

# Edexcel Biology IGCSE

## 2.14B: Enzymes and pH

### Practical notes

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## 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 cm<sup>3</sup> measuring cylinder
- syringes or 10 cm<sup>3</sup> 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 cm<sup>3</sup> 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 cm<sup>3</sup> of amylase into a test tube of buffer solution.
6. Use a syringe to add 2 cm<sup>3</sup> 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 <sup>-1</sup>

### 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.

