomposition under light.

Prepare enzyme catalase by inserting through the centre of the potato, with aid of a cork borer.

Using the scalpel and ruler, cut 1cm pellets of potato that was extracted with the cork borer.

Half-fill a beaker with tap water and add in crushed ice to make water bath for A. Wait for several minutes to allow stabilization of temperature.

Insert test tube A, leave for a 3 minutes before using the 10mL pipette to add 8mL H2O2 to the test tube.

Add the 1cm pellet of potato to the test tube.

Start the timer and record the time required for the first bubbling to occur and the time when the amount of bubbles produced remain constant. Record all observations.

Repeat steps 6-8 for test tubes B to E but without addition of ice. Put them directly into the water baths available in the laboratory.

Repeat steps 4-10 twice, recording all observations and results.

Average the results obtained for test tubes A-E in each experiment (ignoring outliers), and plot your results against temperature.

Extrapolate the graph to determine the optimum temperature for enzyme activity.

Discussion

To achieve results with greater accuracy, we have taken several precautions. Firstly, I was the person who recorded the time throughout the experiment and this could avoid discrepancy caused by different reaction times among individuals. Secondly, the use of cork borer might ensure uniform sizes of potatoes so that the amount of catalase would be relatively the same. Thirdly, stock H2O2 solution was wrapped to reduce unwanted decomposition under light. Fourthly, test tubes with potatoes were put into the water baths for a few minutes before adding H2O2 and this allowed the temperature of the content to reach that of the water baths. Lastly, no temperature was applied to tube E (at room temperature) and it acted as a control to show that the changes in activity of catalase resulted from changes in temperatures.

From our results, enzyme activity is low at very low (0. 5°C) and high (61. 2°C) temperatures. At very low temperatures, substrate and enzyme molecules lack energy for collisions and hence binding to catalase reactions; and at very high temperatures, the alteration of the binding site of enzyme sets in and the denatured enzyme catalyses reactions with decreasing efficiency (Pang 1997, p. 70). The optimum temperature for catalase activity was around 35. 3°C, as indicated by the peak in figure 2. This agrees with

the research conducted by Yumoto et al. (1999, p. 67), in which catalase works the best at about 30°C. However, this does not agree with our findings from figure 1 (optimum temperature at around 40°C), whereas the peak activity occurs at 35. 3°C and 43. 5°C. This might be explained by the fact that first bubbling occurred within a few seconds on addition of H2O2 to potato and it was difficult to measure this time precisely. Therefore, the time taken for bubbling remained constant might be a better representation of our experimental outcomes.

As regards the observations, it is evident that the colourless gas bubbles are oxygen and the reason why potato sank to the bottom might be explained in terms of density. As dilute H2O2 solution was used, the density of solution can be assumed to be equal to water, which is approximately 0. 9970gcm-3 at room temperature (Aylward & Findlay 2008, p. 154). It is reasonable to predict that potato is essentially denser than water and thus it sinks. However, the gaseous oxygen produced on the surface of the potato can produce an upthrust to push the potato upwards (Goodwin 2002). Therefore the potato temporally floats on the surface. When the gaseous oxygen is discharged at the surface, the effect of density takes priority again, causing the potato to sink.

Despite of careful design of our protocol, some experimental errors could have arisen. The 8ml H2O2 was added on a 4ml basis by a pipettor and the timer was started at the first addition. In other words, the measured time could have differed from the actual one by several seconds. This inaccuracy might be improved by the use of graduated pipette so that the 8ml solution could be added via one addition without any delays. Moreover, we forgot to

dry the test tubes completely in some of our trials and this could have caused dilution of the H2O2. This error could be fixed by the use of long cotton sticks to dry the inner parts of the test tubes. Furthermore, the judgements of whether or not the amount of bubbles remained relatively the same might be subjective and this problem could be solved by addressing our focus on the volume of oxygen evolved instead. For example, we might collect the oxygen over water and measure the volume of it every 30 seconds for 5 minutes with a calibrated syringe (Morris 2006). In this way, we might achieve a better measurement of the reaction rate. From this defect in the design, I realized the importance of consulting more sources rather than relying on our own knowledge as we lack experience in experimental design.

Conclusion

In conclusion, enzyme catalase exhibits low activity at low temperatures (0. 5°C) and high temperatures (61. 2°C). Its activity is the greatest at around 35°C. The experimental set-up was generally satisfactory to minimize errors except of some defects such as the methodology in measuring the rate of the reaction. It is suggested that more research should be done in designing the experimental protocol.