3.1 Enzymes and catalysts

What is an enzyme?

Enzymes are proteins that act as biological catalysts. A catalyst is a substance that speeds up the rate of a chemical reaction, but is not itself changed by the reaction. A catalyst can be used over and over again. If you
do Investigation 3.1, you will use manganese(IV) oxide as a catalyst. At the end of the experiment, the manganese(IV) oxide is still there. It has not been used up.

Living organisms have thousands of different chemical reactions, called **metabolic reactions**, taking place inside them. Some of these reactions, such as those involved in respiration, digestion and photosynthesis, are described later in this book. Each of these reactions needs a catalyst to make it happen.

Each kind of enzyme can catalyse only one particular kind of reaction. So our bodies contain many different enzymes, one for each of the many different metabolic reactions that must take place for us to stay alive. Even though the Quarter Horse foals had every kind of enzyme except one, just missing this one was enough to prevent their cells from working.

If you do Investigation 3.1, you will meet an enzyme called **catalase**. This enzyme catalyses the break down of hydrogen peroxide to oxygen and water. Hydrogen peroxide is a waste product of several different metabolic reactions that take place in cells. It is very toxic (poisonous) so it needs to be broken down immediately it is produced. Catalase is one of the fastest working enzymes known. One molecule of catalase can break down thousands of molecules of hydrogen peroxide every second.

### How an enzyme works

Catalase, like all enzymes, is a protein. Figure 3.2 shows how a catalase molecule breaks down a hydrogen peroxide molecule.

An enzyme molecule has a dent in it called the **active site**. The substances taking part in the reaction that the enzyme catalyses fit exactly into this dent. The enzyme pulls on them a little, making them react, and then lets them go again.

The substance that the enzyme works on is called its **substrate**. The new substances that it makes are called the **product**.

This explanation of how an enzyme works is sometimes called the 'lock and key' model. The 'lock' is the enzyme and the substrate is the 'key'. Just as a key must be exactly the right shape to fit into a lock, so must a substrate be exactly the right shape to fit into the enzyme.
The effect of temperature on enzymes

Enzymes can only work if their molecules are exactly the right shape. If the enzyme molecule loses its shape, then its substrates won’t fit into its active site.

If an enzyme molecule gets very hot, it starts to lose its shape. This is called **denaturing**. A denatured enzyme cannot act as a catalyst.

Figure 3.3 shows how the activity of an enzyme from the human body is affected by temperature.

At low temperatures, the enzyme molecules and the substrate molecules are moving very slowly. As the temperature increases, their kinetic energy increases and they move faster. The higher the temperature, the faster they move around and the more frequently they collide with each other. What’s more, they have more energy when they collide and so it is easier for the reaction to take place. So, as temperature rises, the rate of reaction also increases.

But when we get to a temperature above about 40 °C, you can see that the rate of reaction begins to slow down. This is because the enzyme molecules are beginning to be denatured. They have lost their shape and the substrate no longer fits into the active site. By a temperature of 60 °C, the enzyme molecules are all completely denatured and no reaction is taking place at all.

The temperature at which an enzyme works best is called its **optimum temperature**. Most of the enzymes that work inside our bodies have an optimum temperature of around 40 °C. Enzymes from plants often have an optimum temperature quite a bit lower than this, perhaps around 25 °C. Enzymes from bacteria that live in hot springs can have very high optimum temperatures, often well above 70 °C.

### Activity 3.2
The effect of temperature on the rate of breakdown of protein by trypsin

#### 3.2 Amylase is an enzyme found in human saliva. It catalyses the breakdown of starch molecules to maltose molecules.

\[
\text{amylase} \\
\text{starch} \rightarrow \text{maltose}
\]

**a)** Explain what is meant by:
(i) **catalyse**
(ii) **enzyme**

**b)** At what temperature do you think amylase might work fastest? Explain your answer.

c)** Molecules are always moving around. The higher the temperature, the faster they move. Explain why amylase breaks down starch faster at 30 °C than at 0 °C.

d)** Explain why amylase breaks down starch to maltose faster at 30 °C than at 80 °C.
The effect of pH on enzymes

The pH of a solution is a measure of how acid or alkaline it is. The scale runs from 1 to 14. A pH of 7 is neutral. A pH below 7 is acid, and a pH above 7 is alkaline.

The shape, and therefore the activity, of enzyme molecules is affected by pH. For most enzymes, there is a small range of pH in which their molecules are exactly the right shape to catalyse their reaction (Figure 3.4). Above or below this pH, their molecules lose their shape, so their substrates cannot fit into the active sites.

For example, pepsin is a protein-digesting enzyme (protease) that works in the stomach. Here, the pH is very low, because the stomach walls secrete hydrochloric acid. Pepsin works best in these conditions, at a pH of around 2.

Amylase, on the other hand, works in the mouth and in the duodenum, where the pH is much higher. Its optimum pH is around 7 or just above.

Enzymes in seed germination

A seed contains an embryo plant. It also contains a food store on which the embryo will rely when it begins to grow, when the seed germinates. The young plant needs this to carry it through the first few days, before it can begin to make its own food by photosynthesis.

For example, bean seeds contain a lot of starch. Starch is an insoluble carbohydrate. When the seed begins to germinate, the enzyme amylase is secreted (Figure 3.5). This breaks the starch into maltose, which is soluble. The maltose is absorbed by the growing embryo, which can break it down to glucose. The seedling can use the glucose to give it energy, or to make other substances such as cellulose to make its new cell walls.

![Figure 3.4]( UNCORRECTED PROOF COPY)

How pH affects the rate of an enzyme-controlled reaction.

Activity 3.3
The effect of pH on the breakdown of milk protein by trypsin

![Figure 3.5]( UNCORRECTED PROOF COPY)

The role of amylase in seed germination.
Enzymes in washing powders

Washing powders contain detergents. Detergents help to remove greasy dirt from clothes. They help the grease to dissolve in water, so that it can be washed off the cloth.

However, some stains on clothes are not greasy, and so they will not easily wash off with detergents. Stains like this include blood stains and egg yolk stains. These stains contain coloured proteins, which get stuck onto the fibres of the cloth and will not wash out.

To help to get rid of these stains, some kinds of washing powder contain enzymes. They are sometimes called biological washing powders (Figure 3.6). They contain enzymes which break down protein molecules, rather like trypsin does. They break the protein molecules into smaller molecules called amino acids. These amino acids are not coloured, and they easily dissolve in water. So the stain disappears and the amino acids can be washed away.

Some biological washing powders also contain lipase, which helps to break down grease molecules into smaller, soluble ones.

Enzymes in the food industry

Food manufacturers often use enzymes. For example, when juice is squeezed out of apples to make a drink, an enzyme called pectinase is often added. Pectinase is an enzyme that breaks down the substances that hold the cell walls of the apple cells together. This makes it easier to squeeze out more of the juice. Pectinase also helps to break down the substances that make apple juice look cloudy, turning it into a clear liquid that most people prefer (Figure 3.7).

Another example of an enzyme that is often used in the food industry is lactase. This is an enzyme that breaks down the sugar lactose, found in milk, to two other sugars called glucose and galactose. This is done because many people are not able to digest lactose, so lactose can make them ill. If lactase is added to milk, it breaks down the lactose. The milk is then called lactose-reduced milk, and it is safe for people to drink even if they do not have lactase in their digestive system.
A student wanted to find out if using pectinase allows more juice to be extracted from apples.

She took two apples and cut them up into small pieces, each roughly cube-shaped with sides of about 5 mm. She put half of the pieces into one beaker, and the other half into another. She then added 2 cm$^3$ of pectinase to one beaker and 2 cm$^3$ of water to the other. She left the beakers to stand for 20 minutes.

She then filtered the juice from the apple pieces and measured its volume. She found that she obtained 14 cm$^3$ of juice from the apples to which pectinase had been added, and 7 cm$^3$ of juice from the apples to which water had been added.

1. Suggest a hypothesis that is being tested by this investigation.
2. State four variables that the student should have kept constant in her investigation.
3. Suggest two ways in which she could have increased the yield from the apple pieces in both beakers.
4. Suggest one way in which she could increase the reliability of her results.
5. The student decided to see if the pectinase has different effects in different varieties of apples. Outline how she could do this. Your description should include:
   • a hypothesis she could test
   • the variable she should change, and how she should do this
   • the variables she should keep constant, and how she can do this
   • what she will measure, and how she will measure it
   • the results she would expect if her hypothesis is correct.

Using enzymes in microorganisms

Sometimes, rather than using a single kind of enzyme, it is easier to use a microorganism to manufacture a product that we want. The microorganism produces many different enzymes and these act on a substrate to produce a new substance that we can use.

The microorganisms are grown in a container called a fermenter.

Making penicillin

Figure 3.8 shows how a fermenter used for making the antibiotic penicillin.

An antibiotic is a substance that kills bacteria but does not harm our own cells. Many antibiotics are made by fungi. Penicillin is made by a fungus called Penicillium (Figure 3.9).
In a fermenter, the microorganism grows in a liquid called the medium. The medium must contain everything that the microorganism needs to live and to produce the desired product. *Penicillium* needs:

- oxygen for respiration
- a source of carbohydrates, such as sugar or starch
- a source of nitrogen, such as ammonium phosphate
- a suitable temperature – high enough to make sure the reactions happen quickly, but not so high that the fungus’s enzymes are denatured.

The metabolic reactions of the fungus release quite a bit of heat energy, so the fermenter is enclosed in a water-filled jacket to help to keep the temperature stable. It is also important that the pH of the medium is controlled. A stirrer keeps the contents of the fermenter in constant motion, making sure that the nutrients and oxygen are distributed throughout the medium.

**QUESTION**

3.3 a) Before the *Penicillium* or the medium is put into the fermenter, the fermenter is thoroughly cleaned using very hot steam. Suggest why this is necessary.

b) Using what you know about enzymes, explain why it is important to control the pH in the fermenter.

c) Suggest why the air supply to the fermenter comes into the base of it, rather than at the top.

d) Explain why it is important that carbon dioxide is allowed to escape from the fermenter.

**Making enzymes**

Sometimes, the substance that we want from microorganisms in a fermenter is the enzymes that they make. For example, we use microorganisms to make the enzymes that are added to biological washing powders.

For this purpose, the most useful enzymes are ones that have high optimum temperatures. This allows the clothes to be washed at a fairly high temperature, which enables the detergent part of the washing powder
to work better. So we get the enzymes from microorganisms that live at very high temperatures – for example, in hot springs (Figure 3.10). These enzymes can have optimum temperatures as high as 85 °C – a temperature too hot for us to stand.

The microorganisms are grown in fermenters and – just as for the manufacture of penicillin – they are given all the conditions that they need. The liquid around the microorganisms is collected, and the enzymes in the liquid are separated from the liquid and purified before use.

3.4 Suggest two similarities and one difference between the conditions required in a fermenter being used for making enzymes for biological washing powders, and a fermenter being used for making penicillin.

Summary

Now that you have completed this chapter, you should be able to:
- define the terms catalyst and enzyme
- describe how you can investigate the effect of changes in pH and temperature on enzyme activity
- explain the lock and key model of enzyme action
- explain the effect of changes in pH and temperature on enzyme activity
- describe the roles of enzymes in seed germination, and their uses in biological washing powders and the production of fruit juice
- describe how Penicillium is used to make penicillin

Figure 3.10
These orange bacteria are thriving in hot springs.