

Effect of temperature and pH on enzyme activity (amylase)

Investigate and describe the effect of changes in temperature and pH on enzyme activity with reference to optimum temperature and denaturation

For Cambridge IGCSE Biology 0610 (and 0970, which has the same content). Teacher answers start on a new page.

Name

Date

Aim

To investigate how temperature and pH affect the activity of the enzyme amylase by timing how long it takes to break down starch.

Background

Enzymes are proteins that are involved in all metabolic reactions, where they function as biological catalysts. **Amylase** is an enzyme that breaks down starch (the substrate) to maltose. Each enzyme has an **optimum** temperature and an optimum pH at which its activity is highest.

Starch gives a **blue-black** colour with iodine solution. When all the starch has been broken down, a drop of the mixture stays **orange-brown** when it is added to iodine solution. The time taken to reach this **end point** is a measure of enzyme activity: the shorter the time, the faster the reaction. The rate of reaction can be calculated as $1000 \div \text{time}$.

Below the optimum temperature the enzyme and substrate molecules have less kinetic energy, so there are fewer effective collisions and the rate is low. At temperatures above the optimum the shape of the active site changes and the substrate no longer fits: the enzyme is **denatured**. A pH that is too high or too low also changes the shape of the active site and can denature the enzyme.

Hypothesis

The rate of starch breakdown will increase with temperature up to an optimum temperature of about 40 °C and then decrease, because the enzyme is denatured above the optimum. The rate will be highest at about pH 7 and lower at more acidic and more alkaline pH values.

Variables

Independent	Part A: temperature of the water bath / °C. Part B: pH of the buffer solution
Dependent	Time for the starch to be broken down (the iodine stays orange-brown) / s, used to calculate the rate of reaction
Control	- Part A: pH (same buffer), volume and concentration of starch solution, volume and concentration of amylase solution - Part B: temperature of the water bath, volume and concentration of starch solution, volume and concentration of amylase solution - Both parts: size of the drops of mixture and iodine solution; the same time interval between samples

Equipment

- Starch solution, 1%, 100 cm³; amylase solution, 1%, 40 cm³
- Buffer solutions at pH 4, 5, 6, 7, 8 and 9 (20 cm³ of each)
- Iodine in potassium iodide solution (iodine solution) in a dropper bottle
- Spotting tile (or white tile with drops of iodine); dropping pipettes
- Large test-tubes (150 mm × 25 mm) for the starch and buffer, standard test-tubes (125 mm × 15 mm) for the amylase, and a rack; syringes, 5 cm³ and 10 cm³
- Beakers, 250 cm³, to use as water baths; kettle or hot water, and ice; thermometer, -10 °C to +110 °C
- Stop-clock reading to 1 s; glass rod or stirring rod; marker pen; eye protection

Risk assessment

Hazard	Risk	Precaution
Iodine solution	Stains skin and clothes; irritant to the eyes	Wear eye protection and wash off any splashes with water.
Amylase solution	Enzymes can cause an allergic reaction in some people if they get on the skin or are breathed in	Avoid skin contact, wash hands after the experiment and wipe up any spills.
Hot water for the water baths (up to 60 °C) and the glass thermometer	Scalds; broken glass	Use water no hotter than 60 °C (not boiling), take care when carrying beakers, and do not use the thermometer to stir.

Method

1. Put on eye protection. Put a drop of iodine solution in each well of a spotting tile (have a second tile ready, because up to 20 samples may be needed).
2. Use a syringe to put 5 cm³ of starch solution and 5 cm³ of pH 7 buffer into a large test-tube. In a standard test-tube put 2 cm³ of amylase solution.
3. Part A (temperature): set up a water bath in a 250 cm³ beaker at the first temperature (10 °C, using ice). Stand both tubes in the water bath for 5 minutes so they reach the temperature of the bath. Check the temperature with a thermometer.
4. Pour the amylase into the starch and buffer, mix with a clean glass rod and start the stop-clock. Keep the tube in the water bath.
5. After 30 seconds, use a clean dropping pipette to take one drop of the mixture and add it to a drop of iodine solution on the tile. Record the colour.
6. Repeat every 30 seconds with a clean pipette and a new drop of iodine each time until the iodine stays orange-brown. Record this time as the time for the starch to be broken down.
7. If the iodine is still blue-black after 600 s (10 minutes), stop and record the time as '> 600'.
8. Repeat steps 2 to 7 at 20, 30, 40, 50 and 60 °C, using fresh solutions each time. Keep the water bath within 1 °C of the temperature by adding hot or cold water.
9. Part B (pH): use a water bath at 35 °C and repeat steps 2 to 7, using buffers at pH 4, 5, 6, 7, 8 and 9 instead of the pH 7 buffer.
10. Calculate the rate of reaction for each value, using $\text{rate} = 1000 \div \text{time}$ (use a rate of 0 if the end point was not reached).

- L1 . Plot graphs of rate of reaction against temperature and against pH. Use each graph to find the optimum.
- L2 . Repeat the investigation at least twice more and calculate mean times for each value.

Results

Part A: effect of temperature on the time for amylase to break down starch

temperature / °C	time for starch to be broken down / s	rate of reaction (1000 ÷ time) / arbitrary units
10		
20		
30		
40		
50		
60		

Graph

Plot rate of reaction / arbitrary units (y-axis) against temperature / °C (x-axis). Mark points with crosses (x), use more than half of the grid in both directions and draw a single smooth best-fit curve (not straight lines between points). Read the optimum temperature from the highest point of the curve. Do the same for rate of reaction against pH in Part B.



Exam questions 10 questions · 30 marks

- 1** A student investigates the effect of temperature on the breakdown of starch by amylase, [4 marks]
as in Part A. (a) State the independent variable and the dependent variable. [2] (b) State
two variables that must be kept constant. [2]

- 2** The starch solution and the amylase solution are placed in the water bath for 5 minutes [2 marks]
before they are mixed. Explain why.

- 3** Iodine solution is used to find the end point of the reaction. (a) State the colour of the [3 marks]
mixture of iodine solution and a drop from the reaction mixture at the start, and at the end
point. [2] (b) Explain what the colour at the end point shows. [1]

- 4** At 20 °C the time for the starch to be broken down was 300 s. Calculate the rate of [2 marks]
reaction using $\text{rate} = 1000 \div \text{time}$. Give your answer to 1 decimal place.

Answer =

- 5** Part B was carried out at 35 °C. Complete the table by calculating the three missing values of rate of reaction. Give each answer to 1 decimal place. **[3 marks]**

Part B: effect of pH		
pH	time for starch to be broken down / s	rate of reaction (1000 ÷ time) / arbitrary units
4	> 600	
5	270	
6	120	8.3
7	90	11.1
8	180	
9	420	2.4

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- 6** The table shows the rate of reaction at different temperatures. Describe how you would plot a graph of these results to find the optimum temperature. **[4 marks]**

Rate at different temperatures	
temperature / °C	rate of reaction (1000 ÷ time) / arbitrary units
10	1.8
20	3.3
30	6.7
40	11.1
50	4.2
60	0.0

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- 7** (a) Describe the effect of temperature on the rate of reaction shown in the table in question 6. [2] (b) Explain the results at 50 °C and 60 °C. [2] **[4 marks]**

- 8** Use the table in question 5. (a) State the optimum pH for amylase in this investigation. [1] **[3 marks]**
(b) Explain why no starch was broken down at pH 4. [2]

- 9** A student says that the enzyme was denatured at 10 °C because the reaction was so slow. **[2 marks]**
Describe a test to show that the student is wrong, and state the result expected.

- 10** Suggest three ways of improving the investigation so that the optimum temperature is found more precisely and the results are more reliable. **[3 marks]**

Teacher answers

Practical skill 4: Effect of temperature and pH on enzyme activity (amylase). The example results are practice data, not real measurements.

Example results

Part A example results (practice data), pH 7		
temperature / °C	time for starch to be broken down / s	rate of reaction (1000 ÷ time) / arbitrary units
10	570	1.8
20	300	3.3
30	150	6.7
40	90	11.1
50	240	4.2
60	> 600	0.0

Conclusion

Part A (practice data): the rate of reaction increased from 1.8 arbitrary units at 10 °C to 11.1 at 40 °C, then decreased to 4.2 at 50 °C and to 0.0 at 60 °C. The optimum temperature was about 40 °C. Below the optimum, the enzyme and substrate molecules have less kinetic energy, so there are fewer effective collisions and fewer enzyme-substrate complexes form; the enzyme is not denatured at low temperatures. Above the optimum, the bonds holding the enzyme in shape break, so the shape of the active site changes and the starch no longer fits: the enzyme is **denatured**, and at 60 °C no starch was broken down in 10 minutes. Part B (practice data, 35 °C): the times were > 600 s at pH 4 and 270, 120, 90, 180 and 420 s at pH 5, 6, 7, 8 and 9, giving rates of 0.0, 3.7, 8.3, 11.1, 5.6 and 2.4. The optimum pH was about 7. At pH values above and below the optimum the shape of the active site changes, so fewer enzyme-substrate complexes form, and at extreme pH such as 4 the amylase is denatured.

Errors and improvements

Error	Effect on the results	Improvement
Water bath temperature falls (or rises) during the experiment	The enzyme is not working at the stated temperature, so the rate is not correct for that temperature	Use a large volume of water, check the temperature with a thermometer every minute and add hot or cold water to keep it constant.
Starch and amylase are not at the water bath temperature when they are mixed	The reaction starts at the wrong temperature, giving a longer or shorter time	Leave both tubes in the water bath for 5 minutes before they are mixed.
Testing every 30 seconds gives a coarse end point and it is hard to judge the colour	The time is only correct to the nearest 30 s, which is a large percentage error when the time is short	Test every 10 s near the expected end point and judge the colour against a white background.
Using the same pipette or iodine drop for different samples	Carry-over of starch or enzyme gives a false end point	Use a clean pipette and a fresh drop of iodine solution for each sample.
Only one trial at each temperature or pH	An anomalous result may not be noticed and the optimum cannot be located precisely	Repeat at least three times and calculate a mean; add values between 30 and 50 °C to locate the optimum more precisely.

Mark scheme

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

1. [4]

- (a) independent: temperature (of the water bath / mixture) (1); dependent: time taken for the starch to be broken down / for the iodine to stay orange-brown / rate of reaction (1)
- (b) any two from: pH / buffer; volume of starch (solution); concentration of starch; volume of amylase; concentration of amylase; size of the drop of iodine / mixture (2) ignore amount / time of experiment

2. [2]

- so that the enzyme and substrate reach the temperature of the water bath (1)
- so the reaction happens at the stated temperature from the moment they are mixed (1) allow so temperature is controlled / is the independent variable

3. [3]

- (a) start: blue-black (1) allow black
- end point: orange-brown / yellow-brown / brown (1) allow orange / yellow; reject colourless / clear
- (b) all the starch has been broken down / no starch remains (1)

4. [2]

- $1000 \div 300$ (1)
- 3.3 (arbitrary units) (1) allow 3.33; award 2 marks for the correct answer with no working

5. [3]

- pH 4: 0 / 0.0 (1) allow no reaction / less than 1.7
- pH 5: 3.7 (1) allow 3.70
- pH 8: 5.6 (1) allow 5.56

6. [4]

- temperature on the x-axis and rate on the y-axis, both labelled with quantity and unit using a solidus, e.g. temperature / °C and rate of reaction / arbitrary units (1)
- sensible scales using more than half of the grid, with equal intervals (1)
- points plotted correctly as crosses or encircled dots (to within half a small square) (1)
- single smooth best-fit curve, with the optimum read from its highest point (1) reject dot-to-dot straight lines

7. [4]

- (a) rate increases from 10 °C to 40 °C / to the optimum (1) allow data quote, e.g. from 1.8 to 11.1
- then decreases (to zero at 60 °C) (1) allow data quote, e.g. from 11.1 to 0.0
- (b) the enzyme is denatured (1) allow the active site changes shape / the substrate no longer fits / the shape of the enzyme has changed
- so fewer / no enzyme-substrate complexes form (1) allow so no starch is broken down at 60 °C; reject 'the enzyme is killed / dies'

8. [3]

- (a) 7 (1) allow 6.5-7.5
- (b) pH 4 is too acidic / far from the optimum so the enzyme was denatured (1)
- the shape of the active site changed so the starch no longer fitted / no enzyme-substrate complexes formed (1)

9. [2]

- warm the mixture from 10 °C to the optimum temperature / 40 °C (1)
- starch is broken down / the rate increases, showing the enzyme was not denatured (1) allow because low temperatures do not denature enzymes

10. [3]

- use more temperatures between 30 °C and 50 °C / smaller intervals near the optimum (1)
- repeat and calculate a mean (1)
- test samples more often / every 10 s (1)
- use a thermometer to check and keep the water bath at a constant temperature (1)
- use a white tile / background to see the colour more clearly (1)
- Max 3; ignore 'be more careful'; ignore 'use more enzyme'

Exam tips

- Say 'denatured', never 'killed'. A denatured enzyme has a changed shape of the active site, so the substrate no longer fits. A cold enzyme is slow but not denatured.
- For temperature explanations use kinetic energy and effective collisions for the rise, and a change in the shape of the active site for the fall above the optimum.
- The end point of the iodine test is when the colour stays orange-brown. Blue-black means starch is still present.
- Rate = $1000 \div \text{time}$. A shorter time means a faster rate. Show the working and round to the number of decimal places asked for.
- Graph of rate against temperature or pH: independent variable on the x-axis, quantity and unit with a solidus on both axes, and a smooth curve through the points, not a ruler line from point to point.