

Temperature and enzyme activity

investigate how enzyme activity can be affected by changes in temperature

For Pearson Edexcel International GCSE Biology 4BI1 and Science (Double Award) 4SD0. Teacher answers start on a new page.

Name

Date

Aim

To investigate how the temperature affects the time taken for amylase to break down starch.

Background

Enzymes are biological catalysts. **Amylase** is an enzyme that breaks down starch into sugars. Each enzyme has an **active site** that fits its substrate, and the substrate and enzyme must collide for a reaction to happen.

Raising the temperature gives the particles more kinetic energy, so they collide more often and with more energy, and the reaction speeds up. Above the **optimum temperature** the enzyme's shape starts to change, so the active site no longer fits the substrate. The enzyme is **denatured** and stops working.

Iodine solution is orange-brown, and turns blue-black when starch is present. Samples of the reaction mixture are added to iodine solution at regular intervals. The time at which the iodine stays orange-brown shows that all the starch has been broken down. A shorter time means a faster rate.

Hypothesis

As the temperature rises towards the optimum, the starch will be broken down in a shorter time because the particles collide more often and with more energy. Above the optimum the time will increase again because the amylase is denatured.

Variables

Independent	Temperature of the reaction mixture (°C)
Dependent	Time taken for the starch to be completely broken down (min), shown when the iodine stays orange-brown
Control	• pH (the same buffer solution at pH 7 in every tube) • Volume and concentration of the amylase solution • Volume and concentration of the starch solution • Volume of the mixture sampled and the volume of iodine in each well

Equipment

- Starch solution (1%), 2 cm³ per tube
- Amylase solution (1%), 2 cm³ per tube
- Buffer solution at pH 7, 1 cm³ per tube
- Iodine solution in a dropping bottle
- Spotting tile with 6 or more wells
- Water baths (beakers of water, 250 cm³ or larger) set at 10, 20, 30, 40 and 50 °C, using ice, cold tap water and a kettle
- Thermometers (0–100 °C) or water bath thermostats
- Test tubes, test tube rack and marker pen
- Syringes or graduated pipettes (2 cm³ and 5 cm³)

- Dropping pipettes or glass rods for sampling, one per temperature
- Stopwatch or timer
- Eye protection

Risk assessment

Hazard	Risk	Precaution
Hot water (up to 50 °C) and a kettle	Scalds if spilled; the water is hot but not boiling in the water baths	Fill beakers no more than half full, stand them on a heatproof mat away from the edge of the bench, and let the kettle water cool to the required temperature before use.
Iodine solution	Irritates eyes and skin and stains clothes and skin	Wear eye protection; wash splashes off skin with water; clear spills straight away.
Amylase powder or solution	Enzymes can cause an allergic reaction or asthma-like symptoms if the dust is breathed in, and may irritate skin	Use solutions, not powder; avoid contact with skin and eyes; wash hands after the practical.

Method

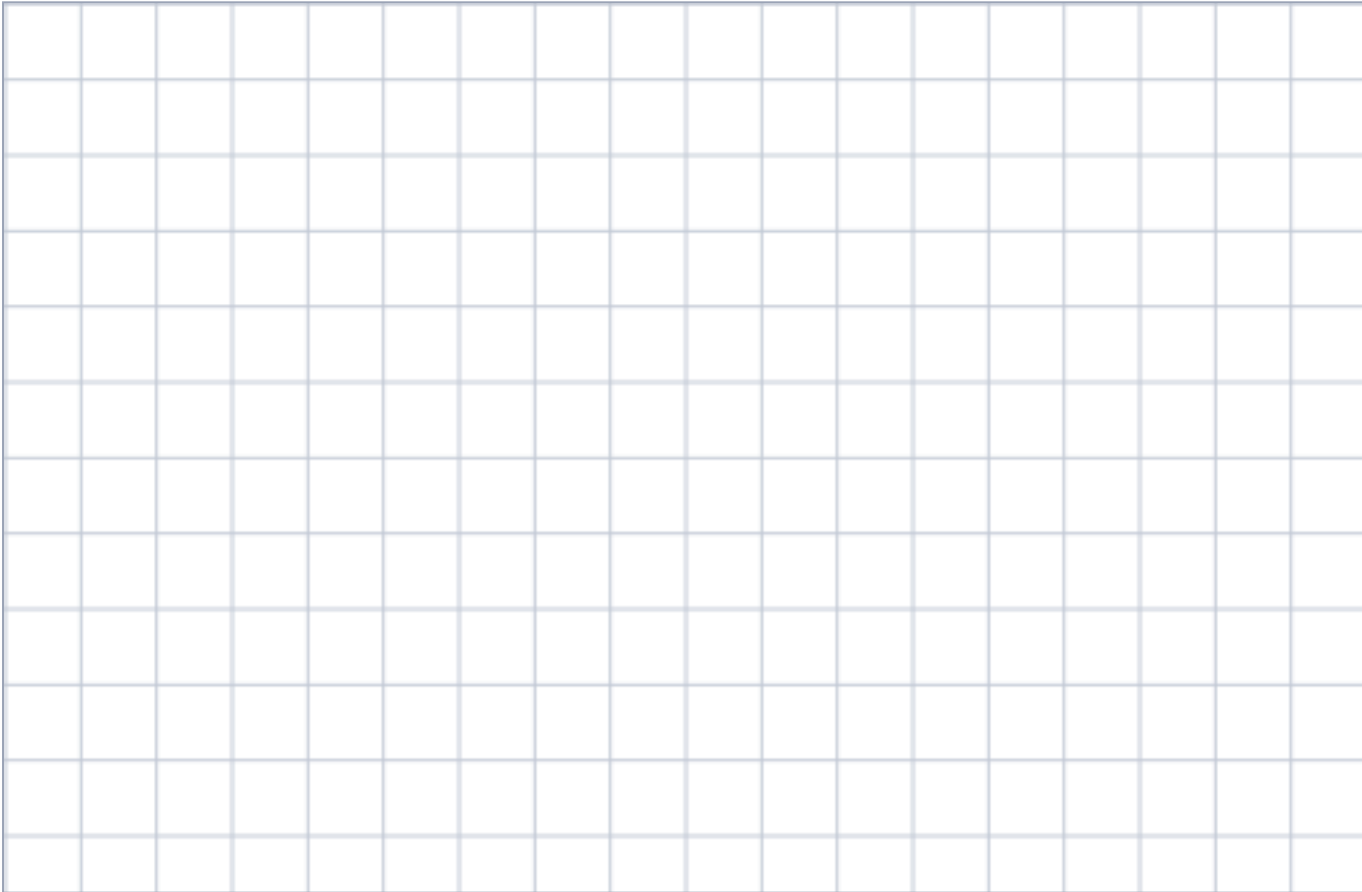
1. Put on eye protection. Set up water baths at 10, 20, 30, 40 and 50 °C and check each temperature with a thermometer.
2. Using a dropping pipette, put one drop of iodine solution into each well of a spotting tile (at least 6 wells per temperature to be tested).
3. Label a test tube for each temperature. Put 2 cm³ of starch solution and 1 cm³ of pH 7 buffer into each of these tubes.
4. Label a second set of tubes and put 2 cm³ of amylase solution in each.
5. Stand one starch tube and one amylase tube in the water bath at the first temperature for 5 minutes so they reach that temperature.
6. Pour the amylase into the starch and buffer tube, mix, and return it to the water bath. Start the stopwatch at once.
7. After 30 seconds, use a clean dropping pipette to transfer one drop of the mixture into the first well of iodine solution. Record whether it is blue-black or orange-brown.
8. Take a further drop every 30 seconds and add it to the next well of iodine solution (or to a cleaned well).
9. Stop sampling when the iodine stays orange-brown. Record this time as the time for all the starch to be broken down.
10. If the iodine is still blue-black after 10 minutes, record the time as '>10 min'.
11. Repeat steps 5–9 for each of the other temperatures, using fresh tubes and a clean pipette each time.
12. Repeat the whole investigation at least twice more, then calculate the mean time at each temperature.
13. Calculate the rate of reaction at each temperature as $1 \div \text{mean time (min}^{-1}\text{)}$.

Results

Time for starch to be broken down by amylase at different temperatures					
Temperature (°C)	Time 1 (min)	Time 2 (min)	Time 3 (min)	Mean time (min)	Rate, 1 ÷ mean time (min ⁻¹)
10					
20					
30					
40					
50					

Graph

Plot rate (1 ÷ mean time, min⁻¹) on the y-axis against temperature (°C) on the x-axis. Mark each point with a cross and draw a smooth curve through the points, or join them with straight lines; do not extend the line beyond the data. Read off the temperature that gives the highest rate: this is the optimum temperature for the amylase, to the accuracy of the temperatures you tested.



Exam questions 10 questions · 30 marks

1 State the independent variable and the dependent variable in this investigation. [2 marks]

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2 Explain why a buffer solution is added to each tube. **[2 marks]**

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3 Describe how the student knows when all the starch has been broken down. **[2 marks]**

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4 Explain why the starch and amylase are placed in the water bath separately before they are mixed. **[2 marks]**

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5 In another experiment, the mean time for the starch to be broken down at 30 °C was 2.5 minutes. Calculate the rate of reaction, using $\text{rate} = 1 \div \text{time}$. Give your answer to 2 decimal places. **[2 marks]**

Answer =

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- 6** Use the table to describe the effect of temperature on the time taken for the starch to be broken down. **[3 marks]**

Time for starch to be broken down	
Temperature (°C)	Mean time (min)
10	9.7
20	5.0
30	2.3
40	1.5
50	6.5

- 7** Explain why the time taken is much longer at 50 °C than at 40 °C. **[3 marks]**

- 8** The student wants to find the optimum temperature of the amylase more accurately. Suggest two improvements to the method. **[2 marks]**

9 Describe how you would investigate the effect of temperature on the rate at which amylase breaks down starch. Explain how you would obtain valid results. **[6 marks]**

[illegible]

10 A student plots a graph of rate against temperature for this practical. The curve rises to a peak at 40 °C, then falls steeply. Explain the shape of the curve. **[6 marks]**

[illegible]

Teacher answers

Practical 2: Temperature and enzyme activity. The example results are practice data, not real measurements.

Example results

Example results (practice data). Rate is calculated from the mean time as shown.

Temperature (°C)	Time 1 (min)	Time 2 (min)	Time 3 (min)	Mean time (min)	Rate, $1 \div \text{mean time (min}^{-1})$
10	10.0	9.5	9.5	9.7	0.10
20	5.0	4.5	5.5	5.0	0.20
30	2.5	2.5	2.0	2.3	0.43
40	1.5	1.0	2.0	1.5	0.67
50	6.5	7.0	6.0	6.5	0.15

Conclusion

The starch was broken down fastest at 40 °C (mean time 1.5 min, rate 0.67 min⁻¹), so the optimum temperature of this amylase is close to 40 °C, although it could lie anywhere between 30 and 50 °C because no other temperatures were tested. Between 10 and 40 °C the rate increased because the enzyme and substrate molecules had more kinetic energy and collided more often with enough energy to react. At 50 °C the time was much longer (6.5 min) because the **active site** had changed shape and the amylase was partly **denatured**, so fewer starch molecules fitted. The results support the hypothesis. Testing at 35 °C and 45 °C would locate the optimum more precisely.

Errors and improvements

Error	Effect on the results	Improvement
Sampling only every 30 seconds, so the end-point time is not exact	Times are only accurate to the nearest 30 s, so differences between fast reactions (at 40 °C) are poorly resolved	Sample every 10 or 15 seconds near the expected end-point, or use a colorimeter to measure the starch remaining.
The water bath temperature drifts, especially at 10 °C and 50 °C	The reaction takes place at a different temperature from the one recorded	Check the temperature with a thermometer throughout and add ice or hot water to keep it constant; use a thermostatically controlled bath.
The enzyme and starch are not at the right temperature when mixed	The reaction starts at the wrong temperature, giving a time that is too long or too short	Leave both solutions in the water bath for at least 5 minutes before mixing.
Judging the colour change by eye at the end-point	Different students may disagree on when the iodine 'stays orange-brown', adding random error	Compare against a standard orange-brown drop on the tile, have the same person judge each time, and repeat and use a mean.
Carry-over of starch or iodine on a pipette between samples	False blue-black results, giving times that are too long	Use a clean pipette (or rinse it) for each sample.

Mark scheme

One mark for each point unless a maximum is given. Words in brackets are not needed for the mark.

1. [2]

- independent: temperature (1)
- dependent: time taken for the starch to be broken down / for the iodine to stay orange-brown (1) allow rate of reaction

2. [2]

- to keep the pH constant / the same in each tube (1)
- because pH affects enzyme activity / so pH does not affect the results (1) allow so only temperature affects the rate

3. [2]

- a drop of the mixture is added to iodine solution (1)
- the iodine stays orange-brown / does not turn blue-black (1)

4. [2]

- so each reaches the temperature of the water bath (1)
- so the reaction starts at the correct temperature (1) allow so the temperature is controlled

5. [2]

- $1 \div 2.5$ (1)
- $0.40 \text{ (min}^{-1}\text{)}$ (1) award 2 marks for the correct answer with no working; 0.4 scores 1 mark only

6. [3]

- time decreases as the temperature rises from 10 to 40 °C (1)
- shortest time at 40 °C (1)
- time increases (again) between 40 and 50 °C (1)
- correct use of data, for example 9.7 min at 10 °C and 1.5 min at 40 °C (1)
- Max 3

7. [3]

- the enzyme / amylase is denatured (1) reject enzyme killed
- the shape of the active site changes (1)
- starch no longer fits (the active site) / fewer enzyme-substrate complexes form (1)

8. [2]

- test more temperatures / smaller intervals between 30 and 50 °C, for example 35 °C and 45 °C (1)
- take samples more often, for example every 10 s (1)
- use a thermostatically controlled water bath / check the temperature with a thermometer throughout (1)
- use a colorimeter to judge the end-point (1)
- Max 2

9. [6]

Level 3 5-6	A clear, logical method that covers measuring the temperature, the enzyme, starch and iodine, the timing, and the use of repeats and a mean, with at least two variables controlled and a reason given for the control
Level 2 3-4	A method with most key steps (several temperatures, iodine used to find the end-point, timing) and some control of variables, but lacking detail or a reason
Level 1 1-2	A basic method with little detail, such as 'heat the starch and amylase and see how long it takes'

Indicative content

- set up water baths at several temperatures and check with a thermometer; put starch, buffer and amylase in separate tubes in the bath to reach temperature; mix and start the stopwatch; take drops every 30 s into iodine on a spotting tile; end-point when the iodine stays orange-brown; record the time; repeat and calculate a mean; control pH with a buffer, volumes and concentrations of enzyme and starch; calculate rate as $1 \div \text{time}$ and plot against temperature

10. [6]

Level 3 5-6	Explains both the rise and the fall in terms of collisions, kinetic energy and denaturation, linking the shape of the active site to the substrate
Level 2 3-4	Explains one part fully, or both parts with some detail missing
Level 1 1-2	Describes the shape with limited or no explanation

Indicative content

- as the temperature rises the particles gain kinetic energy; more frequent collisions between enzyme and substrate; more collisions with enough energy to react, so rate increases; the peak is the optimum temperature; above the optimum the enzyme molecules vibrate more and the bonds holding their shape break; the active site changes shape / enzyme denatured; substrate no longer fits so the rate falls; denaturation is permanent

Exam tips

- Always name the dependent variable as a time taken or a rate, not 'how fast the enzyme works'. Convert time to rate with $1 \div \text{time}$ before plotting a rate graph.
- Use the word 'denatured' and describe the active site changing shape. 'The enzyme dies' or 'the enzyme is killed' gains no credit, because enzymes are not alive.
- Do not say that the enzyme is denatured at low temperatures. It is just working more slowly because there are fewer collisions.
- If asked why a buffer is used, say it keeps the pH constant so that only temperature changes.
- For improvement questions, give a specific change (for example sampling every 10 s) and say what it improves.