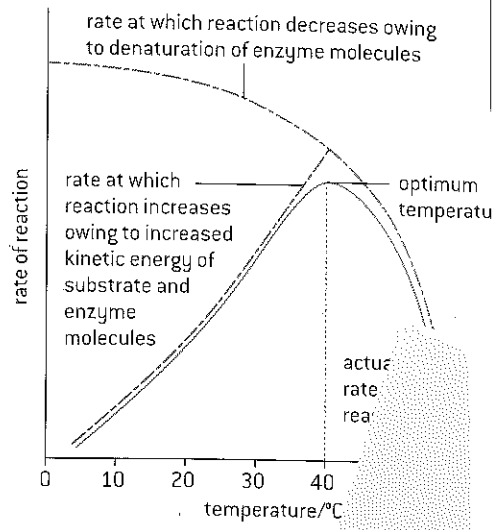
Enzymes are sensitive to pH

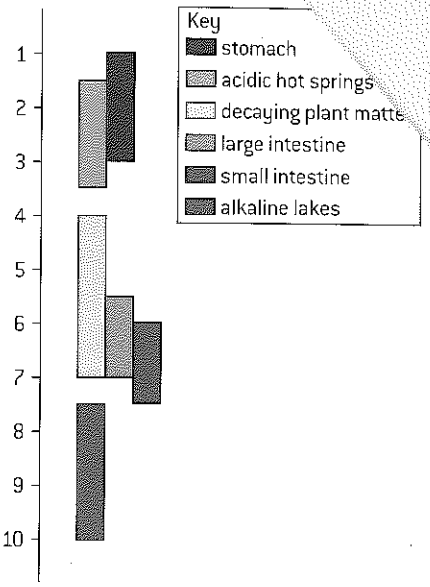
The pH scale is used to measure the acidity or alkalinity of a solution. The lower the pH, the more acid or the less alkaline a solution is. Acidity is due to the presence of hydrogen ions, so the lower the pH, the higher the hydrogen ion concentration. The pH scale is logarithmic. This means that reducing the pH by one unit makes a solution ten times more acidic. A solution at pH 7 is neutral. A solution at pH 6 is slightly acidic; pH 5 is ten times more acidic than pH 6, pH 4 is one hundred times more acidic than pH 6, and so on.

Most enzymes have an optimum pH at which their activity is highest. If the pH is increased or decreased from the optimum, enzyme activity decreases and eventually stops altogether. When the hydrogen ion concentration is higher or lower than the level at which the enzyme naturally works, the structure of the enzyme is altered, including the active site. Beyond a certain pH the structure of the enzyme is irreversibly altered. This is another example of denaturation.

Enzymes do not all have the same pH optimum – in fact, there is a wide range. This reflects the wide range of pH environments in which enzymes work. For example, the protease secreted by *Bacillus licheniformis* has a pH optimum between 9 and 10. This bacterium is cultured to produce its alkaline-tolerant protease for use in biological laundry detergents, which are alkaline. Figure 6 shows the pH range of some of the places where enzymes work. Figure 7 shows the effects of pH on an enzyme that is adapted to work at neutral pH.



▲ Figure 5 Temperature and enz



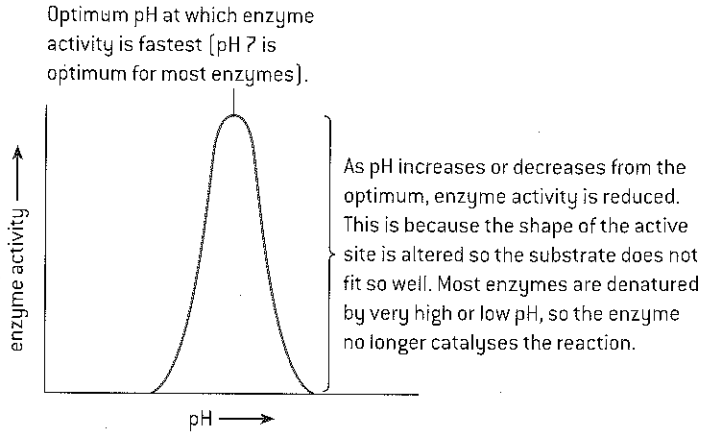
▲ Figure 6

Enzyme activity is affected by substrate concentration

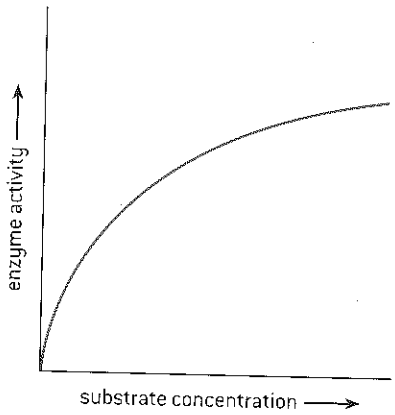
Enzymes cannot catalyse reactions until the substrate binds to the active site. This happens because of the random movements of molecules in liquids that result in collisions between substrates and active sites. If the concentration of substrates is increased, substrate–active site collisions will take place more frequently and the rate at which the enzyme catalyses its reaction increases.

However, there is another trend that needs to be considered. After the binding of a substrate to an active site, the active site is occupied and unavailable to other substrate molecules until products have been formed and released from the active site. As the substrate concentration rises, more and more of the active sites are occupied at any moment. A greater and greater proportion of substrate–active site collisions are therefore blocked. For this reason, the increases in the rate at which enzymes catalyse reactions get smaller and smaller as substrate concentration rises.

If the relationship between substrate concentration and enzyme activity is plotted on a graph, a distinctive curve is seen (figure 8), rising less and less steeply, but never quite reaching a maximum.



▲ Figure 7 pH and enzyme activity



▲ Figure 8 The effect of substrate concentration on enzyme activity

Denaturation

Enzymes can be denatured.

Enzymes are proteins, and like other proteins their structure can be irreversibly altered by certain conditions. This process is denaturation and both high temperatures and either high or low pH can cause it.

When an enzyme has been denatured, the active site is altered so the substrate can no longer bind, or if its binds, the reaction that the enzyme normally catalyses does not occur. In many cases denaturation causes enzymes that were dissolved in water to become insoluble and form a precipitate.

Quantitative experiments

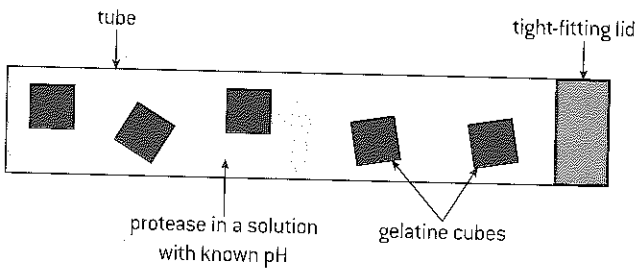
Experimental design: accurate quantitative measurements in enzyme experiments require replicates to ensure reliability.

Our understanding of enzyme activity is based on evidence from experiments. To obtain strong evidence these experiments must be carefully designed and follow some basic principles:

- the results of the experiment should be quantitative, not just descriptive;
- measurements should be accurate, which in science means close to the true value; and
- the experiment should be repeated, so that the replicate results can be compared to assess how reliable they are.

Data-based questions: Digesting jello cubes

Figure 9 shows apparatus that can be used to investigate protein digestion.



▲ Figure 9 Tube used to investigate the rate of digestion of gelatine

If the cubes are made from sugar-free jello (jelly), the colouring that they contain will gradually be released as the protein is digested by the protease. The questions below assume that strawberry-flavoured jello with red colouring has been used!

1 Explain whether these methods of assessing the rate of protein digestion are acceptable:

a) describing whether the solution around the cubes is colourless or a shade of pink or red

b) taking a sample of the solution and measuring its absorbance in a colorimeter

c) finding the mass of the cubes using an electronic balance. [3]

2 If method (c) was chosen, discuss whether it would be better to find the mass of all of the cubes of jello together, or find the mass of each one separately. [2]

3 If the jello cubes have a mass of 0.5 grams, state whether it is accurate enough to measure their mass to:

a) the nearest gram (g)

b) the nearest milligram (mg)

c) the nearest microgram (μg). [3]

4 To obtain accurate mass measurements of the jello cubes, it is necessary to remove them from the tube and dry their surface to ensure that there are no drips of solution from the tube adhering. Explain the reason for drying the surface of the blocks. [2]

Table 1 gives the results that were obtained using sugar-free jello cubes and a protease called papain, extracted from the flesh of fresh pineapples.

5 Discuss whether the results in table 1 are reliable. [2]

6 Most of the results were obtained using an extract of protease from one pineapple, but after this ran out, a second pineapple was used to obtain more protease for use in the experiment.

a) Deduce which results were obtained using the second extract. [1]

b) Suggest how the use of a second extract could have affected the results. [2]

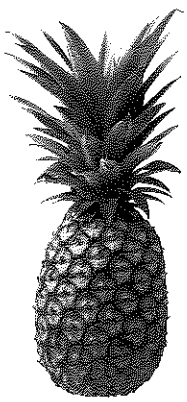
7 Draw a graph of the results in the table. [5]

8 Describe the relationship between pH and papain activity. [3]

9 Discuss the conclusions that can be drawn from this data about the precise optimum pH of papain. [2]

pH	Mass decrease (mg)		
2	80	87	77
3	122	127	131
4	163	166	164
5	171	182	177
6	215	210	213
7	167	163	84
8	157	157	77
9	142	146	73

▲ Table 1



Designing enzyme experiments

Design of experiments to test the effect of temperature, pH and substrate concentration on the activity of enzymes.

1 The factor that you are going to investigate is the **independent variable**. You need to decide:

- how you are going to vary it, for example with substrate concentration you would obtain a solution with the highest concentration and dilute it to get lower concentrations;
- what units should be used for measuring the independent variable, for example temperature is measured in degrees Celsius;
- what range you need for the independent variable, including the highest and lowest levels and the number of intermediate levels.

2 The variable that you measure to find out how fast the enzyme is catalysing the reaction is the **dependent variable**. You need to decide:

- how you are going to measure it, including the choice of meter or other measuring device, for example an electronic stop clock could be used to measure the time taken for a colour change;
- what units should be used for measuring the dependent variable, for example seconds rather than minutes or hours would be used for measuring a rapid colour change;
- how many repeats you need to get reliable enough results.

3 Other factors that could affect the dependent are **control variables**. You need to decide:

- what all the control variables are;
- how each of them can be kept constant;
- what level they should be kept at, for example temperature should be kept at the optimum for the enzyme if pH is being investigated, but factors that might inhibit enzymes should be kept at a minimum level.

Enzyme experiments

Experimental investigation of a factor affecting enzyme activity.

There are many worthwhile enzyme experiments. The method that follows can be used to investigate the effect of substrate concentration on the activity of catalase.

Catalase is one of the most widespread enzymes. It catalyses the conversion of hydrogen peroxide, a toxic by-product of metabolism, into water and oxygen. The apparatus shown in figure 10 can be used to investigate the activity of catalase in yeast.

The experiment could be repeated using the same concentration of yeast, but different hydrogen peroxide concentrations. Another possible investigation would be to assess the catalase concentrations in other cell types, such as liver, kidney or germinating seeds. These tissues would have to be macerated and then mixed with water at the same concentration as the yeast.

1 Describe how the activity of the enzyme catalase could be measured using the apparatus shown in figure 10. [2]

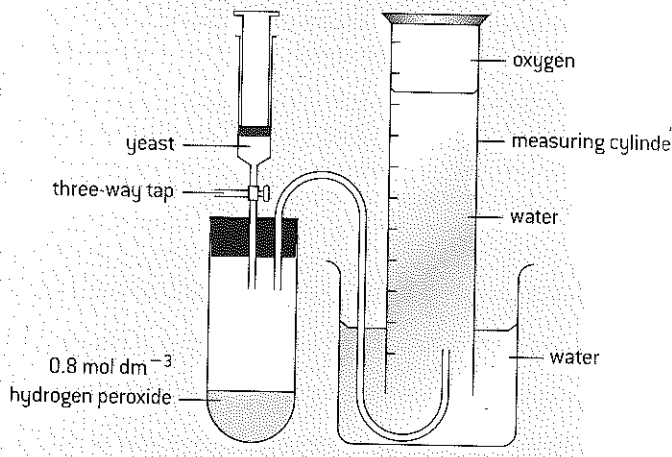
2 Explain why a yeast suspension must always be thoroughly stirred before a sample of it is taken for use in an experiment. [2]

3 State two factors, apart from enzyme concentration, that should be kept constant if investigating the effect of substrate concentration. [2]

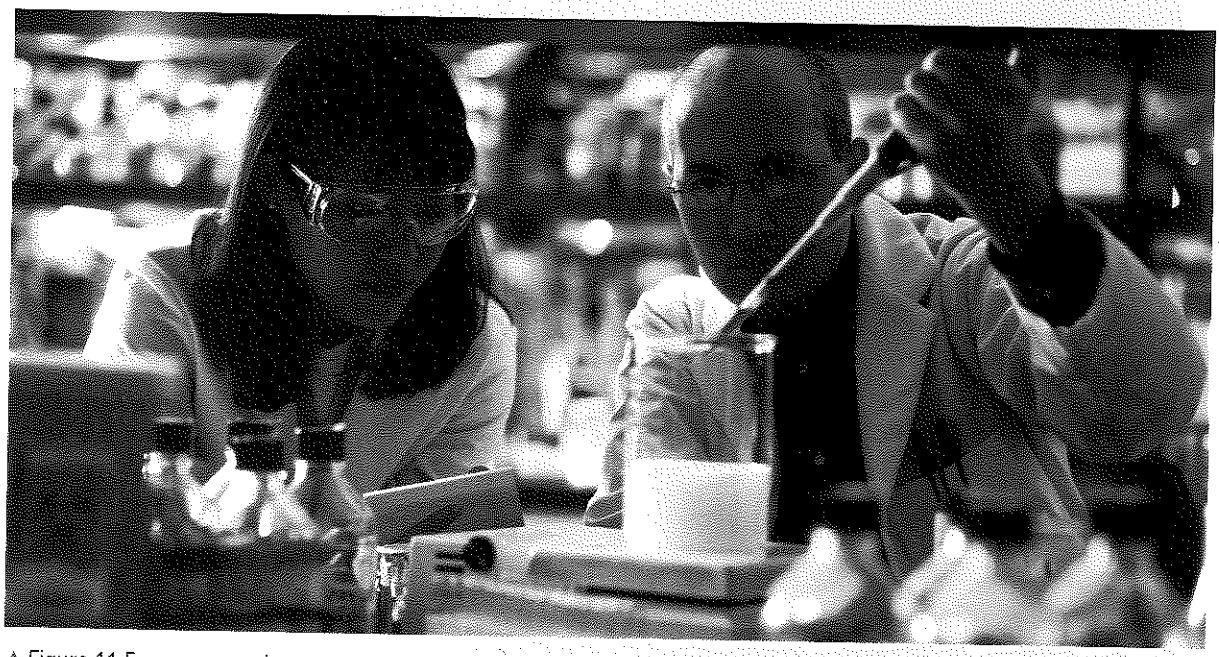
4 Predict whether the enzyme activity will change more if substrate concentration is increased by 0.2 mol dm^{-3} or if it is decreased by the same amount. [2]

5 Explain why tissues such as liver must be macerated before investigating catalase activity in them. [2]

Safety goggles must be worn if this experiment is performed. Care should be taken not to get hydrogen peroxide on the skin.



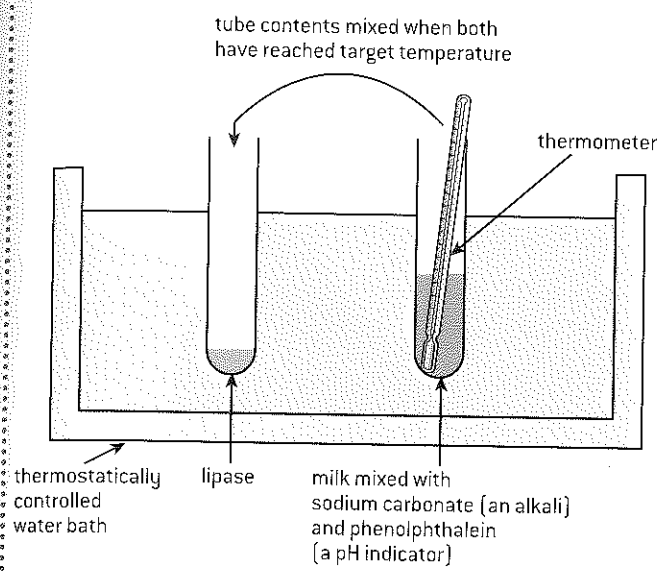
▲ Figure 10 Apparatus for measuring catalase activity



▲ Figure 11 Enzyme experiment

Data-based questions: Designing an experiment to find the effect of temperature on lipase.

Lipase converts fats into fatty acids and glycerol. It therefore causes a decrease in pH. This pH change can be used to measure the activity of lipase. Figure 12 shows suitable apparatus.



▲ Figure 12 Apparatus for investigating the activity of lipase

Phenolphthalein is pink in alkaline conditions, but becomes colourless when the pH drops to 7. The time taken for this colour change can be used to measure the activity of lipase at different temperatures. Alternatively, pH changes could be followed using a pH probe and data-logging software.

1 a) State the independent variable in this experiment and how you would vary it. [2]

b) State the units for measuring the independent variable. [1]

c) State an appropriate range for the independent variable. [2]

2 a) Explain how you would measure the dependent variable accurately. [2]

b) State the units for measuring the dependent variable. [1]

c) Explain the need for at least three replicate results for each temperature in this experiment. [2]

3 a) List the control factors that must be kept constant in this experiment. [3]

b) Explain how these control factors be kept constant.

c) Suggest a suitable level for each control factor.

4 Suggest reasons for:

a) milk being used to provide a sour lipids in this experiment rather th vegetable oil.

b) the thermometer being placed in the tube containing the larger, rather than the smaller, volume of liquid [1]

c) the substrate being added to the enzyme, rather than the enzyme to the substrate. [1]

5 Sketch the shape of graph that you would expect from this experiment, with a temperature range from 0 °C to 80 °C on the x-axis and time taken for the indicator to change colour on the y-axis. [2]

6 Explain whether lipase from human pancreas or from germinating castor oil seeds would be expected to have the higher optimum temperature. [2]

Immobilized enzymes

Immobilized enzymes are widely used in industry.

In 1897 the Buchner brothers, Hans and Eduard, showed that an extract of yeast, containing no yeast cells, would convert sucrose into alcohol. The door was opened to the use of enzymes to catalyse chemical processes outside living cells.

Louis Pasteur had claimed that fermentation of sugars to alcohol could only occur if living cells were present. This was part of the theory of