At Junior Certificate level you carried out two experiments using catalysts – (a) the production of oxygen gas from hydrogen peroxide using the inorganic black powder, manganese dioxide, as the catalyst and (b) the breakdown of starch to maltose sugar using amylase as the organic or biological catalyst.

A **catalyst** is a substance that alters the rate of a chemical reaction without being used up in that reaction. A biological catalyst is known as an **enzyme**. Substances that slow down the rate of a reaction are known as inhibitors or negative catalysts.

Enzymes are positive catalysts speeding up reactions by as much as a hundred million million times. They are very selective; they typically affect only one specific reaction; for example, \( \alpha \)-amylase breaks starch into glucose subunits.

Enzymes are essential for life and an understanding of how they operate is important for medicine and industrial biochemistry.

### How enzymes operate – the active site theory of enzyme action

1. The active site of the enzyme has a shape closely complementary to the substrate.
2. The substrate locks into the enzyme’s active site.
3. The shape of the active site changes slightly gripping the substrate more tightly and straining it; an enzyme-substrate complex is formed.
4. The substrate undergoes a chemical change – the new substance (the product) is released from the active site. The active site springs back to its original shape and is ready to receive fresh substrate.

Note: For enzymes to operate they must be able to make contact with the substrate; the enzyme or substrate or both must be in solution, i.e. in rapid random motion in water. Our cells are about 90% water and water is the medium in which most of our biochemistry takes place.

### Six enzyme practicals using catalase to decompose hydrogen peroxide

\[
2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2
\]

Catalase is present in the cells of fungi, plants and animals.

Hydrogen peroxide is poisonous and is a dangerous waste product of many different biochemical reactions that occur in our cells. Our cells produce the enzyme catalase which decomposes hydrogen peroxide to water and oxygen. Catalase operates very quickly – one molecule of catalase can break down 40 million hydrogen peroxide molecules a second.

The six enzyme practicals are easy – and very similar. The first one is to demonstrate the action of an enzyme; here you will show that catalase greatly speeds up the decomposition of hydrogen peroxide.

Then you will discover the effect of:

- a) **temperature** – repeating the same experiment at different temperatures.
- b) **\( \text{pH} \)** – repeating the same experiment at different **\( \text{pH} \)** values.
- c) **enzyme concentration** – the same experiment but using different quantities of catalase.
- d) **substrate concentration** – the same experiment but using different concentrations of hydrogen peroxide.
- e) **heat denaturation** – the same experiment but using catalase that has been boiled.

### Requirements (materials and apparatus)

A potato, a cork borer, a blade, a Petri dish, hydrogen peroxide solution (20% and other concentrations), a range of **\( \text{pH} \)** buffer solutions, a water bath, thermometer, several identical boiling tubes and a ruler (or 50-100 mL graduated cylinders), a beaker or test tube rack.

1. **To show that catalase speeds up the decomposition of hydrogen peroxide**...

### Preparing the catalase

**Fresh potato tissue contains** catalase.

1. Remove a solid cylinder of fresh potato tissue using a cork borer.

2. Using a sharp one-sided blade slice the cylinder into discs 1 millimetre thick and place them in a Petri dish of water. Ten potato discs will be needed for each boiling tube.

### Running the practical

Set up the apparatus as shown in the diagram including the control.

### The apparatus set up for catalase

![Diagram showing setup for catalase experiment]

**Record the procedure using labelled diagrams.**

The substrate hydrogen peroxide solution (20%); the same volume is used in each test tube. The **\( \text{pH} \)** is constant – the same volume of **\( \text{pH} 7 \)** buffer is used in both. The temperature is kept constant at 25°C using a heated water bath.

The control is essential – it is the same set up as the experiment but without the raw potato discs; it is intended to show that only the enzyme is responsible for the rapid decomposition of hydrogen peroxide.
Results
Experiment (catalase present): a good height of froth is formed. Control (catalase absent): no froth.
Conclusion: catalase greatly speeds up the decomposition of hydrogen peroxide.

2. To determine the effect of temperature on the rate of enzyme action.
Repeat practical 1 at different temperatures.
- 0°C – use a large beaker of crushed ice to surround the water bath, 20°C – room temperature, use a water bath that has warmed up to room temperature.
- Use a heated water bath for other temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C).

Keep the other factors constant.
After a minute measure and record the height of froth produced in each boiling tube; the greater the height, the faster the reaction. Record the results for a range of temperatures.

Results
Control results: no froth. Experiment results: record the height of froth for each pH.
Graph the results – temperature on x-axis; height on the y-axis.

3. To determine the effect of pH on the rate of enzyme action.
Repeat practical at 25°C and at a range of pH values (pH 2, 4, 6, 7, 8, 10 and 12) but keeping other factors the same.
After a minute measure and record the height of froth at each pH.
Repeat at a range of temperatures to verify the results. Record the height of froth in each case. Graph the results – pH on x-axis; height of froth on the y-axis.

4. To determine the effect of substrate concentration on the rate of enzyme action.
Repeat practical 1 using different concentrations of hydrogen peroxide solution – 5%, 10%, 20%, 30%, 40%, and 50%. Keep the other factors constant. Graph the results putting substrate concentration on the x-axis.

5. To determine the effect of enzyme concentration on the rate of enzyme action.
Repeat practical 1 using different concentrations of catalase and different numbers of potato discs – 2, 5, 10, 15, 20 and 25 discs. Keep the substrate, temperature and pH fixed. Repeat the experiment a number of times to ensure that the results are consistent. Graph the results putting enzyme concentration on the x-axis.

6. To determine the effect of heat denaturation on the rate of enzyme action.
Repeat practical 1 using potato discs that had been boiled for 3 minutes. Use fresh potato discs in the control.
Repeat the experiment a number of times to verify the results. Conclusion: boiling (excessive temperature) destroys the catalytic effect of the enzyme.
Catalase is an enzyme that rapidly speeds up the decomposition of hydrogen peroxide to water and oxygen.

**Learning Outcomes**

On completing this section, the student will be able to:

- Define catalyst and enzyme
- Outline the ‘active site theory’ of enzyme action
- Explain how enzymes are specific
- List at least four factors that affect the rate of enzyme action
- Describe an experiment to show the action of a named enzyme.
- Appreciate the necessity for a ‘control’ experiment.
- Carry out an enzyme experiment at different temperatures, pH values, enzyme concentrations and substrate concentrations.
- Graph the experimental results
- Explain what is meant by heat denaturation of an enzyme.

**True/False Questions**

(a) An enzyme is a positive biological catalyst.  
(b) Enzymes slow down the rate of biochemical reactions.  
(c) Each particular type of biochemical reaction has its own enzyme.  
(d) Catalase speeds up the decomposition of hydrogen peroxide.  
(e) The rate of enzyme action is not affected by temperature.  
(f) pH is kept constant by use of a buffer.  
(g) Catalase operates best at pH 2.  
(h) Enzyme action decreases with increase of enzyme concentration.  
(i) Catalase operates fastest at approx 40°C.  
(j) Excessive temperature destroys the catalytic ability of enzymes.  

Check your answers to these questions on www.sta.ie

**Examination Questions**

**Leaving Certificate Biology, 2007 Higher Level**

Q7 (a)

(i) What is meant by an enzyme?  
(ii) Give an example of a protein that has a structural role.

Q7 (b)  Answer the following questions in relation to an investigation that you have carried out to determine the effect of temperature on enzyme action.
(i) Name the enzyme that you used.
(ii) Name the substrate of the enzyme.
(iii) State one factor that you kept constant during the investigation.
(iv) How did you keep this factor constant?
(v) How did you vary the temperature?
(vi) How did you measure the rate of activity of the enzyme?
(vii) What was the result of your investigation?

Q11 (c) Enzymes can be immobilised and then used in bioprocessing.

(i) What is meant by immobilising?
(ii) Name a substance that is used to immobilise enzymes.
(iii) Give two advantages of using immobilised enzymes.
(iv) Give one application of a named immobilised enzyme. In your answer, refer to substrate, enzyme and product.

Leaving Certificate Biology, 2006 Higher Level

Q3 The graph shows how the rate of reaction of a carbohydrate-digesting enzyme in the human alimentary canal varies with pH.

(a) Name a carbohydrate-digesting enzyme in the human alimentary canal.
(b) Where in the alimentary canal does this enzyme act?
(c) State the enzyme’s product(s).
(d) What is the pH at A?
(e) A is said to be the enzyme’s ......................... pH.
(f) Suggest a temperature at which human enzymes work best.
(g) What term describes the shape of an enzyme?

Junior Certificate Science, 2007 Higher Level

Q2 (b) In the small intestine starch is broken down to maltose by amylase. Identify the enzyme, and the substrate named in the reaction above.

Junior Certificate Science, 2006 Ordinary Level

Q2 (c)

(iii) Salivary amylase found in the mouth acts on starch in the food we eat. This action can be investigated in the laboratory.

Name the chemical used to test for the presence of starch at the beginning of the experiment.

When the salivary amylase is added to starch solution and the mixture placed in a water bath at 37°C for 5 minutes, a new product is formed.

Name the product formed.

Another chemical is used to test for the presence of this new product. The chemical reacts with the new product to produce a brick-red colour when they are heated together in a hot water bath for 5 minutes. Name this chemical.

Did You Know?

A typical human cell, which is about a billionth the size of a drop of water, has about 3,000 different types of enzymes operating at any given time. So in each of our hundred trillion cells about 3,000 different biochemical reactions are running in perfect coordination. Imagine the ‘fun’ you would have trying to run 10 different experiments at the same time in the lab; living cells are amazing chemical factories.

Bacteria use special enzymes to protect themselves against viruses. They have special digestive enzymes to chop up the DNA injected into them by the virus attackers. By using a special chemical ‘trick’ the bacteria protect their own DNA from these special protective enzymes. Molecular biologist and genetic engineers use these bacterial enzymes to produce genetically modified organisms by ‘cutting and pasting’ new genes or sections of DNA into the DNA of the cells of the host organism.

Most of the more than 5,000 known genetic diseases are due to the absence of a particular enzyme because the gene carrying the information for that enzyme is faulty. Haemophilia, in which blood does not clot, is mostly due to the absence of a particular enzyme in the sequence of chemical changes needed to bring about the conversion of a spherical soluble protein (fibrinogen) in blood plasma to an insoluble thread-like protein (fibrin).

Biographical Notes

Important scientists in the history of enzymes are:

Anselme Payen the French chemist who in 1833 showed that a substance from a malt (germinated barley or other grain) extract speeded up the conversion of starch to sugar. He named this substance diastase and it was the first enzyme.

Theodore Schwann the German physiologist extracted a substance from animal stomach tissue that speeded up the dissolving of meat. He called the substance pepsin form the Greek word pepsis meaning ‘digestion.’

Eduard Buchner who brought about alcoholic fermentation using material extracted from dead yeast.

James Sumner the American biochemist in 1926 proved that enzymes were proteins by isolating the enzyme urease in pure form.

Emil Fisher in 1894 put forward the ‘lock and key’ hypothesis of enzyme action now modified as the ‘active site theory of enzyme action.

Read more about other famous scientists at www.sta.ie

Revise The Terms

Can you recall the meaning of the following terms?

Experiment, catalyst, inorganic, organic, biological, rate, chemical reaction, enzyme, inhibitor, biochemistry, industrial biochemistry, active site, substrate, enzyme-substrate complex, product, in solution, medium, cell, fungi, plant, animal, poisonous, temperature, pH, concentration, denaturation, buffer, water bath, experiment, control, apparatus, graph.

Check the Glossary of Terms for this lesson at www.sta.ie

For further examples of past paper questions check www.sta.ie