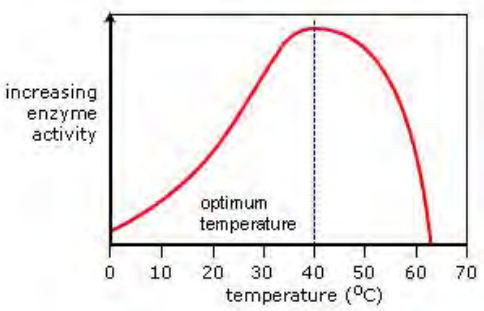
OCR (A) Biology A-level

Module 1: Development of practical skills in Biology

PAG 4: Rates of Enzyme Controlled Reactions

Please note: You only need to do one from each PAG, and you don't need to do the PAGs listed here, as long as you show the same skills that these are testing (see 5f of the specification for more information). However, you need to at least be able to design your own method for most of these experiments in the exam.

Investigating the effect of temperature on catalase activity:

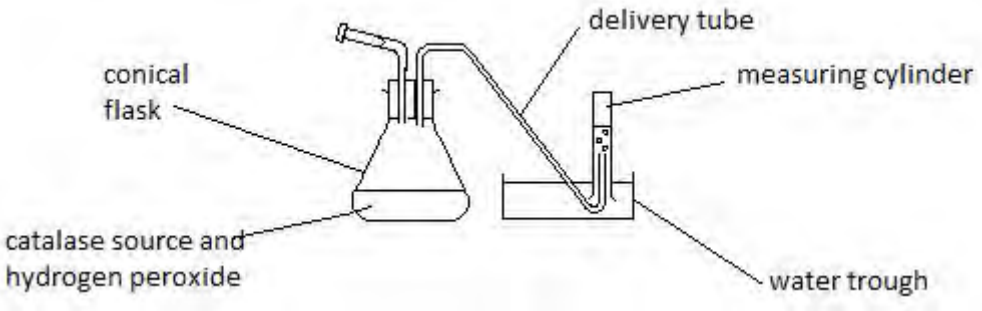


The reaction investigated in this practical is the breakdown of hydrogen peroxide by catalase.

Independent variable = temperature

Dependent variable = rate of reaction

1. Use your knowledge of factors that affect enzyme activity to suggest a hypothesis for this investigation.
2. Set up water baths at temperatures of 40°C, 60°C and 80°C. Also, set up an ice bath, try and keep this at a constant temperature. Use a thermometer to check the temperatures.
3. Set up 4 boiling tubes containing the same volume and concentration of hydrogen peroxide solution. Measure the volumes using measuring cylinders or 10cm^3 syringes.
4. To keep pH constant, add equal volumes of a suitable buffer solution to each boiling tube.
5. Set up the apparatus with a bung attached to an upside-down measuring cylinder in a trough of water by a delivery tube (see diagram).
6. Put each boiling tube in a water bath/ice bath of a different temperature. Allow the apparatus to equilibrate for 5 minutes.
7. Use a 5cm^3 syringe to add the same volume and concentration of catalase to each boiling tube.
8. Quickly put the bung on and start the timer.
9. Measure the amount of oxygen produced every 30 seconds for 3 minutes. Record this in a results table.
10. Repeat the experiment at each temperature at least 2 more times and calculate a mean.
11. Calculate the rate of gas production in cm^3 min^{-1} for each temperature.
12. Draw a graph of rate of reaction against temperature.



Investigating the effect of enzyme (amylase) concentration on the rate of reaction:

The reaction investigated in this practical is the breakdown of starch by amylase.

Independent variable = the concentration of amylase (enzyme concentration)

Dependent variable = rate of reaction

1. Put a drop of iodine in potassium iodide solution into each well onto a spotting tile. Label the wells to help read the results.
2. Prepare boiling tubes with different concentrations of amylase using the **serial dilutions** method (see: serial dilutions).
3. Add the same concentration and volume of starch to the first boiling tube. At the same time, start the timer.
4. Use a dropping pipette to put a drop of this mixture into one of the wells containing the iodine solution at regular intervals (e.g. at every 10 seconds).
5. Observe the resulting colour. The iodine solution goes **dark blue-black when starch is present** but **remains orange-brown when there's no starch**.
6. **Time how long it takes for the colour to no longer turn blue-black** when the starch/amylase solution is added.
7. Repeat at least 2 more times and calculate the mean time taken.
8. Repeat the experiment for each concentration of amylase.
9. Calculate the rate of reaction in mol dm^{-3} s^{-1} by dividing the concentration of starch by the time taken.
10. Plot a graph of rate of reaction against amylase concentration.

The effect of substrate concentration on the rate of an enzyme-controlled reaction:

Independent variable = the concentration of hydrogen peroxide (substrate concentration)

Dependent variable = rate of reaction

1. Set up 5 beakers with varying concentrations of hydrogen peroxide using the **serial dilutions** technique (see: serial dilutions). Transfer each solution to a conical flask.
2. Set up the apparatus with a bung attached to an upside-down measuring cylinder in a trough of water by a delivery tube (see diagram).
3. Use a syringe to add the same volume and concentration of catalase to the first conical flask.
4. Quickly put the bung on and start the timer.
5. Record how much oxygen is produced every 30 seconds for 3 minutes. Record this in a results table.
6. Repeat steps 2-5 for each concentration.
7. Repeat at least 2 more times then calculate the mean and standard deviation for each concentration.
8. Calculate the rate of gas production in cm^3 min^{-1} for each concentration of hydrogen peroxide.
9. Draw a graph of rate of reaction against substrate concentration.

Serial dilutions:

The following method has a dilution factor of 2, meaning with each dilution the concentration halves.

1. Set up 5 boiling tubes.
2. Starting with a 2.00% catalase solution, add 4cm^3 to the first boiling tube.
3. Using a syringe, add 2cm^3 of the solution in the first tube to the next tube. This makes a 1.00% catalase solution.
4. Repeat this process to make 0.50%, 0.25% and 0.13% solutions.