Bio Factsheet

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Investigating Catalase

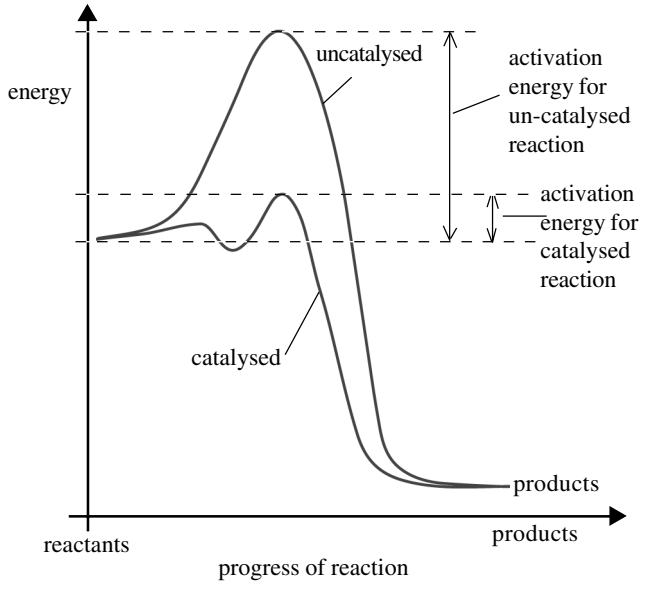
This Factsheet
- Outlines the importance of catalase;
- Describes how catalase can be used to investigate the general properties of enzymes;
- Analyses common errors that students make in carrying out and writing up investigations on catalase.

The enzyme catalase is found in both plant and animal cells. Catalase breaks down hydrogen peroxide – a toxic by-product of metabolism - into water and oxygen.

$$\underset{\text{Reactant}}{2H_2O_2} \xrightarrow{\text{catalase}} \underset{\text{Products}}{2H_2O + O_2}$$

Hydrogen peroxide is a powerful oxidising agent and will kill cells unless it is broken down rapidly. Catalase is extremely fast-acting: in human cells, catalase can break down 600,000 molecules of hydrogen peroxide per second. Like all enzymes, catalase speeds up reactions by lowering the amount of energy needed to activate the reacting molecules - the activation energy (Fig 1).

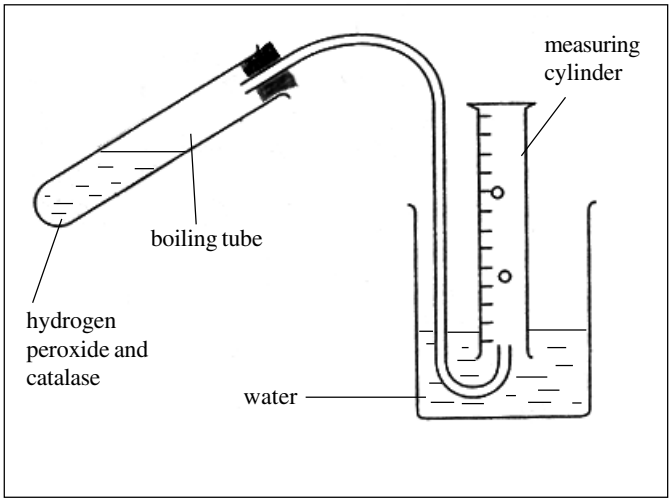
Fig 1. Activation energy



Metabolic reactions that produce **hydrogen peroxide**
Oxidation of lactic acid in liver cells
Photorespiration in mesophyll cells

It is easy to practically demonstrate the activity of catalase.

1. Hydrogen peroxide and catalase can be mixed in a boiling tube connected to an inverted measuring cylinder. The volume of oxygen evolved can then be easily recorded.



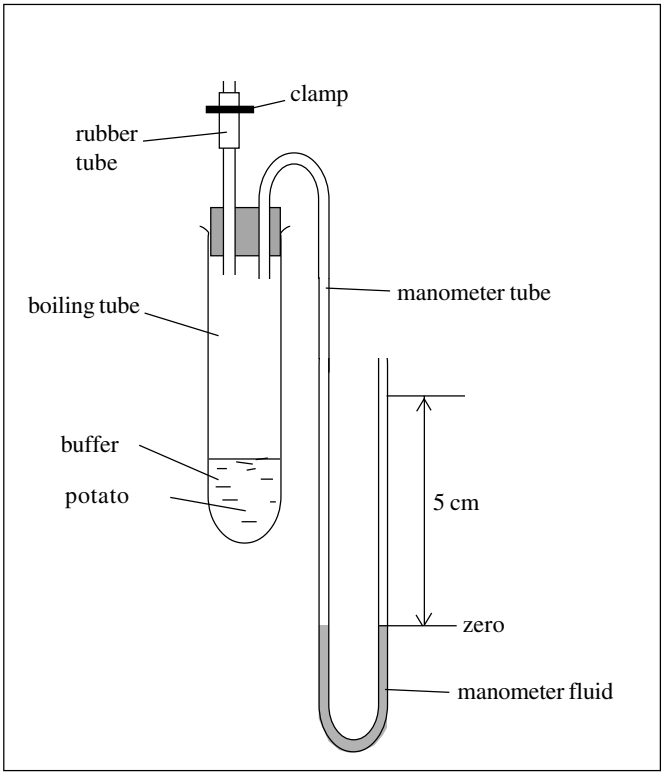
2. This apparatus can also be used to investigate:

   - the effect of pH or a specific enzyme inhibitor, for example, on the rate of catalase activity
   - the relative concentration of catalase in different tissues. Potato, apple, turnip and liver are commonly used. If pieces of tissue from liver, potato, turnip and apple, for example, are each treated with hydrogen peroxide, the resulting effervescence indicates oxygen production as the catalase in the tissue breaks the hydrogen peroxide down. The relative amount of catalase in each tissue can then be estimated by:
     - measuring the height of the effervescence
     - measuring the rate at which oxygen is released by using a manometer
     - measuring the temperature increase (the reaction is strongly exothermic)
     - titrating the enzyme solution with potassium permanganate – this is an assay, whereby the amount of potassium permanganate used is proportional to the amount of hydrogen peroxide remaining

Student investigation

A student carried out an investigation to determine the relative concentrations of catalase in three tissues: potato, apple and liver. The student's method is shown below. Read the method and then answer the questions that follow.

Equal portions of each tissue were prepared. Each tissue was ground up using a pestle and mortar and then placed in a boiling tube which had a simple manometer attached.



5cm^3 of hydrogen peroxide was then added to the boiling tube containing the tissue using a pipette. The tissue fizzed. The level of the liquid in the left - hand arm of the manometer went down and the time taken for the level in the right arm to move up by 5cm was recorded.

This procedure was repeated with each tissue. The results are shown in the table below.

Tissue	Time taken for liquid in manometer to rise by 5cm (secs)
Potato	65
Apple	80
Liver	15

(a) Suggest why the student crushed each tissue [2]

(b) Explain the movement of the liquid in the manometer [2]

(c) Suggest three ways in which the student's method could be improved [3]

(d) Suggest an explanation for the relative concentration of catalase in the three tissue samples [3]

Peroxisomes: organelles with a single boundary membrane that contain high concentrations of enzymes that break down fatty acids and amino acids. The breakdown of these substances produces hydrogen peroxide and for this reason peroxisomes also contain high concentrations of catalase. Peroxisomes are most abundant in humans in liver and kidney cells but they may also be found in large numbers in leaves and seeds.

Nice 3-d structure of catalase http://crystal.uah.edu/~carter/enzyme/catalase.htm

Enzyme activity theory

Increasing temperature usually increases the rate of an enzymatic reaction because the enzyme and substrate molecules have greater kinetic energy and therefore have more collisions, some of which result in reaction. Above a certain temperature however, the bonds responsible for the tertiary structure of the enzyme are destroyed and thus the shape of the **active site** is changed. The enzyme cannot then form enzyme-substrate complexes and is said to be **denatured**.

Changing the pH away from the enzyme's optimum will also quickly reduce the rate of reaction. This is because changing pH will change the ionic charge of the amino and carboxyl groups of the amino acids that make up the active site. Again, this will change the shape of the active site.

Increasing the concentration of substrate – in this case, the hydrogen peroxide – will increase the rate of reaction because more of the enzyme's active sites will be occupied faster. Initially then, the graph of rate of reaction against substrate concentration is a straight line and this tells us that the rate of reaction is not limited by the concentration of the substrate. However, at a certain substrate concentration the rate will level off and further increases in the concentration of the substrate will have no effect on the rate of reaction. This is because there is now so much substrate – hydrogen peroxide – that all of the enzyme's active sites are full so the reaction cannot proceed any faster.

Increasing the concentration of enzyme will usually increase the rate of reaction because there are more active sites.

Answers

(a) Break open cells to release catalase;
Speed up investigation;

(b) Oxygen evolved as catalase in tissue breaks down the hydrogen peroxide;
Increased pressure pushes liquid down in left arm and up in right arm;

(c) Equal mass of each tissue;
Macerate in distilled water and filter;
Repeat test for each tissue e.g. three samples of apple etc to get average;
Remove skin on apple and potato;
Shake samples to ensure sample mixes with hydrogen peroxide
Measure temperature change to see if consistent with oxygen evolution;

(d) Liver has greatest concentration of catalase because metabolically very active and therefore cells produce a lot of hydrogen peroxide;
Potato intermediate level of catalase – synthesis/hydrolysis of starch will generate hydrogen peroxide;
Apple least because least metabolically active – function of fruit is simply to attract organisms to eat it in order to bring about seed dispersal.

Acknowledgements:
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