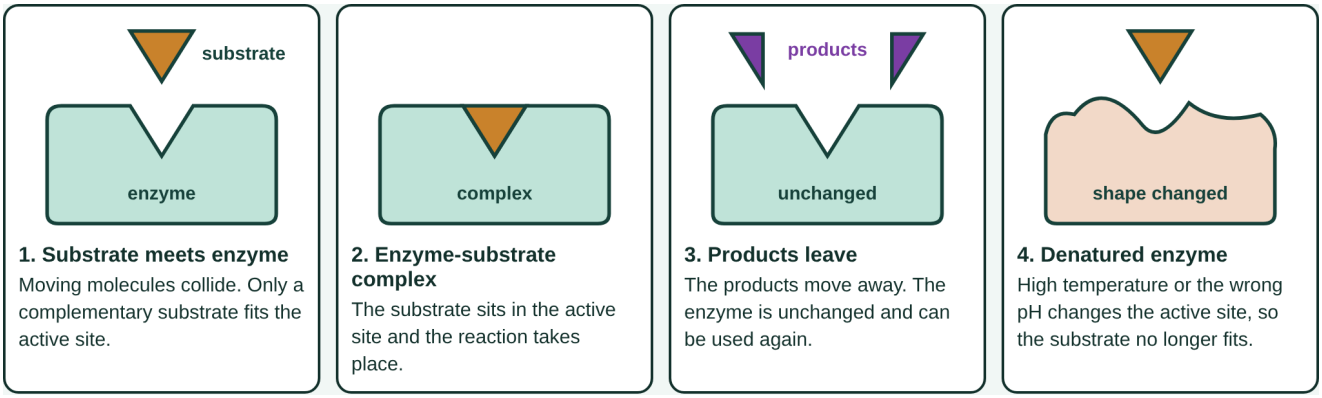
IGCSE and O Level Biology enzymes

Notes, practice questions and answers for Cambridge IGCSE Biology 0610 and O Level Biology 5090. Free from numrahedu.com.

How an enzyme works



1. Substrate meets enzyme
Moving molecules collide. Only a complementary substrate fits the active site.

2. Enzyme-substrate complex
The substrate sits in the active site and the reaction takes place.

3. Products leave
The products move away. The enzyme is unchanged and can be used again.

4. Denatured enzyme
High temperature or the wrong pH changes the active site, so the substrate no longer fits.

Enzyme key words

Term	Meaning
Catalyst	A substance that increases the rate of a chemical reaction and is not changed by the reaction.
Enzyme	A protein that works as a biological catalyst.
Substrate	The molecule an enzyme acts on, such as starch for amylase.
Product	The molecule made by the reaction, such as maltose from starch.
Active site	The part of the enzyme where the substrate fits. Its shape is complementary to the substrate.
Enzyme-substrate complex	The enzyme and substrate joined together while the reaction takes place.
Specific	Each enzyme usually acts on one substrate, because only that substrate fits its active site.
Lock and key hypothesis	A model of enzyme action: the substrate fits the active site as a key fits a lock.
Optimum	The temperature or pH at which the enzyme works fastest.
Denatured	The shape of the enzyme and its active site has changed, so the substrate no longer fits.

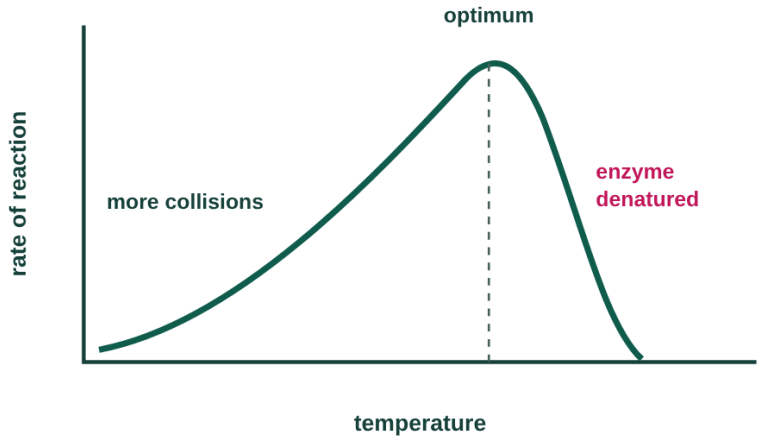
The lock and key hypothesis

This model explains why enzymes are specific. The enzyme is the lock, its active site is the keyhole, and the substrate is the key. Only a key with the right shape fits, just as only a substrate with a complementary shape fits the active site.

The model also helps with denaturing: a denatured enzyme is like a lock with a bent keyhole, so the key no longer fits. It has one limit. A real key comes out unchanged, but the substrate is changed into products. The enzyme is the part that stays unchanged.

The 5090 syllabus names the lock and key hypothesis. The 0610 syllabus asks for the complementary shape of the active site, so 0610 students should always use those words in an answer.

Effect of temperature on enzymes



Below the optimum

As temperature rises, enzyme and substrate molecules have more kinetic energy. They move faster and collide more often, so more effective collisions happen and the rate increases. Cold slows an enzyme but does not denature it.

At the optimum

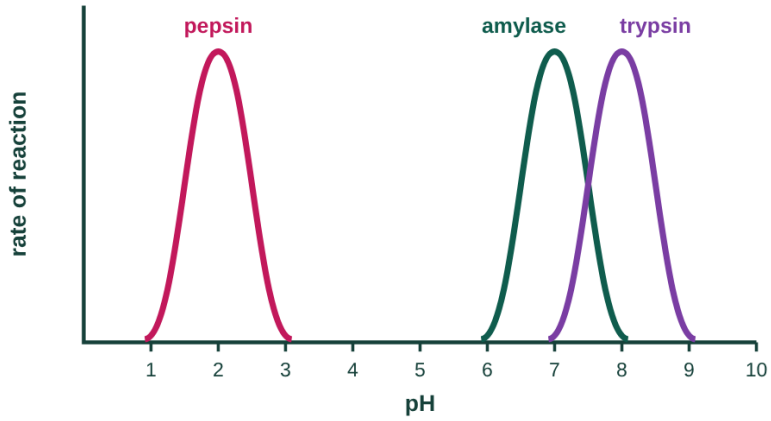
The rate is at its highest. Many human enzymes have an optimum near 37 °C, but other organisms have enzymes with different optimums.

Above the optimum

The enzyme starts to lose its shape. The active site changes and fewer substrate molecules fit, so the rate falls quickly. Once the enzyme is denatured, cooling it does not bring the activity back.

Numrahedu.com: the graph shows the shape to learn, not real data.

Effect of pH on enzymes



Each enzyme has its own optimum pH

Amylase in the mouth works best at about pH 7. Pepsin in the acidic stomach works best at about pH 2. Trypsin in the alkaline small intestine works best at about pH 8.

Away from the optimum

A pH that is too high or too low changes the shape of the active site, so the substrate fits less well and the rate falls. A pH far from the optimum denatures the enzyme.

Numrahedu.com: the graph shows approximate optimums, not real data.

Enzyme experiments to know

Effect of temperature on amylase

1. Put iodine solution in each well of a spotting tile.
2. Put a test-tube of starch solution and a separate test-tube of amylase solution in a water bath at the chosen temperature for a few minutes.
3. Mix them and start the stop-clock.
4. Every minute, use a clean pipette to add a drop of the mixture to a new well.
5. Record the time when the iodine stays orange-brown. This shows that the starch has gone.
6. Repeat at other temperatures, using the same volumes and concentrations. Repeat each temperature and calculate a mean.

Control. Use boiled and cooled amylase in place of amylase. The iodine should stay blue-black.

Remember. Warm the two solutions separately first, so the reaction starts at the chosen temperature.

Effect of pH on amylase

1. Set up the same spotting tile method.
2. Add a buffer solution of a known pH to the starch solution. A buffer keeps the pH constant.
3. Keep the mixture in a water bath at one temperature, such as 37 °C.
4. Add the amylase, start the stop-clock and test a drop every minute.
5. Record the time when the iodine stays orange-brown.
6. Repeat with buffers of other pH values, keeping everything else the same.

Control. Use boiled and cooled amylase at each pH.

Remember. Change only the pH. Temperature, volumes and concentrations stay the same.

Measuring gas from hydrogen peroxide

1. Put hydrogen peroxide solution in a large test-tube. An enzyme in liver or potato breaks it down to water and oxygen.
2. Add a measured mass of fresh liver or potato and start the stop-clock.
3. Measure the height of the foam after a set time, or collect the gas and measure its volume.
4. Repeat with one factor changed, such as temperature or pH.

Control. Use boiled liver or potato. Little or no gas should form.

Remember. Use the same mass and surface area of tissue each time, since a larger surface area exposes more enzyme.

Calculating a rate

Rate can be worked out from the time a reaction takes: **rate = 1 ÷ time**.

Example with made-up values: the starch is gone after 4 minutes, so the rate is 1 ÷ 4 = 0.25 per minute. A shorter time means a higher rate.

How to write your answers

Avoid	Write
The enzyme was killed.	The enzyme was denatured. Enzymes are proteins, not living things.
The enzyme was used up.	The enzyme is not changed by the reaction and can be used again.
The active site is the same shape as the substrate.	The active site is complementary to the substrate.
The cold denatured the enzyme.	At low temperature the molecules have less kinetic energy and collide less often.
The enzyme works best when it is hot.	The enzyme works fastest at its optimum temperature.
The rate went down after that.	The rate decreased above the optimum because the enzyme was denatured. Say why.
The starch went orange-brown.	The iodine solution stayed orange-brown, so no starch was present.

Fair tests and evaluation

- **Keep other variables the same.** Keep the volumes and concentrations of enzyme and substrate the same. Keep the temperature the same when testing pH, and keep the pH the same when testing temperature.
- **Use a control.** Use boiled and cooled enzyme. It shows that the change is caused by the active enzyme.
- **Repeat and find a mean.** Repeats help you spot an anomalous result and make the results more reliable.
- **Test more values.** To find an optimum more accurately, test smaller steps of temperature or pH close to the highest rate.
- **Sample more often.** Testing every minute gives the end point only to the nearest minute. Shorter intervals give a more accurate time.
- **Know the limit of colour.** The end of the iodine test is judged by eye, so two people may choose different times.

Hazards and precautions

What	Hazard	Precaution
Enzyme solutions	Can irritate skin and airways	Avoid contact with skin and wash off any spill.
Hydrogen peroxide	Can harm skin and eyes	Wear eye protection and rinse any spill with water.
Very hot water baths	Can scald skin	Use a test-tube holder and handle tubes with care.
Acid or alkali buffers	Can irritate skin and eyes	Wear eye protection and rinse any spill with water.

Enzymes questions

12 original questions, 43 marks. Answer on paper, then mark your work with the answers at the end.

Q1. What is an enzyme? [2 marks]

Describe what is meant by an enzyme.

Q2. Why enzymes are specific [3 marks]

Protease breaks down protein but does not break down starch. Explain why.

Q3. Reading temperature results [7 marks]

Made-up results for the time taken for amylase to break down starch: 10 °C, more than 15 minutes. 25 °C, 8 minutes. 37 °C, 3 minutes. 50 °C, 6 minutes. 70 °C, starch never gone. (a) State the temperature with the highest rate. [1] (b) Explain the difference between the results at 25 °C and 37 °C. [2] (c) Explain the result at 70 °C. [3] (d) Suggest how to find the optimum more accurately. [1]

Q4. Calculating a rate [3 marks]

Reaction A finished in 8 minutes and reaction B finished in 2 minutes. (a) Calculate the rate of each reaction using rate = 1 ÷ time. [2] (b) How many times faster is reaction B than reaction A? [1]

Q5. Cold or hot? [4 marks]

One tube of amylase is kept at 5 °C and another is heated to 90 °C. Both are then moved to 37 °C and starch is added. Predict and explain what happens in each tube.

Q6. pH in the gut [3 marks]

Pepsin has an optimum of about pH 2. Explain why it stops working when it moves from the stomach into the alkaline small intestine.

Q7. Why warm the tubes first? [2 marks]

A student warms starch and amylase in separate tubes in a water bath before mixing them. Explain why.

Q8. Using a control [2 marks]

A student repeats an experiment using boiled and cooled amylase. State the expected iodine result and explain why this control is useful.

Q9. Gas from hydrogen peroxide [5 marks]

Fresh liver is added to hydrogen peroxide solution and foam forms. (a) Name the gas in the foam. [1] (b) Explain why boiled liver gives little or no foam. [2] (c) State two variables to keep the same when comparing liver at different temperatures. [2]

Q10. Spot the mistakes [3 marks]

Read this student's account: "At 70 °C the enzyme was killed and used up, because the active site was the same shape as the starch." Give three corrections.

Q11. Evaluating a method [3 marks]

A student tests a drop of the mixture with iodine every 3 minutes. Suggest three ways to improve the accuracy or reliability of the results.

Q12. Planning [6 marks]

Plan an experiment to find the optimum pH of amylase, using starch solution and iodine solution.

Answers

Q1. What is an enzyme?

- One mark for a protein.
- One mark for a biological catalyst, or a substance that increases the rate of a reaction without being changed.

Q2. Why enzymes are specific

- One mark because the active site has a specific shape.
- One mark because the shape is complementary to protein molecules.
- One mark because starch does not fit the active site, so no enzyme-substrate complex forms.

Q3. Reading temperature results

- (a) One mark for 37 °C.
- (b) One mark for more kinetic energy at 37 °C.
- (b) One mark for more frequent collisions between enzyme and substrate.
- (c) One mark for the enzyme being denatured.
- (c) One mark for the shape of the active site changing.
- (c) One mark for the starch no longer fitting the active site.
- (d) One mark for testing smaller temperature steps close to 37 °C.

Q4. Calculating a rate

- (a) One mark for A: 0.125 per minute.
- (a) One mark for B: 0.5 per minute.
- (b) One mark for 4 times.

Q5. Cold or hot?

- One mark for the 5 °C tube breaking down the starch.
- One mark because the enzyme was not denatured by cold.
- One mark for the 90 °C tube not breaking down the starch.
- One mark because the enzyme was denatured and this does not reverse on cooling.

Q6. pH in the gut

- One mark for the pH being far above its optimum.
- One mark for the shape of the active site changing, or the enzyme being denatured.
- One mark for protein no longer fitting the active site.

Q7. Why warm the tubes first?

- One mark for both solutions reaching the chosen temperature.
- One mark so the reaction takes place at that temperature from the start.

Q8. Using a control

- One mark for the iodine staying blue-black.
- One mark for showing that the starch breakdown is caused by the active enzyme.

Q9. Gas from hydrogen peroxide

- (a) One mark for oxygen.
- (b) One mark for boiling denaturing the enzyme.
- (b) One mark because hydrogen peroxide no longer fits the active site.
- (c) One mark each, up to two, for the mass of liver, surface area of liver, volume of hydrogen peroxide, concentration of hydrogen peroxide or pH.

Q10. Spot the mistakes

- One mark for denatured, not killed.
- One mark because an enzyme is not used up in a reaction.
- One mark because a working active site is complementary to the starch, and at 70 °C its shape has changed.

Q11. Evaluating a method

- One mark for testing at shorter intervals.
- One mark for repeating and calculating a mean.
- One mark for a further improvement, such as a clean pipette for each drop, a thermostatic water bath, or comparing colours against a white background.

Q12. Planning

- Any six of these, one mark each:
- Use buffer solutions to give a range of pH values.
- Mix starch solution with a buffer in a test-tube.
- Keep the tube in a water bath at one temperature.
- Add amylase and start a stop-clock.
- Add a drop of the mixture to iodine solution at regular intervals.
- Record the time when the iodine stays orange-brown.
- Keep the volumes and concentrations of starch and amylase the same.
- Use boiled and cooled amylase as a control.
- Repeat and calculate a mean time, then choose the pH with the shortest time.

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