

Edexcel Biology IGCSE

2.14B: Enzymes and pH

Practical notes



Effect of pH on enzyme activity

Aim

Investigate the effect of changes in pH on amylase activity, measured by the rate of disappearance of substrate (starch).

Amylase catalyses the reaction below:

Starch → Maltose

Equipment

- test tubes
- a test tube rack
- water baths (electrical or Bunsen burners and beakers)
- spotting tiles
- a 5 cm³ measuring cylinder
- syringes or 10 cm³ measuring cylinders
- a glass rod
- a stopwatch
- starch solution
- amylase solution
- buffered solutions
- iodine solution
- thermometer

Method for pH

1. On a tile, label each well with the time (from 0 onwards) and add a drop of iodine solution to each well.
2. Add 2 cm³ of each buffer solution (ranging from pH 3.0 to 7.0) into each labelled test tube.
3. Immerse the starch solution, amylase solution, and the test tubes of buffer solution in a water bath at 25°C.
4. Allow a few minutes for the temperature to equilibrate.
5. Use a syringe to add 2 cm³ of amylase into a test tube of buffer solution.
6. Use a syringe to add 2 cm³ of starch into the same test tube and start timing immediately.
7. Use the glass rod to transfer a drop of the mixture to the well labelled '0' on the tile.
8. Repeat step 6 every minute, rinsing the glass rod in between every test, until the iodine solution remains brown and does not turn blue-black.
9. Calculate the rate of enzyme reaction by using 1/ time taken for iodine solution to remain brown.
10. Repeat steps 2-8 for buffer solutions with different pH values.
11. Plot a graph of the rate of enzyme reaction against pH.



pH	Time taken for amylase to completely break down all the starch / s	Rate of reaction / s ⁻¹

Sources of error

The intervals between testing samples may be too long to accurately measure the time taken for the starch to be completely broken down.

Potential Hazards

Be careful using hot water.

If using a Bunsen burner tie long hair back and wear goggles.

Wear safety goggles when using iodine solution, amylase solution and hot water.

