Many factors affect the activity of enzymes and it is very easy to investigate these factors using common enzymes.

Enzymes work at their optimum temperature and pH. Any changes in these may affect the ability of enzymes to carry out their function, as they may work at a slower rate, become denatured, or not work at all. Other factors such as concentration of enzymes and substrates affect the rate of reaction. All these factors will be investigated in this topic guide.

On successful completion of this topic you will:
- be able to use biochemical practical skills and cognate techniques (LO4).

To achieve a Pass in this unit you need to show that you can:
- determine experimentally the characteristics of an enzyme, using safe practices (4.4).
1 The effect of temperature on the rate of milk clearing

Before you start
If you find some parts of this unit challenging, remember you are working at a higher level than you may be used to. In this unit it is important that you fully understand the following themes and topics before you begin:

• structure and function of biological molecules
• enzyme structure and function
• aerobic respiration.

If you need to check your understanding of proteins, carbohydrates, lipids and nucleic acids, Unit 2 Module 1 of OCR AS Biology (P. Kennedy and F. Sochacki, 2008), offers a good introduction to the topic.

If you need to check your understanding of aerobic respiration and the stages of glycolysis, link reaction, the Krebs cycle and the electron transport chain, you may find Unit 1 Module 4 of OCR A2 Biology (S. Hocking, 2008) useful.

Casein is a milk protein that makes the milk appear white and opaque (does not allow light through). As it is digested (broken down into smaller molecules) by protease, the milk changes from white to clear because the casein protein is being broken down into amino acids and therefore loses the ability to make milk appear white or opaque.

We can investigate the effect that temperature has on the enzyme protease (an enzyme that breaks protein into amino acids) by using water baths at different temperatures, each with a beaker of milk. If you draw a cross at the bottom of each beaker, you should notice at the start that the cross is not visible because the milk is opaque. However, as the enzyme affects the casein, it turns the milk transparent and the cross becomes visible.

Safety and COSHH requirements
• glassware
• water baths
• wear safety glasses

Method
1 Label five 50 cm³ beakers with the water bath temperatures (20 °C, 30 °C, 40 °C, 50 °C and 60 °C) and draw a black ‘X’ with a permanent OHP pen on the bottom of each beaker.

2 Put 10 g of milk powder into a 150 cm³ beaker and add 100 cm³ distilled water, ensuring you mix well.

3 Add 10 cm³ of the made-up milk solution to each of the labelled 50 cm³ beakers and add 1 cm³ of pH buffer (any pH, to control the pH of the solution) to each beaker, then stir with a glass rod. Make sure the glass rod is rinsed and wiped between each stirring.
4 Once stirred, place the beaker labelled ‘20 °C’ and the beaker containing 10 cm³ of the enzyme protease in the 20 °C water bath for 5 minutes.

5 After 5 minutes add 1 cm³ of the enzyme protease into the beaker of milk and place it on a white tile. Start the stopwatch.

6 Observe the milk solution every 30 seconds, looking for the black cross to appear. Record the time the cross is seen.

7 Repeat steps 4 to 6 for each temperature, standing both beakers in the appropriate water baths each time.

2 The effect of pH on the rate of catalase activity

Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide to oxygen and water. The symbol equation below shows the decomposition of hydrogen peroxide:

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

Enzymes are affected by changes in pH and catalase works best at its optimum pH of 7. We can investigate the effects of differing pHs in the investigation below. See Figure 1.4.1 to help set up the apparatus.

![Figure 1.4.1: Diagram of apparatus for investigating effect of pH on rate of catalase activity.](image)

Safety and COSHH requirements

- glassware
- hydrogen peroxide is irritant and catalase is a biological hazard
- wear safety glasses

Method

1 Set up five inverted 100 cm³ measuring cylinders by filling with water and inverting in a large beaker.

2 Insert the delivery tube into the open end of the measuring cylinder under water.

3 Label five test tubes with the pH buffers 4, 5, 6, 7 and 8.
4 Add 1 cm$^3$ of the correct pH buffer to the appropriately-labelled tubes using a clean 1 cm$^3$ syringe each time. Add 10 cm$^3$ of hydrogen peroxide to each tube.

5 Quickly add 1 cm$^3$ of the enzyme (catalase) to each tube and insert the rubber bungs and side-arm delivery tubes. Start the stopwatch. The other end of each delivery tube should be completely under the inverted measuring cylinder.

6 As the gas is given off the water will be pushed from the inverted measuring cylinders. After 10 minutes read the volume of gas collected for each tube and record the results in a table.

3 **The effect of substrate concentration on catalase activity**

Rate of reaction can be increased when there are more substrate molecules available to bind with the active site of the catalase enzyme. The effect of substrate concentration can be investigated using the following investigation.

**Safety and COSHH requirements**

- glassware
- eye protection must be worn as hydrogen peroxide ($H_2O_2$) is harmful and catalase is a biological hazard
- disposable gloves are advised as $H_2O_2$ can damage the skin

**Method**

1 Make up six different dilutions of hydrogen peroxide in boiling tubes, using one 5 cm$^3$ syringe for distilled water and another for hydrogen peroxide, to make different concentrations of hydrogen peroxide (substrate). The dilutions should be as in Table 1.4.1.

<table>
<thead>
<tr>
<th>Conc. $H_2O_2$ (%)</th>
<th>Volume of water (cm$^3$)</th>
<th>Volume of 20 volume $H_2O_2$ (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.0</td>
<td>10.0</td>
</tr>
<tr>
<td>80</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>60</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>40</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>20</td>
<td>8.0</td>
<td>2.0</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

2 Each tube should now contain 10 cm$^3$ of a hydrogen peroxide dilution. Label the tubes appropriately.

3 Using the 1 cm$^3$ syringe, put 1 cm$^3$ of celery extract (celery contains large amounts of catalase – this is going to break the hydrogen peroxide into oxygen and water) into a watch glass and soak three discs of filter paper in it for 5 minutes.
4 Use forceps to remove one of the paper discs and gently shake to remove any enzyme drips.

5 Drop the paper disc into the first hydrogen peroxide dilution. Use a clean glass rod to push the disc to the bottom if necessary. Start the stopwatch as soon as the disc hits the bottom of the tube and time until the disc rises to the surface of the hydrogen peroxide.

6 Remove the disc and repeat with the other two discs in the same dilution.

7 Discard the celery extract and put another 1 cm³ of fresh celery extract into the watch glass.

8 Soak three more discs in the fresh celery extract and, after 5 minutes, remove one and drop into the second dilution of hydrogen peroxide.

9 Repeat the procedure with three discs for each hydrogen peroxide dilution.

10 Record your results in a table and calculate the average time for the disc to rise for each dilution.

4 The effect of enzyme concentration on the rate of an enzyme-catalysed reaction

Sucrase is an enzyme that catalyses the hydrolysis of sucrose to fructose and glucose.

Safety and COSHH requirements

- glassware
- Benedict’s solution is irritant
- water baths
- wear safety glasses

Method

1 Set up a water bath to 35–40 °C. Label six test tubes with each of the enzyme concentrations (see Table 1.4.2) and keep the tubes in order of concentration.

<table>
<thead>
<tr>
<th>Sucrase concentration (% volume)</th>
<th>Volume of sucrose (cm³)</th>
<th>Volume of distilled water (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>80</td>
<td>8.0</td>
<td>2.0</td>
</tr>
<tr>
<td>60</td>
<td>6.0</td>
<td>4.0</td>
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<tr>
<td>40</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>20</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

2 Add 5 cm³ of sucrose solution to each of the test tubes and place all the tubes in the water bath for 2 minutes.
3 Label and fill a set of 1 cm³ syringes so that you have one of each enzyme concentration. This will help you carry out step 4 fast.

4 Add in quick succession 1 cm³ of the correct enzyme concentration to the labelled tubes of sucrose using the syringes. Start the stopwatch and leave the tubes in the water bath for 10 minutes. Be careful the water bath does not rise above 40 °C.

5 After 10 minutes heat the water bath to 80 °C and leave the tubes for a further 2 minutes.

6 Fill the 10 cm³ syringe with Benedict’s solution and quickly add 1 cm³ to each of the sucrose/enzyme tubes (starting with the lowest concentration and finishing with the highest) and start the stopwatch.

7 Observe the tubes for the first signs of green cloudiness and record the time taken for the first sign of green cloudiness to appear in each tube. After 10 minutes stop the experiment.

**Explanation**

Sucrose is not a reducing sugar (it does not have the ability to reduce other components). However, sucrase breaks sucrose into fructose and glucose. Glucose is a reducing sugar – it reduces (gives electrons to) the Benedict’s solution and a colour change is observed. Therefore, once the colour change is observed we know a reducing sugar is present.

**Portfolio activity (4.4)**

You can generate evidence for your portfolio by carrying out practical work on enzymes:

- carry out suitable experiments investigating temperature change, pH change, substrate concentration and enzyme concentration
- record data in suitable tables
- plot suitable graphs that will enable you to identify correlations
- explain what your results tell you about the enzyme activity under different conditions.

**Further reading**


**Link**

Enzymes in relation to drug action are introduced in *Unit S: Chemistry for applied biologists*.

**Acknowledgements**

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