A-LEVEL BIOLOGY • EXERCISE SHEET

# 3.2 Factors that affect enzyme action

Name: _____ Class: _____ Date: _____ **Total: 17 marks**

## Objective

Build the skills to answer exam questions on the **factors that affect enzyme action** —temperature, pH, enzyme and substrate concentration, **inhibitors** 抑制剂, the **Michaelis–Menten constant** 米氏常数, and **immobilised enzymes** 固定化酶.

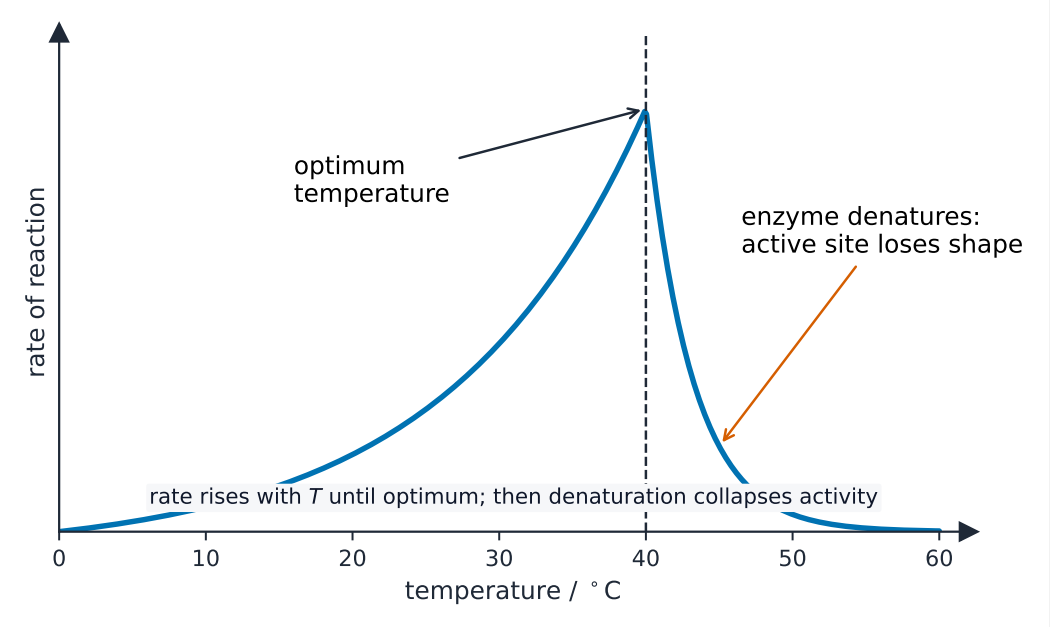
**You must be able to:**

- explain the effect of temperature, pH, enzyme concentration and substrate concentration on rate
- explain $V_{max}$ and $K_m$ and use $K_m$ to compare affinity
- explain competitive and non-competitive inhibition, and state the advantages of immobilised enzymes

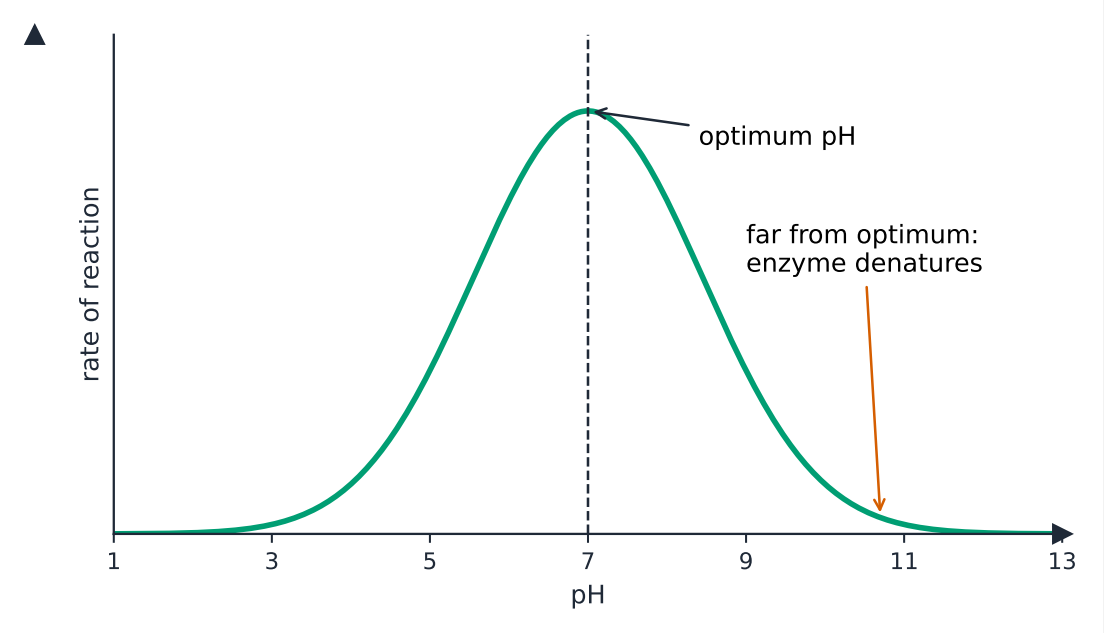
## 1 Worked examples

### Temperature and pH

Rate rises with temperature (more **kinetic energy** 动能, more collisions) up to the **optimum**; above it the enzyme **denatures** 变性—bonds holding its shape break, the active site changes, and rate falls fast. Each enzyme also has an optimum pH; far from it the enzyme denatures.



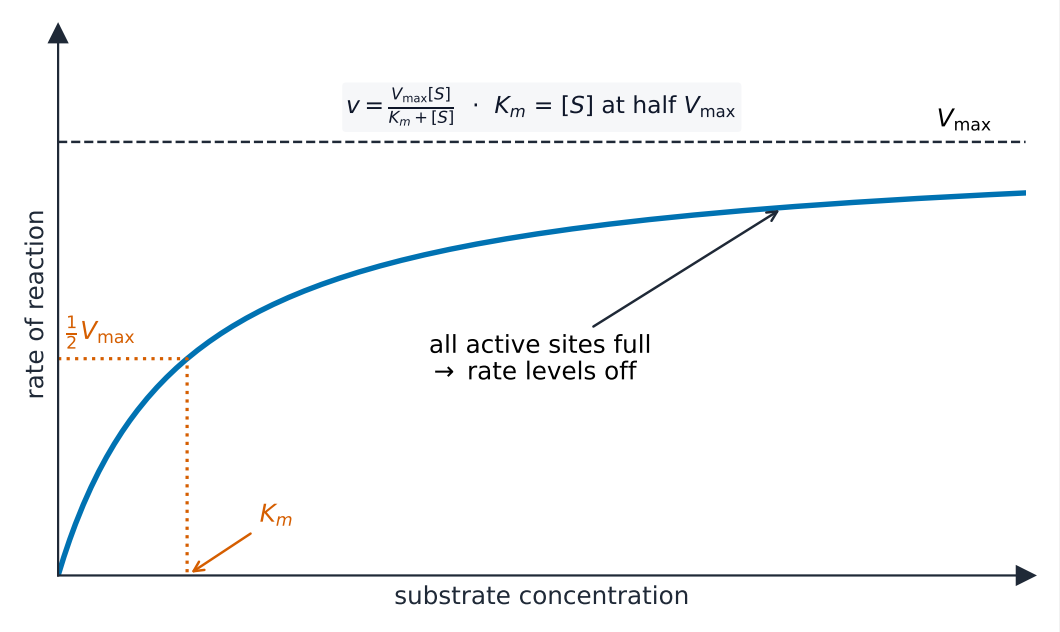

*Rate rises to the optimum, then falls fast as the enzyme denatures*




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

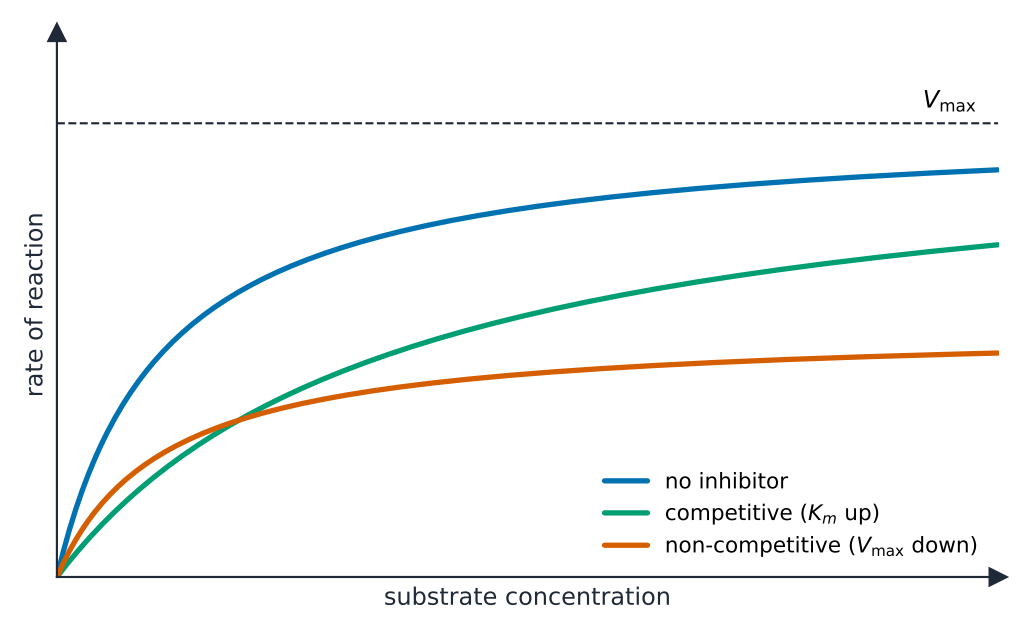
### ■ Substrate concentration, V_max and K_m

Adding substrate speeds the reaction until every active site is busy; then rate levels off at the maximum, $V_{\max}$. The **Michaelis–Menten constant** $K_{\mathrm{m}}$ is the substrate concentration that gives **half** of $V_{\max}$. A **low** $K_{\mathrm{m}}$ = **high affinity** 亲和力 (reaches half-speed at low substrate).




*$K_m$ is the substrate concentration giving half $V_{max}$ —a lower $K_m$ means higher affinity*

## Reversible inhibitors

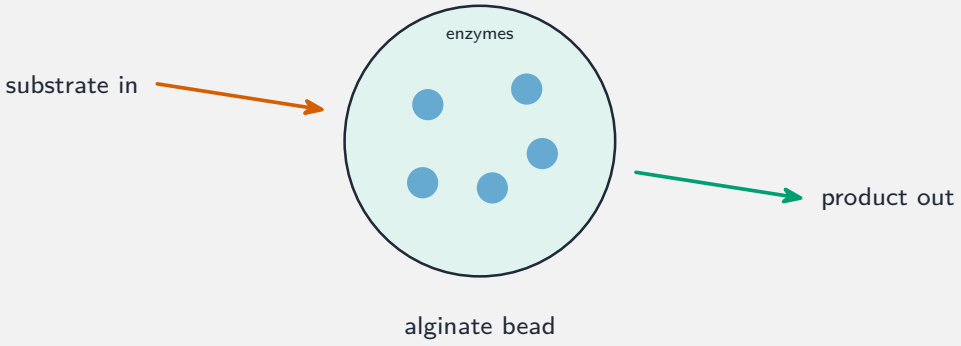



*Competitive inhibition raises $K_m$ (more substrate overcomes it); non-competitive lowers $V_{max}$*

- **competitive** 竞争性: binds the active site (similar shape to substrate); more substrate overcomes it → $V_{\max}$ unchanged, $K_{\mathrm{m}}$ rises.
- **non-competitive** 非竞争性: binds elsewhere, changing the active site; more substrate does not help → $V_{\max}$ falls.

## Immobilised enzymes

An **immobilised enzyme** is fixed in place (e.g. trapped in **alginate** 海藻酸盐 beads).




*Enzymes stay trapped while substrate flows in and product out —so they are not washed away*

Advantages: re-used many times; pure product; more stable to temperature/pH; can run continuously.

## 2 Practice

**2.1** Explain why the rate of an enzyme reaction falls above the optimum temperature. [3]

**2.2** State **two** advantages of using an immobilised enzyme rather than a free one. [2]

## 3 Exam-style questions

**3.1** A competitive inhibitor is added to an enzyme reaction. Compared with no inhibitor, what happens to $V_{max}$ and $K_m$? [1]

- **A** $V_{max}$ unchanged; $K_m$ increases
- **B** $V_{max}$ decreases; $K_m$ unchanged
- **C** both increase
- **D** both decrease

**3.2** Enzyme Y has a lower $K_m$ than enzyme Z for the same substrate. This means that enzyme Y: [1]

- **A** has a higher affinity for the substrate
- **B** has a higher $V_{max}$
- **C** is denatured more easily
- **D** works at a higher optimum pH

**3.3** A student investigates how temperature affects the rate at which catalase breaks down hydrogen peroxide. She measures the volume of oxygen produced in the first minute at five temperatures.

(a) State **two** variables she must control to make this a fair test. [2]

(b) Describe and explain the results she would expect between 10 °C and 60 °C. [4]

(c) Explain why a buffer solution would be used in a similar experiment that varied pH. [2]

**3.4** The graph of rate against substrate concentration for an enzyme levels off at high substrate concentration. Explain why. [2]

## 4 Go further

You are now ready for the real exam questions on this subtopic. Open the **3.2 Factors that affect enzyme action** past-paper sheet in the Library, or try these in **Practice mode**:

- 9700/12 N25 —Q14 (factors affecting rate)
- 9700/13 N25 —Q16 (inhibitors)
- 9700/31 N25 —Q1 (enzyme practical, structured)

## Solutions

**2.1** heat breaks the bonds (hydrogen/ionic) holding the enzyme's shape; the active site changes shape / denatures; so the substrate no longer fits and fewer enzyme–substrate complexes form.

**2.2** Any two: re-used many times / product is pure (no enzyme) / more stable to temperature and pH / allows continuous flow.

**3.1 A**. A competitive inhibitor raises $K_m$ but leaves $V_{max}$ unchanged (more substrate overcomes it).

**3.2 A**. A lower $K_m$ means half $V_{max}$ is reached at lower substrate concentration —higher affinity.

**3.3** (a) any two: substrate (hydrogen peroxide) concentration / volume / enzyme concentration / pH / time.
(b) rate rises from 10 °C towards the optimum as molecules gain kinetic energy and collide more often; above the optimum the enzyme denatures, so the rate falls.
(c) a buffer keeps pH constant so that pH is not an uncontrolled variable affecting the rate.

**3.4** at high substrate concentration every active site is occupied / saturated, so adding more substrate cannot increase the rate further —it is at $V_{max}$.