Effect of enzyme catalase on hydrogen peroxide

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Introduction

In this experiment, I am going to determine the effect of different concentration of enzyme catalase on the rate of reaction of decomposition of hydrogen peroxide. Normally, hydrogen peroxide is produced naturally in human or plant cell. Hydrogen peroxide is the by-product of respiration. As an oxidizer, it will decompose to form oxygen and water. The chemical equation for the decomposition of hydrogen peroxide is 2 H2O2 → 2 H2O + O2. The reaction is speeded up by the presence of enzyme, namely catalase which is used in this experiment. This mechanism is important in living organisms' cells and body system particularly in human. This is because the corrosive characteristic of hydrogen peroxide may damage the wall of liver where it is largely produced during cellular respiration process. When it is present in high concentration, it is an aggressive and powerful oxidizer, whereby it is unstable and also hazardous as it will corrode many substances including human skin. Therefore, concentration of hydrogen peroxide in the cell should be constantly regulated. When hydrogen peroxide is used for the purpose of experiment, this highly corrosive material should be kept in a container made up of non-reactive material such as glass. However, at low concentration, hydrogen peroxide can be used as disinfectant and antiseptic for medicinal uses.

In this context, catalase, a teramer of four polypeptide chains is made up of over 500 amino acids long. It is also categorised as globular protein in which the polypeptide chain is highly folded into a compact spherical shape. There is also active site available to bind to the hydrogen peroxide substrate to form enzyme-substrate complex. It is further adapted with four porphyrin

heme groups to react with hydrogen peroxide. Besides, the enzyme catalase is known to be one of the enzymes that possess a high turnover number. Its turnover number can be up to 600 000 whereby one molecule of enzyme catalase can catalyse the decomposition of 600 000 molecules of hydrogen peroxide to oxygen and water at body temperature. This reaction is known as catabolic reaction as the hydrogen peroxide molecule is broken down into oxygen and water which are comparatively smaller. Sometimes, catalase also uses hydrogen peroxide to oxidise toxins including Phenols, Formic Acid, Formaldehyde and Alcohols. In this experiment, potato is chosen to be tested due to the presence of catalase in it. However, other organisms such as fungi or yeast can be used as well as they are producers of enzyme catalase.

Enzyme is used to speed up the rate of reaction by lowering the activation energy of a reaction. Activation energy or free energy of activation, is the initial investment of energy for starting a reaction – the energy required to contort the reactant molecules so the bond can break for a reaction to occur. Enzyme functions as biological catalyst in many chemical reactions that occur inside our body. For example, saliva secretes enzyme amylase which catalyses the hydrolysis of carbohydrates in the mouth. Not only does enzyme play an important role in maintaining efficient function of body system, it is largely used in industrial field as well to speed up the production rate. For example, protease is commonly used in biological detergent for domestic washing and rennin is used in manufacture of cheese. For an enzyme to carry out its function effectively, active site should present on the surface of the polypeptide chain. An active site is a groove or pocket formed by the folding pattern of the protein. This active site has particular chemical

composition and electrical charges on the amino acids, which make up the specificity of the enzyme, in which it allows only certain substances to bind to it. When the substrates bind to the active site, here the working mechanism of enzyme starts. The binding of the substrate to the active site bring the substrates closer and thus aids in bond formation in anabolic reaction. In catabolic reaction, the active site may distort the shape of substrate to break its bond. When the products are formed, the substances no longer fit into the specific shape of the enzyme and will leave the active site of the enzyme. The enzyme is free to bind to another substrate and catalyse another reaction. The enzyme is not altered at the end of reaction.

As enzyme contains specific shape and charge on its active site, its activity is easily affected by the changes in the surrounding conditions. Generally, different pH, temperature, concentration of substrate or concentration of enzyme has a large impact on its efficiency in carrying out its function. Whenever the changes in surrounding such as change in pH or temperature alter the bonding between the R group of the amino acids in the polypeptide chain which form the active site, the shape of active site will change and thus the substrate will no longer bind to the site. At this point, the enzyme is said to be denatured. On the other side, when the temperature or pH is optimum for the reaction, the rate of reaction is the highest. Although the optimum pH and temperature may vary from one another, optimum temperature for most enzymes functioning in human body system is often 37 °C. However, the presence of inhibitors or cofactors may alter the enzyme activity as well. In this experiment, the effect of enzyme concentration is chosen to be investigated on the rate of reaction catalysed by enzyme

catalase. An increase in enzyme concentration will increase the active site available and thus increase the rate of reaction until it reaches maximum velocity when all active sites of the enzyme molecules are engaged.

Problem Statement:

Do different concentrations of enzyme affect the rate of reaction?

Objectives:

- To investigate the effect of different concentrations of catalase on the rate of reaction to catalyse the decomposition reaction of hydrogen peroxide
- To determine the presence of catalase on the rate of reaction of hydrogen peroxide.
- To develop effective experimental skills throughout the experiment

Aim:

To determine the effect of different concentrations of enzyme on the enzyme activity

Hypothesis:

The higher the concentration of enzyme, the higher the rate of reaction until a maximum velocity is reached.

Techniques:

Use a water displacement technique to determine the volume of oxygen gas evolved

Calculate the rate of reaction by using the gradient of the graph

Materials:

Freshly mashed or blended potato, 3. 0 % hydrogen peroxide solution, buffer solution (pH 6. 5), distilled water

Apparatus:

Boiling tubes, graduated tubes, 500 ml beaker, weighing balance, spatula, delivery tube, stop watch, measuring cylinder, dropper, rubber bung, weighing dish

Variables:

Variable

How the variable is determined

1. Manipulated

Concentration of catalase

By using different mass of blended potato at 1g, 2g, 3g and 4g. Different masses of blended potato indicate the difference in concentration of catalase in its content.

2. Responding

The volume of oxygen gas released

By recording down the reading on the graduated tubes at 30 seconds interval.

3. Constant

pH

Volume and Concentration of hydrogen peroxide

By using buffer solutions at pH 6. 8 throughout the experiments

By using the same volume and concentration of hydrogen peroxide, which is 2. 5cm3of 3. 0 % hydrogen peroxide throughout the experiment

Procedure:

1 g of the freshly prepared or blended potato is transferred into a boiling tube.

5 cm3 of buffer solution is added into the tube and it is swirled to mix the substrate.

A graduated tube is filled with water to the brim.

It is placed carefully into a beaker of water. One end of the delivery tube is placed into the graduated tube with the other end with rubber bung ready to fix with boiling tube.

2. 5 cm3 of hydrogen peroxide solution is measured and it is added into the boiling tube containing the potato and buffer solution.

The tube is immediately closed with a rubber bung connected to the delivery tube. A stopwatch is started by one member of the pairs in conducting this experiment.

The volume of gas released is measured for every 30 seconds for 5 minutes or until the gas evolution stops.

The experiment is repeated using 2g, 3g and 4g of freshly blended potato.

The results obtained are recorded in a table.

Graphs for volume of gas released against time is plotted for each concentration or amount of enzyme used.

The initial rate of reaction for each concentrations of enzyme used are worked out.

Discussion:

Based on the above experiment, the effect of different concentrations of enzyme on the rate of reaction is successfully determined. Five graphs are plotted based on the results obtained in the experiment to show the data in a clearer way and provides a better mean for analysing. The results show that the rate of reaction is increased by an increase in enzyme concentration. In this experiment, potato is used as source of catalyse. The first four graphs showing oxygen gas evolved against time are drawn based on respective mass of blended potato used. The initial rate of reaction is measured from each graph by obtaining the gradient of the graph. A predicted line is drawn on each graph. Generally, the longer the time taken, the higher the volume of oxygen gas evolved. In the beginning, all graphs show an rapid increase , the speed is the slow down as some of the substrates are converted to products. For the substrate at 1 and 2 g of bended potato used, the maximum volume of oxygen gas evolved has reached within 300 seconds and a plateau is obtained. This is because the reaction has completed for all substrates. Theoretically, the maximum volume of oxygen gas released should takes a shorter time as compared to 1g and 2 g of potato as more active site are offered. However, In the 3 and 4

g of blended potato which react, the maximum volume of oxygen is unable to be obtained within 300 seconds. This is probably due to some errors conducted throughout the experiment, particularly due to the vigorous and rapid reaction and in the process of changing the graduated tube. The errors will be discussed later. The initial rate is taken because the rate of reaction is rapid as the collision between the substrate and enzyme is the highest. The rate of reaction may not be reliable to be compared between data if readings are taken in the middle of the experiment because some reactions have reached the maximum rate. The initial rate of reaction for hydrogen peroxide with 1g, 2g, 3g and 4g of blended potatoes are 0. 0611, 0. 2895, 0. 6579 and 0. 7000 cm3/ s respectively.

The initial rate of reactions for all the experiments are then compiled into the fifth graph. This shows a clearer picture on the effect of concentration of substrate on the rate of reaction. Initially, there is an increase in the rate of reaction when the mass of blended potato increases. This is because the increase in the concentration of enzyme offers more active site for the binding of substrate. Then, the slope of increasing line becomes less steep with further increase in concentration of enzyme. This is because the active site has been occupied by the substrates or it is said to be saturated whereby the increase in substrate has no further effect on the rate of reaction. Theoretically, the graph should reach a maximum velocity where the plateau occurs in the graph. However, in this experiment, the plateau is not shown because most probably the concentration of enzyme is not high enough to bind to all the 3. 0 % of hydrogen peroxide substrate.

However, throughout the experiment some errors might occur in which the real values may not be obtained. Firstly, there is a high tendency for the reading obtained from water displacement method to be inaccurate especially when the volume of oxygen gas evolved are too much that the first graduated tube is fully filled with oxygen gas and when the delivery tube has to be transferred to the next prior-prepared graduated tube. The delivery tube transferring process may consume some time particularly if a rubber delivery tube is used instead of a glass delivery tube. This will cause some of the oxygen gas to escape into the water during the process. Next, parallax error may occur as well when the reading is taken from the graduated tube on the volume of oxygen gas evolved. This is because oxygen gas is a colourless gas, in which its level is not so clearly seen on the calibration of the graduated tube. To minimise the errors, the experiment is repeated twice and the mean reading is obtained. To further increase the accuracy of the results, a piece of white paper can be placed behind the graduated tube to make the reading easier. Next, the possible error is greater if the experiment is carried out individually. This is due to the human limited ability to record the reading and at the same time watch over the time. Inaccuracy may arise. In this case, a pair work is preferred in this experiment as one of the members times and the other one record the readings obtained. Next, when the mashed potato is poured into the boiling tube from the weighing dish, some potato may be left in the weighing dish. To minimise this error, a few drops of distilled water can be used to rinse the weighing dish to ensure there is no residue left.

Consequently, there are a few precautions that ought to be taken to increase the accuracy of the results obtained. For each experiment, the potato used must be freshly mashed or blended. If the potato is prepared in a container, the lid of the container should be kept closed after the desired mass of blended potato is scooped out for each and every experiment. The preparation of blended potato in a beaker which is exposed to the air should be prevented because oxidation will occur and this may affect the activity of enzyme catalase in it. Changes in surrounding such as temperature may also induce changes in the enzyme. A blended potato is used instead of discs of potato so that it will react easier. Its viscosity should be reduced so that it is easier to use. Next, hydrogen peroxide has to be stored in an opaque container as it breaks down quickly when exposed to light. The lid of the container that contains hydrogen peroxide solution should be kept closed after each desired sample is taken out using a dropper as the oxygen in the surrounding air may oxidise its content and causes the results to be inaccurate. A buffer solution is used to ensure the pH is kept constant throughout the experiment. The buffer solution of citric acid sodium phosphate solution which has a pH of 6. 8 is used because this is the optimum pH for the enzyme catalase. Furthermore, a water bath is preferable as the surrounding temperature may change throughout the experiment. In addition, as the rubber bung of the delivery tube should be of the same size as the boiling tube to ensure all the opening of the boiling tube containing enzyme and substrate is fit tightly, it should be pushed and twisted with care. It should also be checked from time to time to ensure there is no leakage of product in gaseous form to the surrounding. Besides, the other open end of delivery tube should be placed in water all the time for

the bubble of gas to form and rise to its surface. The presence of air bubbles ensure that the rubber bung is still in contact with the boiling tube unless the substrate and enzyme has completely reacted. To fix the graduated tube in place, a retort stand and clamp can be used. Besides, the boiling tube containing reactants and enzyme ought to be swirled throughout the experiment to ensure the substrate and enzyme react. This may increase the rate of collision between the reactants and enzymes and thus fasten the time taken for the reaction to complete.

Throughout the experiment, some safety measures should be abided by. As the substrate used in this experiment which is hydrogen peroxide is highly corrosive, rubber glove should be used to protect the skin. After the hydrogen peroxide is used, it should be disposed off and not to be returned to stock bottles as any contaminants may result in decomposition and explosion may occur. The blended potatoes have to be handled carefully as well as it will irritate some people’s skin. A lab coat should be put on. The glass wares and the delivery tube used should be handled carefully as they are fragile.

Conclusion:

The hypothesis is accepted. The presence of enzyme increases the rate of reaction of hydrogen peroxide. When the concentration of enzyme increases, the rate of reaction increases until a maximum velocity is reached.

Limitations:

The species of potato

- Different species of potato may contain various concentration sof catalase

The age of potato

- An older potato may have lower concentration of catalase

The freshness of potato

- The concentration of catalase may vary in different potatoes which are stored in different ways before experiment. Storage at high temperature may cause the enzyme to denature

Part of potato used

- Different parts on the potato may have different amount of catalase.

Further Work:

- The effect of temperature on the enzyme activity
- The effect of different concentrations of substrate on the enzyme activity
- The effect of pH on the enzyme activity
- The effect of concentrations of enzyme on activity of other type of enzyme such as amylase on starch
- The effect on the rate of reaction of hydrogen peroxide by using different concentration of fungi as the source of catalase