5 Enzymes

Focus

In the previous chapter you developed your knowledge and understanding of biological molecules. You discovered the importance of carbon atoms in their formation. You now know that the properties of key biological molecules make them suitable for many different uses in the organism. For example, some protein molecules form enzymes. In this chapter you will find out about the properties of enzymes and how they work. You will meet the terms *complementary shape* and *active site*. In what ways are enzymes similar to, and different from, catalysts? Why does an enzyme only work on one chemical? Once you understand how enzymes work, you will be able to answer these questions.

FOCUS POINT

- What is a catalyst?
- What are enzymes and why are they important?
- How do enzymes catalyse reactions?
- What are the effects of temperature and pH on enzyme activity?

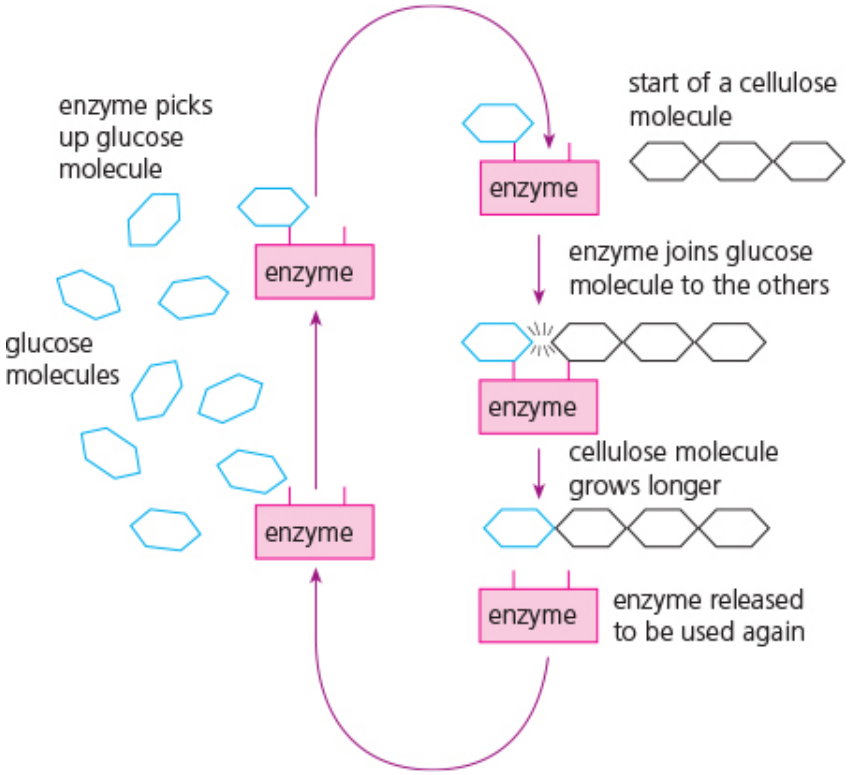
- Why are enzymes specific to only one reaction?
- Why do pH and temperature affect enzyme activity?

Key definitions

A **catalyst** is a substance that increases the rate of a chemical reaction and is not changed by the reaction.

Enzymes are proteins that function as **biological catalysts** and are involved in all metabolic reactions.

Enzymes are proteins that act as catalysts. They are made in all living cells. Enzymes, like all catalysts, can be used repeatedly because they are not used up during the reaction. Also, only small amounts are needed to speed the reaction up (Figure 5.1). They are important because they control the reactions in the cell. They make sure that these reactions occur quickly enough for the cell to function.



▲ **Figure 5.1** Building up a cellulose molecule

Enzyme action

An enzyme-controlled reaction involves a **substrate**, an enzyme and a **product**. The substrate and product may be two or more different molecules:

$$\text{substrate} \xrightarrow{\text{enzyme}} \text{product}$$

The substance on which an enzyme works is called its **substrate** and the molecules produced are called the products. For example, the enzyme sucrase works on the substrate sucrose to produce the monosaccharide products glucose and fructose.

The part of an enzyme molecule that is responsible for combining with a substrate is called the **active site**.

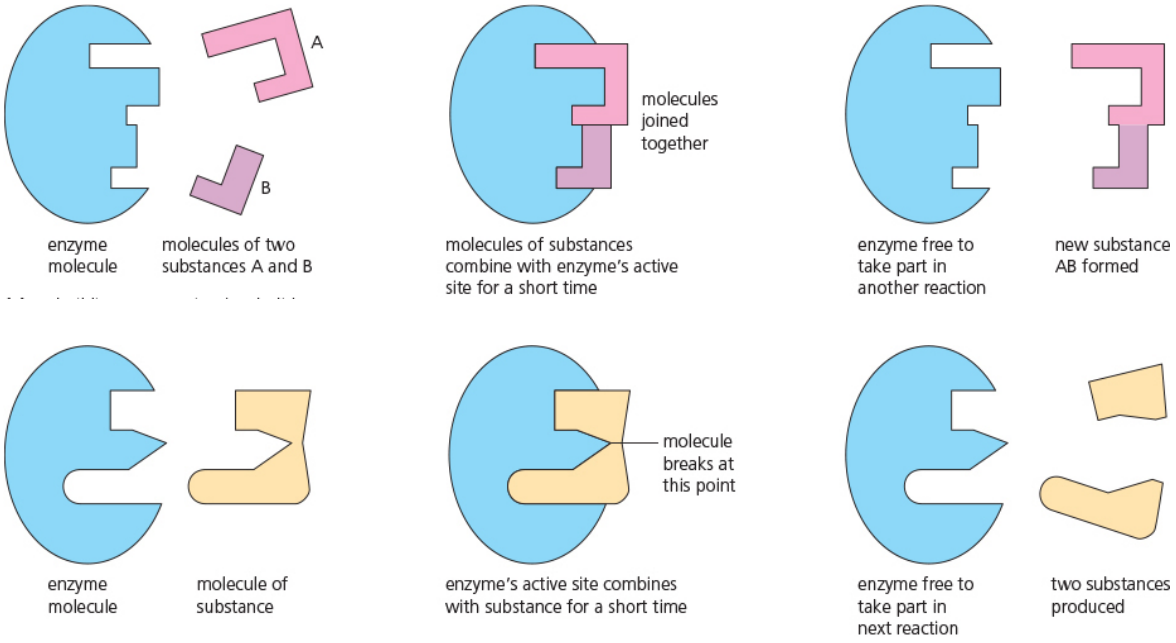
Figure 5.2 shows how an enzyme molecule might work to join two other molecules together (the substrates) to form a more complicated substance (the product).

An example of an enzyme-controlled reaction like this is the joining up of two glucose molecules to make a molecule of maltose. You can see that the enzyme's active site and the substrate molecules have **complementary** shapes (like pieces of a jigsaw puzzle that are next to each other) so they fit together. Other substrate molecules would not fit into this enzyme as they would have the wrong shape. For example, the substrate molecule in Figure 5.2(b) would not fit the enzyme molecule in Figure 5.2(a). The product (substance AB in Figure 5.2(a)) is released from the enzyme's active site and the enzyme is then free to repeat the reaction with more substrate molecules. Molecules of the two substances can combine without the enzyme being present, but the process would be very slow (it could take hours or days to happen without the enzyme: too slow to keep an organism alive). By bringing the substances close together, the enzyme molecule makes the reaction take place much more rapidly. The process can be extremely fast: catalase, a very common enzyme found in most cells, can break down 40 000 molecules of hydrogen peroxide every second! A complete chemical reaction takes only a few seconds when the right enzyme is present.

Try chewing a piece of bread but keep it in your mouth without swallowing it. Eventually you should detect the food tasting sweeter, as maltose sugar is formed. If starch is

mixed with water, it will break down very slowly to sugar. The process takes years. In your saliva there is an enzyme called **amylase**. This can break down starch to sugar in minutes or seconds. In cells, many of the enzymes are helping to break down glucose to carbon dioxide and water to release energy (Chapter 12).

As well as enzymes being responsible for joining two substrate molecules together, like two glucose molecules to form maltose, they can also make long chains. For example, hundreds of glucose molecules can be joined, end to end, to make a long molecule of starch. This is stored in the plastid of a plant cell. The glucose molecules can also be built up into a molecule of cellulose, to be added to the cell wall. Protein molecules are built up by enzymes, which join tens or hundreds of amino acid molecules. These proteins are added to the cell membrane, to the cytoplasm or to the nucleus of the cell. They may also become the proteins that work as enzymes.



▲ **Figure 5.2** Possible explanation of enzyme action

Enzymes and temperature

A rise in temperature increases the rate of most chemical reactions; a fall in temperature slows them down. However, above 50°C most enzymes, being proteins, are denatured and stop working.

Figure 5.2 shows how the shape of an enzyme's active site could be very important if it must fit the substrates on which it works. Above 50°C the shape of an enzyme is permanently changed. So, the active site becomes deformed and the enzyme molecule can no longer combine with the substrates. It is denatured.

This is one of the reasons why organisms may be killed by continued exposure to high temperatures. The enzymes in their cells are denatured and the chemical reactions happen too slowly to keep the organism alive.

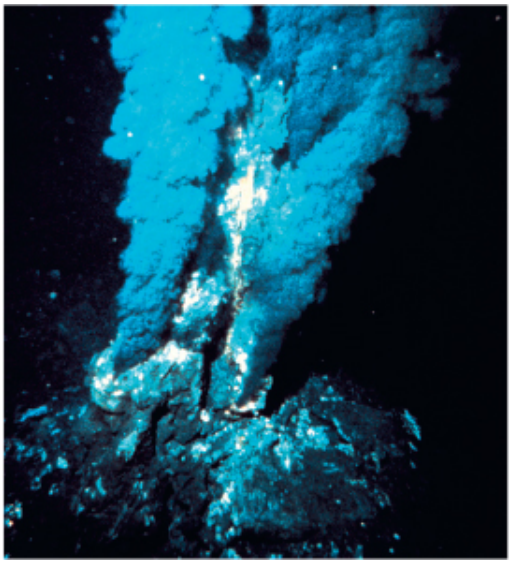
Egg white is a protein. When it is heated, its molecules change shape. The egg white goes from a clear, runny liquid to a white solid. It cannot be changed back again. The egg white protein, albumen, has been denatured by heat.

Proteins make enzymes and many of the structures in the cell. So, if they are denatured the enzymes and the cell structures will stop working. The cell will die. Whole organisms may stay alive for a time above 50°C. This depends on the temperature, the period of exposure and the proportion of the cells that are damaged.

One way to test if a substance is an enzyme is to heat it to boiling point. If it can still carry out its reactions after this, it cannot be an enzyme. This technique is used as a **control** (see 'Aerobic respiration' in Chapter 12) in enzyme experiments.

Scientists are starting to discover exceptions to the ways some enzymes are affected by high temperatures. There are some bacteria that live in an environment where the temperature is very high. Examples include species that live

successfully in hot springs and around hydrothermal vents in the deep oceans (Figure 5.3). They have enzymes that are made of very stable proteins; their active sites are not deformed by temperatures above 50°C. Scientists are very interested in these enzymes. They could be used in industrial applications where high temperatures are needed. For example, biological washing powders containing these enzymes could be used at a high temperature to remove difficult stains. Normally, the enzymes would be denatured.



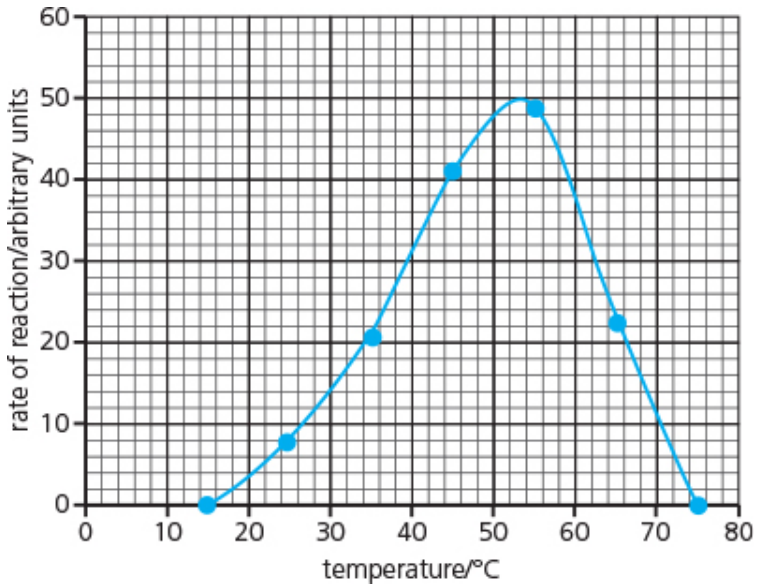
▲ **Figure 5.3** A hydrothermal vent, made as a result of volcanic activity on the sea floor. It is a good habitat for marine organisms including bacteria and invertebrates because the water is so rich in nutrients. However, these organisms need to survive at very high temperatures

Worked example

The table shows the results of an experiment investigating the effect of temperature on an enzyme reaction.

temp °C	rate of reaction /arbitrary units
15	0
25	8
35	21
45	41
55	49
65	23
75	0

1 a Plot a graph to show the effect of temperature on the rate of reaction.



Your graph should have the correct axis, with the independent variable (temp) as the x-axis and the dependent 1 (rate of reaction) as the y-axis.

b What was the optimum temperature for this reaction? 55°C. This is the temperature at which the rate of reaction is greatest.

c Calculate the percentage increase in reaction rate between 20 and 40°C.

The rate at 20°C = 4, the rate at 40°C = 32 increase in rate = 32 – 4 = 28

percentage increase in reaction rate = increase/starting rate × 100

= 28/4 × 100 = 700%

2 Calculate the percentage change in reaction rate between 60 and 70°C.

At 60°C the rate is 38, at 70°C the rate is 11. The change in rate is 38 – 11 = 27.

The percentage change = 27/38 × 100 = 71%

3 a At which two temperatures was the reaction rate 30? 39°C and 62°C

b Suggest why the rate was the same even though the temperatures were different.

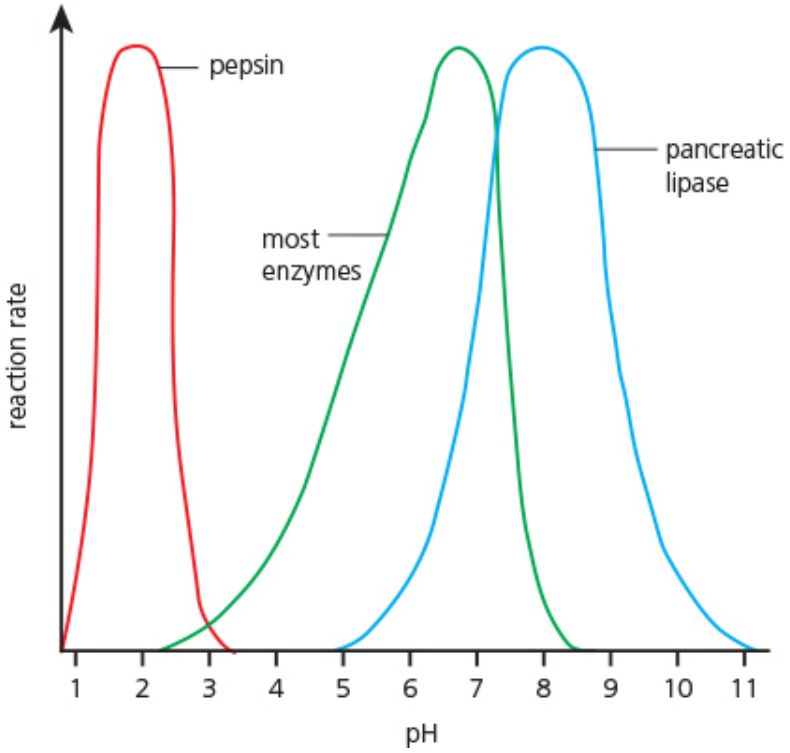
At 39°C, the temperature is not quite the optimum, so the number of collisions is still limited, affecting the rate of reaction. At 62°C, although the temperature is higher and so there will be more collisions, some of the enzyme molecules will have been denatured, so the rate of reaction is limited.

Enzymes and pH

Acid or alkaline conditions alter the chemical properties of proteins, including enzymes. Most enzymes work best at a particular level of acidity or alkalinity (pH), as shown in Figure 5.4.

The protein-digesting enzyme in your stomach, for example, works well at an acidity of pH 2. At this pH, the enzyme amylase, from your saliva, cannot work at all. Inside the cells, most enzymes will work best in neutral conditions (pH 7). The pH or temperature at which an enzyme works best is called its **optimum** pH or temperature. Conditions in the **duodenum** are slightly alkaline: the optimum pH for pancreatic lipase is pH 8.

Although changes in pH affect the activity of enzymes, these effects are usually reversible, i.e. an enzyme that is disabled by a low pH will restart its normal activity when its optimum pH is met again.



▲ **Figure 5.4** The effect of pH on digestive enzymes

Going further

Intracellular and extracellular enzymes All enzymes are made inside cells. Most of them remain inside the cell to speed up reactions in the cytoplasm and nucleus. These are called **intracellular enzymes** (‘intra’ means ‘inside’). Some enzymes made in the cells are let out of the cell to do their work outside. These are **extracellular enzymes** (‘extra’ means ‘outside’). Fungi and bacteria (see ‘Features of organisms’ in Chapter 1) release extracellular enzymes to digest their food. A mould growing on a piece of bread releases starch-digesting enzymes into the bread and absorbs the soluble sugars that the enzyme produces from the bread. In the

digestive systems of animals (‘Alimentary canal’ in Chapter 7), extracellular enzymes are released into the stomach and intestines to digest the food.

Test yourself

1 Copy and complete the table using a tick (✔) or a cross (✗) to show which of the following statements apply to enzymes and/or any other catalysts.

statement	enzymes	any other catalysts
Their activity is stopped by high temperature.		
They speed up chemical reactions.		
They are proteins.		
They are not used up during the reaction.		

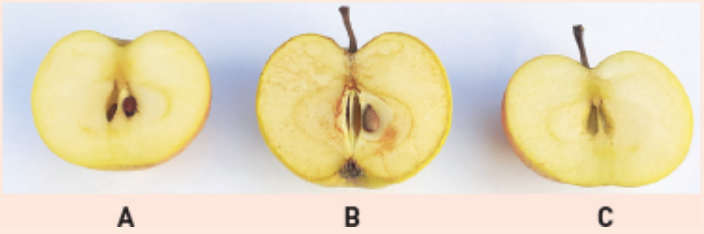
2 How would you expect the rate of an enzyme-controlled reaction to change if the temperature was raised

1 from 20°C to 30°C

2 from 35°C to 55°C?

Explain your answers.

- Apple cells contain an enzyme that turns the tissues brown when an apple is peeled and left for a time. Apple dipped in boiling water does not go brown (Figure 5.5). Explain why the boiled apple behaves differently.



▲ **Figure 5.5** Experiment to investigate enzyme activity in an apple. Slice A has been freshly cut. B and C were cut 2 days earlier, but C was dipped immediately in boiling water for 1 minute

- Suggest why amylase from the mouth does not work in the stomach.
- Would **pepsin** from the stomach work effectively in the small intestine? Explain your answer.

Explaining enzyme action

In some reactions, large molecules are built up from smaller molecules (Figure 5.2(a)). When the enzyme joins with the substrate, an **enzyme– substrate complex** is formed temporarily.

Figure 5.2(b) shows an enzyme speeding up a chemical change, but this time the molecule of a substance is split into smaller molecules. Again, when the enzyme joins with the substrate, an enzyme–substrate complex is formed temporarily.

Enzymes are specific

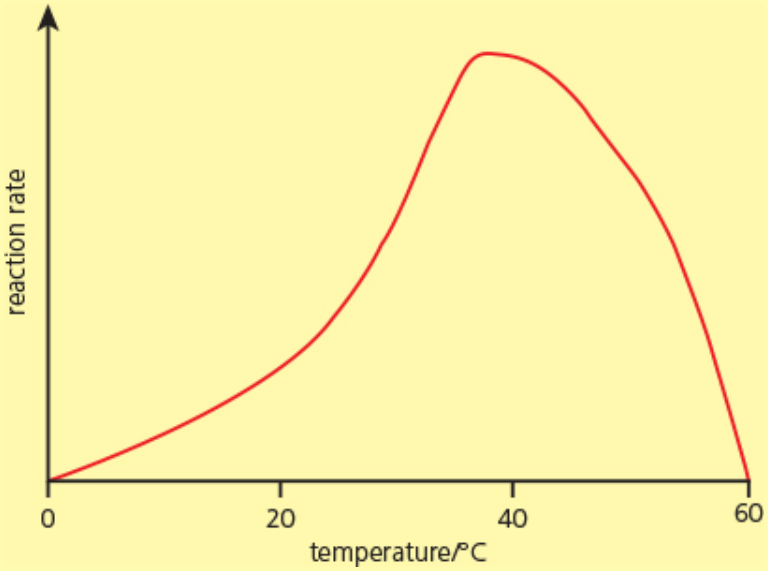
Specificity simply means that an enzyme which normally works on one substrate will not act on a different one. Figure 5.2(a) shows how the shape of an enzyme can control what substrates it joins with. The enzyme in Figure 5.2(a) has a shape called the active site, which exactly fits the substrates on which it works but which will not fit the substrate in Figure 5.2(b). So, the shape of the active site of the enzyme molecule and the substrate molecule are complementary. So, an enzyme which breaks down starch to maltose will not also break down proteins to amino acids. Also, if a reaction takes place in stages, for example, a different enzyme is needed for each stage.

The names of enzymes usually end with *-ase* and they are named according to the substrate on which they work or the reaction which they speed up. For example, an enzyme

that works on proteins may be called a **protease**; one that removes hydrogen from a substance is a **dehydrogenase**.

Enzymes and temperature

Figure 5.6 shows the effect of temperature on an enzyme-controlled reaction.



▲ **Figure 5.6** Graph showing the effect of temperature on the rate of an enzyme-controlled reaction

Usually, a rise of 10°C will double the rate of an enzyme-controlled reaction in a cell, up to an optimum temperature of about 37°C (body temperature).

This is because the enzyme and substrate molecules are constantly moving, using kinetic energy. The reaction only happens when the enzyme and substrate molecules collide with each other. As the temperature is increased, the molecules gain more kinetic energy, so they move faster and there is a greater chance of **collisions** happening. So, the rate of reaction increases. Above the optimum temperature the molecules gain even more kinetic energy, but the reaction will slow down. This is because enzyme molecules are proteins. Protein molecules start to lose their

shape at higher temperatures, so the shape of the active site changes. As a result, although there are more collisions due to the molecules having greater kinetic energy, the number of effective collisions reduces. Substrate molecules cannot fit together with the enzyme, stopping the reaction. Not all the enzyme molecules are affected straight away, so the reaction does not suddenly stop – it is a gradual process as the temperature increases above 37°C. Denaturation is a permanent change in the shape of the enzyme molecule. Once it has happened the enzyme will not work any more, even if the temperature is reduced below 37°C. You experience an example of a protein denaturing if you cook egg white (made of the protein albumin). As you heat it, the albumin turns from clear and runny to solid and opaque and white. You cannot change it back to what it was like before cooking.

Enzymes and pH

Extremes of pH can denature some enzymes. This is because the shape of the active site of the enzyme molecule is changed (as it does when exposed to high temperatures). As a result, the enzyme and substrate molecules no longer have complementary shapes and so will not fit together.

Test yourself

6 With reference to enzymes, explain the meaning of the terms
- **7** complementary
- **8** active site.

• Explain why, in an enzyme-controlled reaction in the human body, the rate of reaction starts to go down at temperatures above 40°C, even though there is more kinetic energy available for the molecules.

Practical work

Tests for proteins are described in Chapter 4. Experiments on the digestive enzymes amylase and pepsin are described in Chapter 7.

For safe experiments/demonstrations which are related to this chapter, please refer to the *Biology Practical Skills Workbook* that is also part of this series.

Safety

- Eye protection must be worn.

1 Extracting and testing an enzyme from living cells

In this experiment, catalase is the enzyme to be extracted and tested and the substrate is hydrogen peroxide (H_2O_2).

Some reactions in the cell produce hydrogen peroxide, which is poisonous. Catalase makes the hydrogen peroxide harmless by breaking it down to water and oxygen.

- Grind a small piece of liver with about 20 cm^3 water and a little sand in a mortar. This will break open the liver cells and release their contents.
- Filter the mixture and share it between two test tubes, A and B. The filtrate will contain many substances dissolved out from the cytoplasm of the liver cells, including enzymes. However, only one of these, catalase, will work on hydrogen peroxide because enzymes are specific.
- Add some drops of the filtrate from test tube A to a few cm^3 of hydrogen peroxide in another test tube. You will see a strong reaction as the hydrogen peroxide breaks down to produce oxygen. (The oxygen can be tested with a glowing splint.)
- Now boil the filtrate in tube B for about 30 seconds. Add a few drops of the boiled filtrate to a fresh sample of

hydrogen peroxide. There will be no reaction because boiling has denatured the catalase.

- Next, shake a little manganese(IV) oxide powder (***CARE**: manganese(IV) oxide powder is harmful) in a test tube with some water and pour this into some hydrogen peroxide. There will be a vigorous reaction like the one with the liver extract.
- Now boil some manganese(IV) oxide with water and add this to hydrogen peroxide. The reaction will still occur. Manganese(IV) oxide is a catalyst, but you know it is not an enzyme because heating has not changed its catalytic properties.
- The experiment can be repeated with a piece of potato to compare its catalase content with catalase in liver. Make the piece of potato about the same size as the liver sample.

Going further

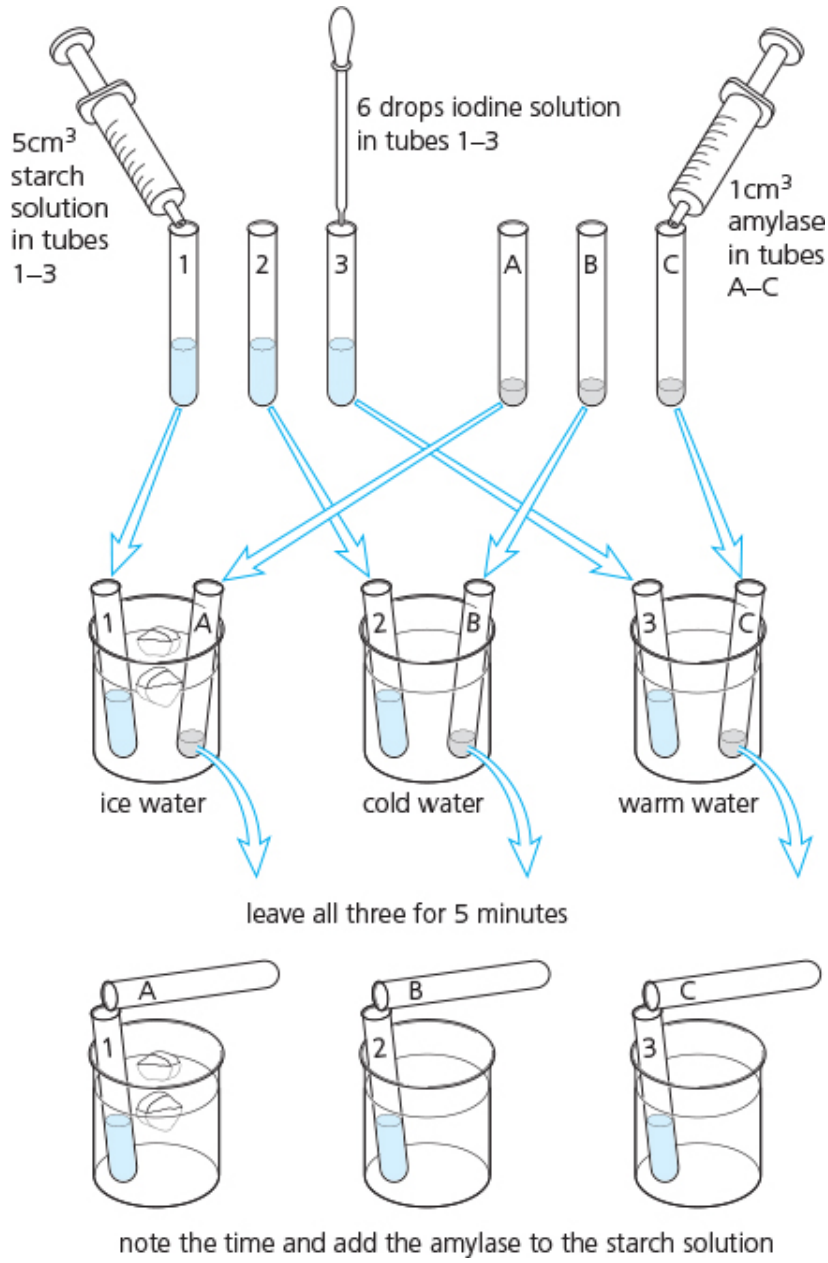
Investigate a range of plant tissues to find out which is the best source of catalase. Decide how to make **quantitative** comparisons (observations which involve measurements). Possible plant tissues include cassava, potato, celery, apple and carrot.

The effect of temperature on an enzyme reaction

- Amylase is an enzyme that breaks down starch to a sugar (maltose).
- Use a plastic syringe (or graduated pipette) to measure 5 cm^3 of 5% amylase solution and place 1cm^3 in each of three test tubes labelled A, B and C.
- Rinse the syringe thoroughly and use it to place 5 cm^3 of a 1% starch solution in each of three test tubes labelled 1, 2 and 3.

- Using a dropping pipette, add six drops of dilute iodine solution to each of the tubes 1 to 3.
- Prepare three water-baths by half filling beakers or jars with
  1. ice and water. Keep adding ice during the experiment to keep the temperature at about 10°C
  2. water from the cold tap at about 20°C (if you are working in a hot room, add some ice as needed)
  3. warm water at about 35°C by mixing hot and cold water.

- Place tubes 1 and A in the cold water-bath, tubes 2 and B in the water at 20°C, and tubes 3 and C in the warm water.
- Leave the three beakers for 5 minutes to reach the temperature of the water they are each in (Figure 5.6).
- After 5 minutes, take the temperature of each water-bath, then pour the amylase from tube A into the starch solution in tube 1. Then put tube 1 back in the water-bath.
- Repeat this with tubes 2 and B, and 3 and C.
- As the amylase breaks down the starch, it will cause the blue colour to disappear. Make a note of how long this takes in each case.



▲ **Figure 5.7** Experiment to investigate the effect of temperature on an enzyme reaction

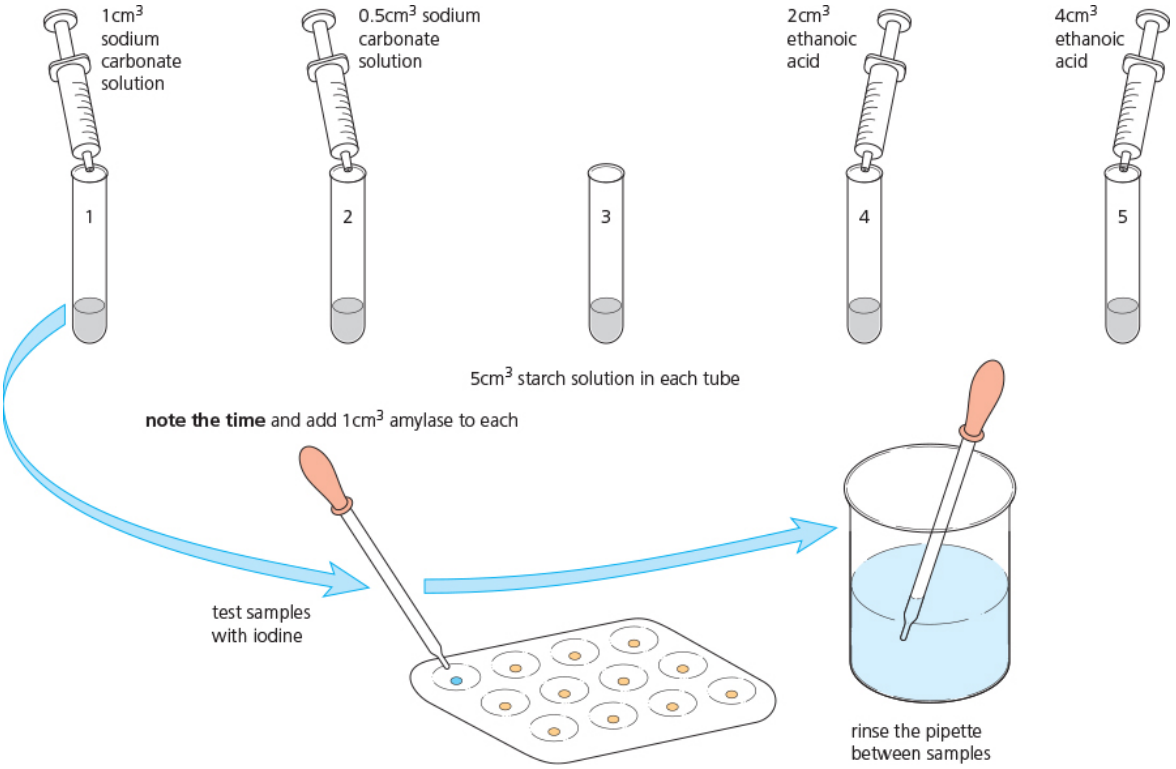
The effect of pH on an enzyme reaction

- Label five test tubes 1 to 5 and use a plastic syringe (or graduated pipette) to place 5 cm^3 of a 1% starch solution in each tube.

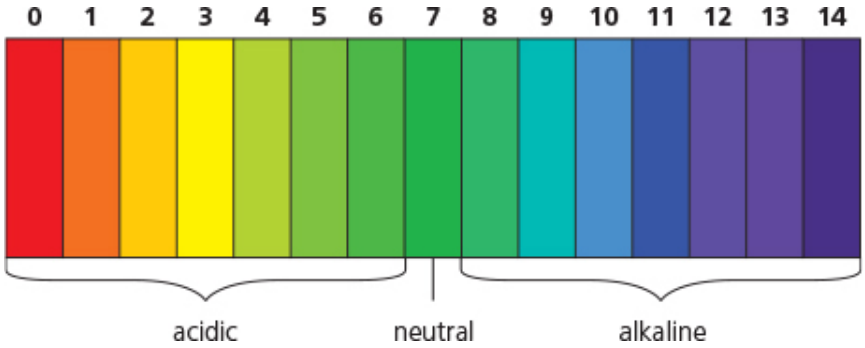
Tube	Chemical	Approximate pH	
1	1 cm^3 sodium carbonate solution (0.05 mol dm^{-3})	9	(alkaline)
2	0.5 cm^3 sodium carbonate solution (0.05 mol dm^{-3})	7–8	(slightly alkaline)
3	nothing	6–7	(neutral)
4	2 cm^3 ethanoic (acetic) acid (0.1 mol dm^{-3})	6	(slightly acid)
5	4 cm^3 ethanoic (acetic) acid (0.1 mol dm^{-3})	3	(acid)

- Add acid or alkali to each tube as shown in the table below. Rinse the syringe when changing from sodium carbonate to acid.
- Place several rows of iodine solution drops in a cavity tile.
- Place 5 cm^3 of 5% amylase solution in a clean syringe and place 1 cm^3 in the first tube. Shake the tube and note the time (Figure 5.8).
- Using a clean dropping pipette, remove a small sample from the tube and add one drop to one of the iodine drops in the cavity tile. Rinse the pipette in a beaker of water between each sample. Keep on sampling in this way every 30 seconds.
- When any of the samples does not give a blue colour, this means that the starch in that tube has been completely broken down to sugar by the amylase. Note the time when this happens for the tube. Stop taking samples from that tube.
- Repeat with each of the remaining tubes.
- Stop sampling the remaining tubes after about 15 minutes. Put a drop from each tube on to a piece of pH paper or mix

with a few drops of universal indicator solution in a cavity tile. Compare the colour produced with a colour chart of pH values.



▲ **Figure 5.8** Experiment to investigate the effect of pH on an enzyme reaction



▲ **Figure 5.9** A colour chart for Universal Indicator

Revision checklist

After studying Chapter 5 you should know and understand the following:

- Catalysts are substances that increase the rate of chemical reactions and are not changed by the reaction.
- Enzymes are proteins that function as biological catalysts.
- Enzymes are important in all organisms because they maintain a reaction speed needed to sustain life.
- The substance on which an enzyme works is called the substrate. After the reaction, a product is formed.
- An enzyme and its substrate have complementary shapes.
- Enzymes are affected by pH and temperature and are usually denatured above 50°C.

- ✔ Enzymes tend to be very specific in the reactions they catalyse due to the complementary shape of the enzyme and its substrate.
- ✔ When an enzyme catalyses a reaction, it forms a temporary enzyme–substrate complex before the product is released.
- ✔ Changes in temperature affect the kinetic energy of enzyme molecules and their shape.
- ✔ Enzymes can be denatured by changes in temperature and pH.
- ✔ Changes in pH affect the shape of enzyme molecules.

Exam-style questions

1. 1. i) Explain the term **enzyme**. [2]

   ii) State **two** ways in which an inorganic catalyst is different from an enzyme. [2]

2. Explain how the speed of an enzyme-controlled reaction is affected by changes in
   1. temperature
   2. pH.

   Support your answers using sketch graphs. [6]

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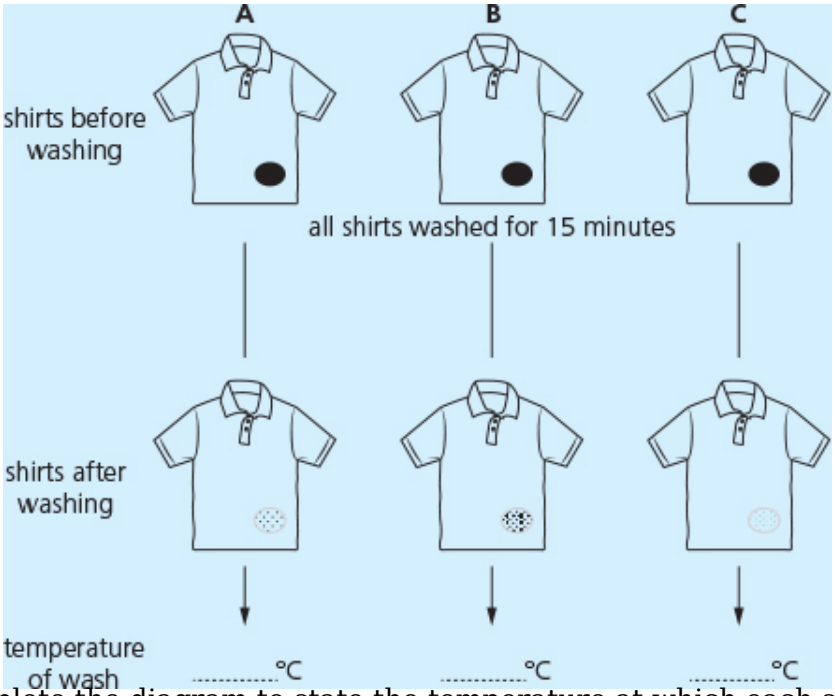
2 The diagram shows an experiment to investigate the effect of temperature on removing fat stains using an enzyme-containing washing powder. Three identical T shirts with identical fat stains were washed with the washing powder at three different temperatures, 15°C, 35°C and 70°C.



a Complete the diagram to state the temperature at which each shirt was washed. [1]

b Explain the result for shirts A, B and C. [1]

c Suggest two changes to the method which could have resulted in the complete removal of the stain from shirt C. [2]

d **i)** State the name of the type of enzyme that was present in the washing powder. [1]

ii) Another shirt had blood stains. Suggest an enzyme that would need to be present in the washing powder to remove these stains.

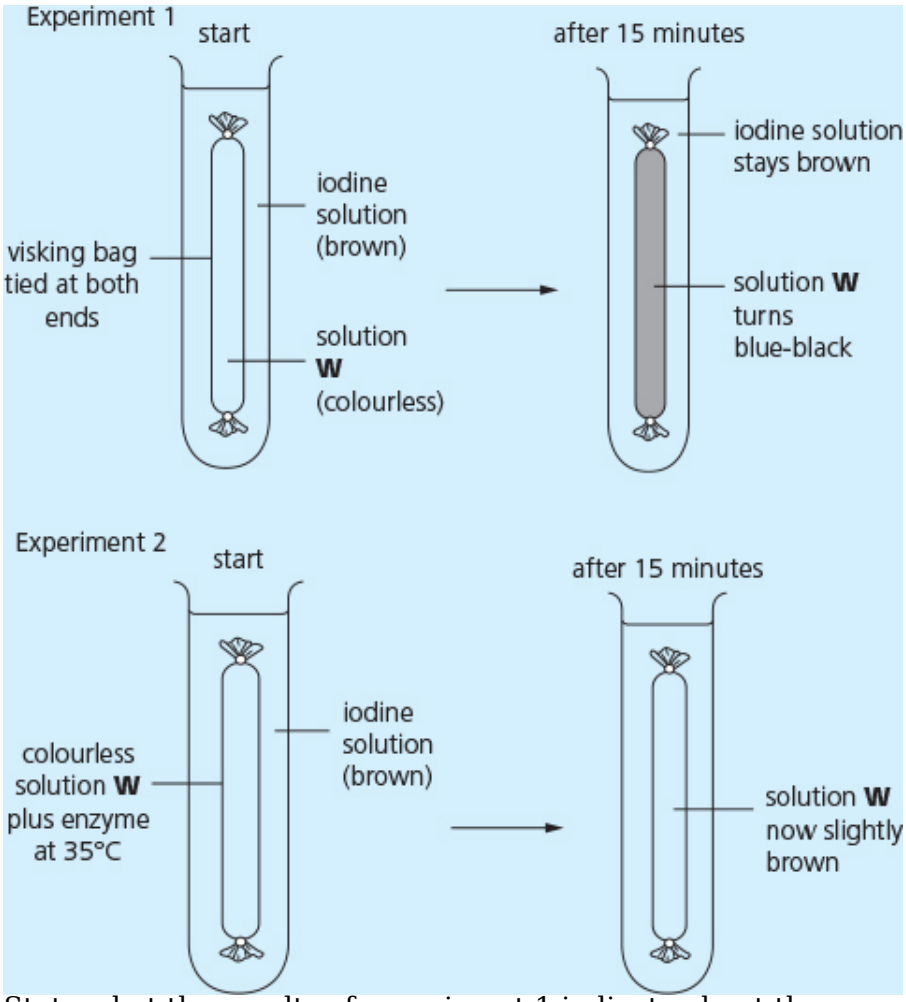
3 **a** **i)** Describe how you would carry out an investigation to show the effect of changing temperature on the reaction between a piece of vegetable and hydrogen peroxide. [6]

ii

) State two safety precautions you would take when carrying out this investigation. [2]

b Explain why digestion of starch in bread, started in the mouth, stops when the bread reaches the stomach. [2]

4 The diagram shows an investigation into the permeability of Visking tubing and the action of an enzyme on an unknown solution.



a **i)** State what the results of experiment 1 indicate about the permeability of the Visking bag to iodine. [1]

ii [1]

) Suggest what nutrient was present in the solution.

ii [1]

i) Suggest why the iodine solution surrounding the bag stayed brown.

b In experiment 2,

i) Suggest why the enzyme in the bag was kept at 35°C. [1]

ii [2]

) Explain why the solution in the bag was still brown after 15 minutes.

5 Two groups of students carried out an investigation into the effect of temperature on the reaction between hydrogen peroxide and the enzyme catalase in sweet potato extract. The reaction produces oxygen, which was collected for 10 minutes at each temperature. The results are shown in the table.

temperature/°C	volume of oxygen produced/cm^3	
	group 1	group 2
20	9	7
30	38	36
40	52	50
50	35	33
60	10	8

a Calculate the mean volume of oxygen produced at each temperature. [2]

b Plot a graph of the mean results. Label the axes. [4]

c **i)** Suggest the optimum temperature for the reaction. [1]

ii [1]

) Predict what the volume of oxygen produced would be at 70°C.

ii

i) The students expected that the enzyme would be denatured at temperatures above body temperature. Explain why the volume of oxygen produced at 50°C was not zero. [2]

d For the reaction in the investigation, state the name(s) of

a the substrate [1]

b the products. [2]