

Enzymes

A-Level Biology

What enzymes are

Enzymes are **globular proteins** – a type of **protein** with a rounded, soluble shape. They are biological catalysts: they **catalyse** (speed up) the chemical reactions in living things, and they are not used up, so each enzyme works again and again.

Enzymes work in two places:

- **intracellular** enzymes work inside the **cell** that made them. An example is **catalase**, which breaks down harmful hydrogen peroxide.
- **extracellular** enzymes are **secreted** (sent out) to work outside the cell. An example is **amylase**, which is released into the gut to **digest starch**.



Yeast enzymes ferment sugar, giving off bubbles of carbon dioxide

How enzymes work

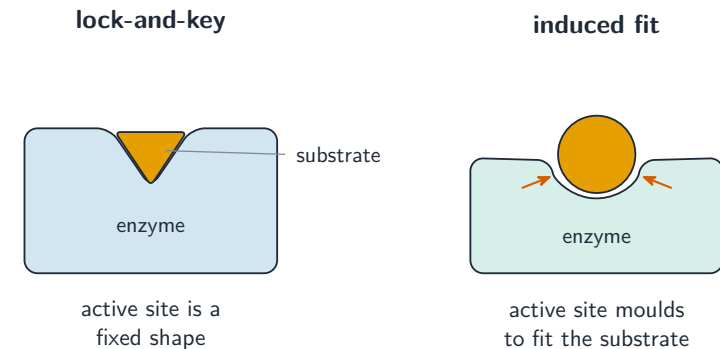
Each enzyme has a special pocket called the **active site**. The molecule it acts on is its **substrate**. The substrate fits into the active site to form an **enzyme-substrate complex**. The reaction then happens, and the **products** leave, freeing the active site for the next substrate.

■ Specificity

An enzyme is **specific**: it usually works on only one substrate. This is because the shape of the active site is **complementary** to (fits) the shape of that substrate and no other. We call this **specificity**.

Two ideas explain how the substrate fits:

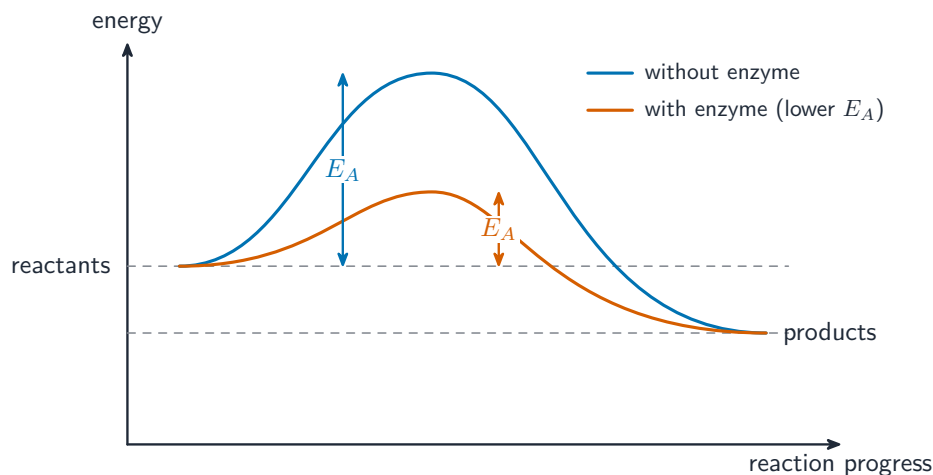
- the **lock-and-key hypothesis** – the active site is a fixed shape, and only a substrate with the matching shape fits, like a key in a lock.
- the **induced-fit hypothesis** – the active site is not quite the right shape at first. When the substrate binds, the active site changes shape a little to wrap around it tightly. This idea fits the evidence better.



Lock-and-key: a fixed active site. Induced fit: the active site changes shape to grip the substrate

■ Lowering activation energy

Every reaction needs a small “push” of energy to start, called the **activation energy**. An enzyme lowers the activation energy. This lets the reaction go quickly at the cell's normal **temperature**, instead of needing high heat.



The enzyme route has a lower activation energy (E_A), so more molecules can react

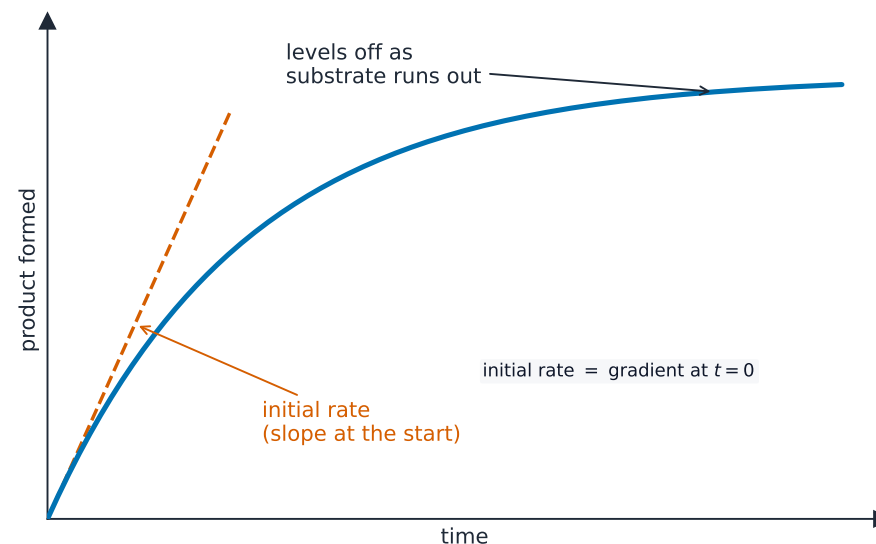
Measuring the rate of a reaction

You can follow an enzyme reaction in two ways:

- measure how fast **product** is made. With catalase, oxygen gas is a product, so you collect the gas and measure its volume over time.
- measure how fast substrate disappears. With amylase, you remove samples and use the iodine test; the blue-black colour fades as the starch is used up.

A **colorimeter** makes this exact. It shines light through the tube and measures how much light is absorbed, so a colour change becomes a number you can plot.

The **rate of reaction** is steepest at the start (most substrate present), so the **initial rate** (the slope at time zero) is the fairest value to compare.



Product builds fastest at the start; the initial rate is the slope of the tangent at time zero – the fairest value to compare

Worked example. In the first 20 seconds of a catalase reaction, 16 cm³ of oxygen is collected. Estimate the rate of reaction.

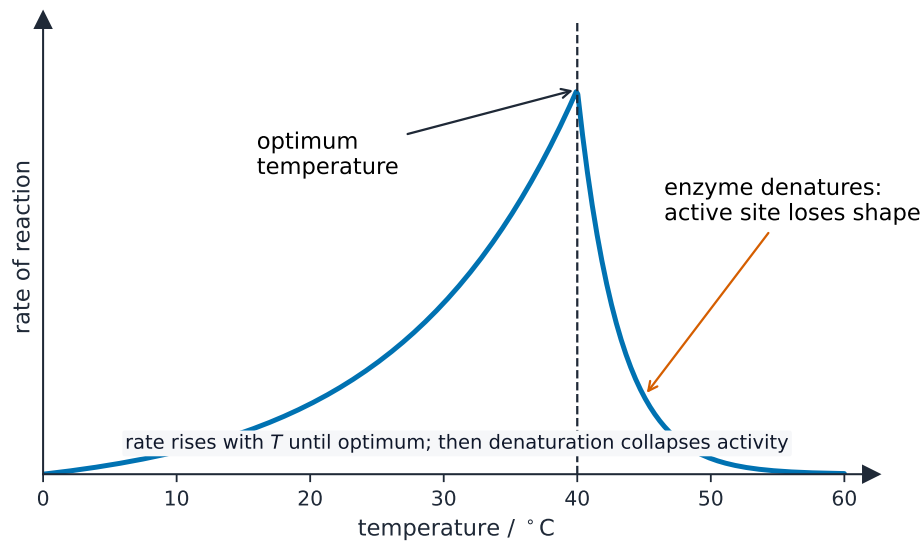
$$\text{rate} = \frac{\text{volume of product}}{\text{time}} = \frac{16}{20} = 0.8 \text{ cm}^3 \text{ s}^{-1}.$$

Because the reaction is fastest at the start, measuring over this short early interval gives a value close to the initial rate; averaging over a longer time would include the slower later stages and underestimate it.

Factors that affect enzyme activity

■ Temperature

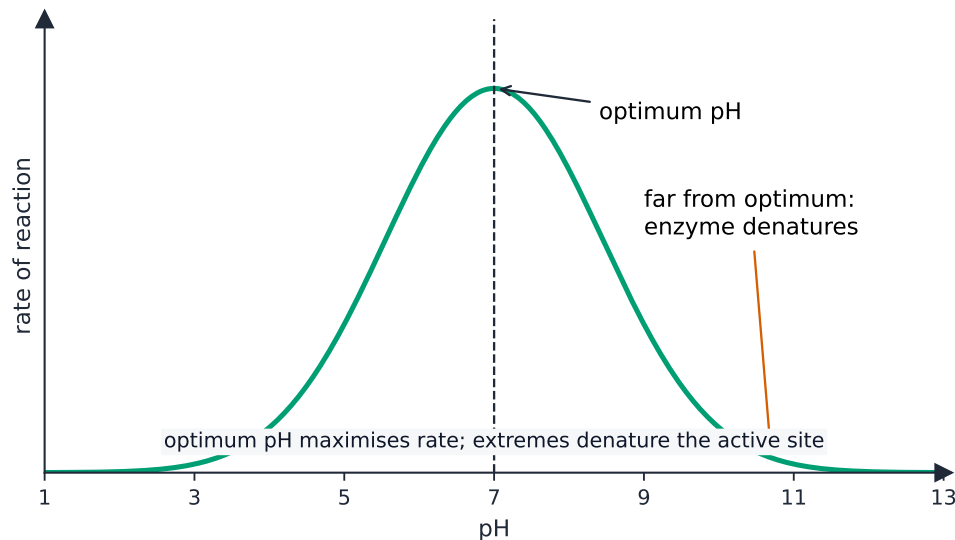
As temperature rises, molecules gain more **kinetic energy** and **collide** more often, so the rate rises. But above the **optimum temperature** the enzyme begins to **denature**: the heat breaks the bonds holding its shape, so the active site changes and no longer fits the substrate. The rate then falls quickly.



Rate rises to the optimum, then falls fast as the enzyme denatures

■ pH

Each enzyme has an optimum pH. If the pH moves too far from it, the enzyme denatures and the rate drops. To study pH fairly, you keep it steady with a **buffer solution**.



The rate peaks at the optimum pH and falls away on either side

■ Enzyme concentration

With plenty of substrate, more enzyme means more active sites, so the rate goes up in proportion to enzyme concentration.

■ Substrate concentration

At first, adding more substrate speeds the reaction. But once every active site is busy, adding more makes no difference – the rate levels off at a maximum.

■ Inhibitor concentration

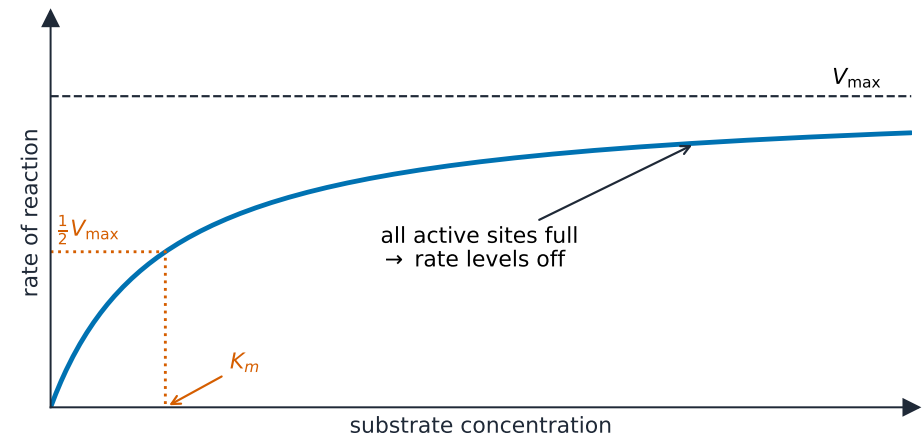
An **inhibitor** is a molecule that slows an enzyme. The more inhibitor present, the lower the rate.

$V_{\max}$ and the Michaelis–Menten constant

The levelling-off rate, when all active sites are full, is the maximum rate, written $V_{\max}$. The **Michaelis–Menten constant** (K_m) is the substrate concentration that gives **half** of $V_{\max}$. It tells you about the enzyme's **affinity** (pulling power) for its substrate:

- a **low** K_m means the enzyme reaches half-speed at a low substrate concentration, so it has a **high** affinity.
- a **high** K_m means a **low** affinity.

So K_m lets you compare how strongly different enzymes hold their substrates.



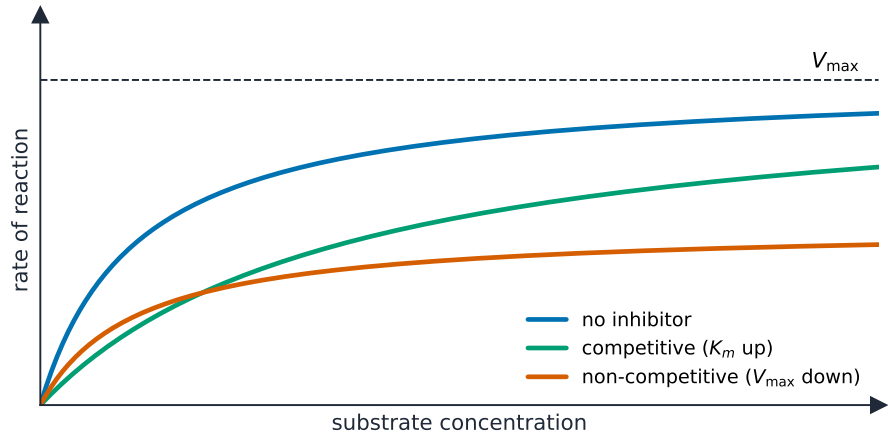
$$v = \frac{V_{\max}[S]}{K_m + [S]} \quad \cdot \quad K_m = [S] \text{ at half } V_{\max}$$

Rate climbs to $V_{\max}$ when all active sites are full; K_m is the substrate concentration giving half $V_{\max}$

Reversible inhibitors

Some inhibitors are **reversible** : they can leave the enzyme again. There are two types.

Type	Where it binds	Effect of adding more substrate	Effect on $V_{\max}$ and K_m
competitive inhibitor	in the active site (it has a similar shape to the substrate)	more substrate out-competes it, so its effect is reduced	$V_{\max}$ unchanged; K_m rises
non-competitive inhibitor	at another site, changing the active site's shape	adding more substrate does not help	$V_{\max}$ falls; K_m unchanged

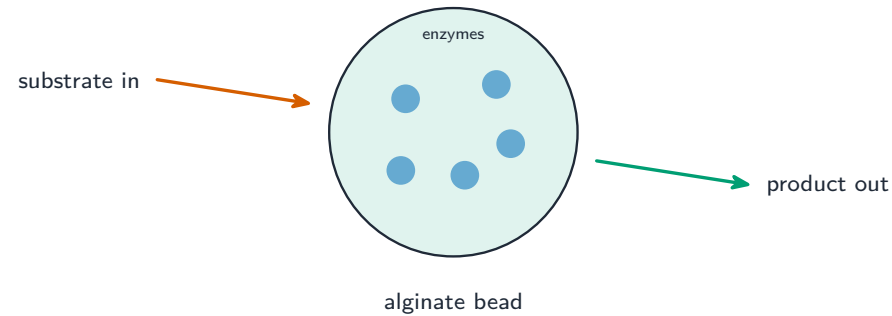


competitive: $\uparrow K_m$, same $V_{\max}$ · non-competitive: $\downarrow V_{\max}$

A competitive inhibitor raises K_m (more substrate overcomes it); a non-competitive one lowers $V_{\max}$

Immobilised enzymes

An **immobilised enzyme** is fixed in place – for example, trapped inside small beads of **alginate** – instead of floating free in solution. The substrate solution flows past the beads.



The enzymes stay trapped in the bead while substrate flows in and product flows out – so the enzyme is never washed away

A free enzyme usually works a little faster, because the substrate can reach it easily. But immobilised enzymes have big practical advantages:

- the enzyme is not washed away, so it can be used again and again.
- the product is pure – it is not mixed with enzyme.
- the enzyme is more stable, so it survives changes in temperature and pH better.
- the process can run continuously, with substrate flowing in and product flowing out.



Biological washing powders contain enzymes that digest food and blood stains at low temperatures

Exam tips

- Explain enzyme action with the **induced-fit** model (the active site moulds around the substrate) – now preferred to lock-and-key.
- Above the optimum the enzyme **denatures** (hydrogen bonds and tertiary structure break) – write “denatures”, never “dies” or “is killed”.
- Distinguish inhibitors: **competitive** binds the active site (overcome by more substrate, same V_{max}); **non-competitive** binds elsewhere (lower V_{max}).
- Compare rates using the **initial rate** (tangent at time zero) – the fairest measure, before substrate becomes limiting.