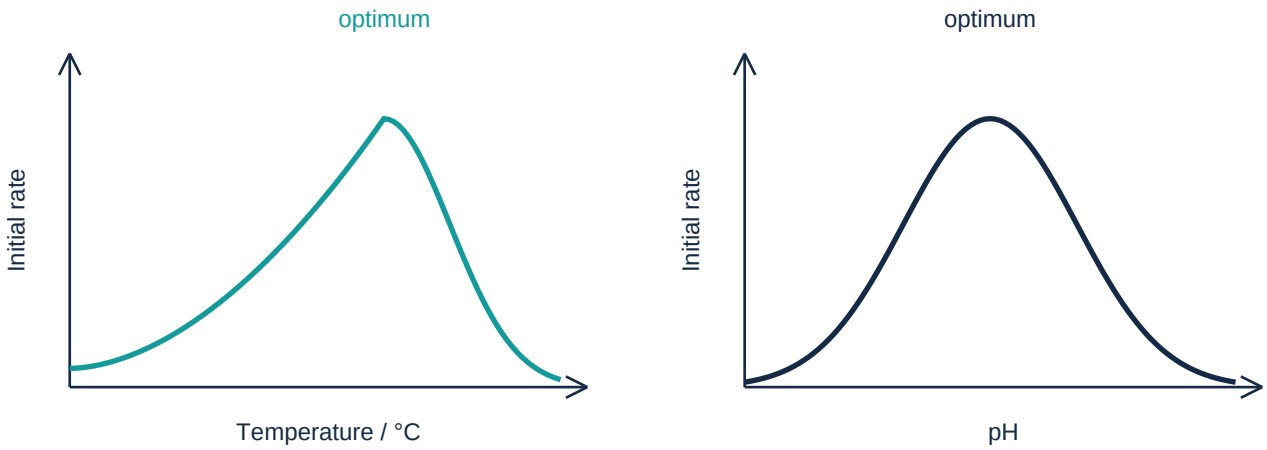
Temperature and pH

Reaction rate depends on molecular encounters and a functional active site. Changing the conditions can alter either, so faster molecular movement does not always mean a faster reaction.

SCHEMATIC CURVES: ONE ENZYME, FIXED ASSAY CONDITIONS



1. TEMPERATURE: TWO COMPETING EFFECTS

Warming initially increases kinetic energy and successful enzyme-substrate encounters. At sufficiently high temperatures, disruption of interactions stabilising the protein changes its active site; denaturation lowers activity. Cooling usually slows catalysis without denaturing the enzyme.

2. pH: CHARGES AND SHAPE MATTER

Changing pH changes the ionisation of amino acid side chains. This can alter substrate binding, catalytic groups and interactions maintaining the active site. Large pH changes can denature the enzyme. A buffer resists pH change and helps maintain the chosen pH.

Optima vary between enzymes and experimental conditions; do not assume every enzyme works best at 37 °C or pH 7. A lower rate away from optimum pH does not by itself prove permanent denaturation.

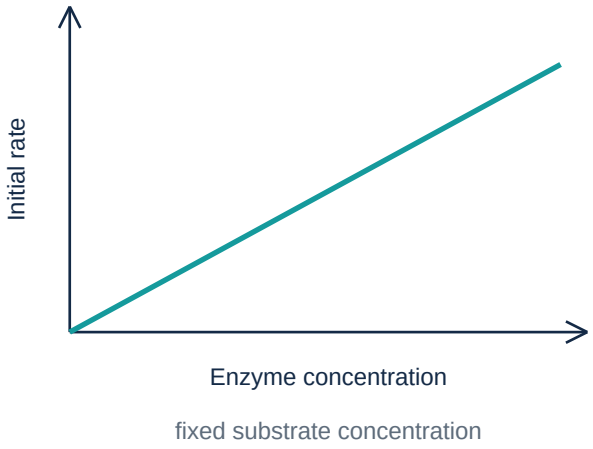
Supporting reading: OpenStax Biology 2e, 6.5 Enzymes.

Scope: Cambridge Biology 9700 syllabus, section 3.2, outcome 1, p. 20.

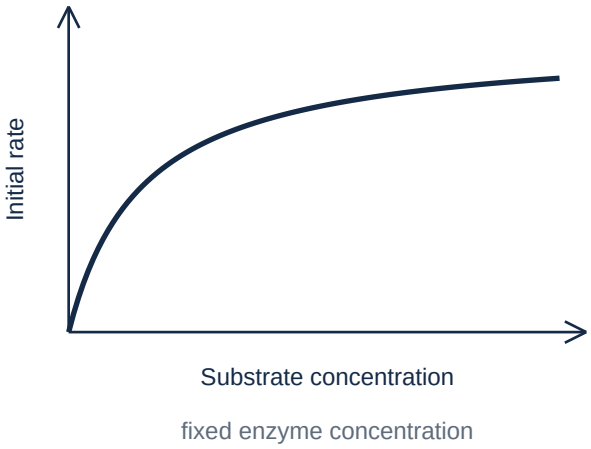
Enzyme versus substrate

These concentrations change different parts of the system. More enzyme supplies more catalytic sites; more substrate increases how frequently the available sites can be occupied.

SCHEMATIC INITIAL-RATE COMPARISONS



fixed substrate concentration



fixed enzyme concentration

3. INCREASE ENZYME CONCENTRATION

At the same substrate concentration and under suitable initial-rate conditions, more active enzyme provides more active sites, so rate is proportional to enzyme concentration. For example, doubling active enzyme approximately doubles initial rate.

4. INCREASE SUBSTRATE CONCENTRATION

Initially, substrate encounters active sites more frequently, so more enzyme-substrate complexes form per second. At high substrate concentration, active sites are occupied for most of the time and rate approaches a maximum. Adding more substrate then has little effect.

An enzyme-concentration investigation may depart from a straight line if substrate is depleted during measurement. Measure the early rate. A substrate-concentration plateau means enzyme capacity is limiting, not that enzymes have been used up.

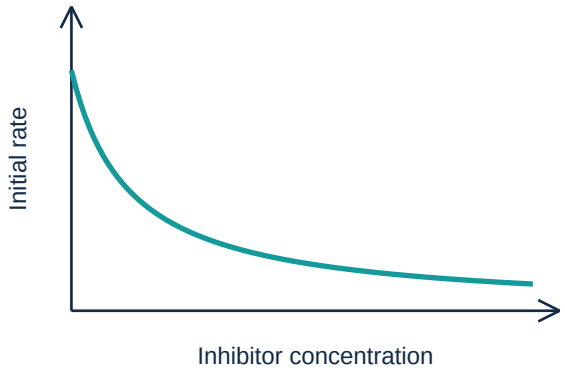
Supporting reading: Worthington Biochemical, Enzyme Concentration.
Supporting reading: Worthington Biochemical, Substrate Concentration.

Scope: Cambridge Biology 9700 syllabus, section 3.2, outcome 1, p. 20.

Inhibitors and fair investigations

An inhibitor reduces enzyme activity. To investigate any factor, change it systematically and measure rate while controlling the other conditions.

5. INCREASE INHIBITOR CONCENTRATION



At fixed enzyme and substrate concentrations, increasing inhibitor concentration usually reduces rate. Binding can obstruct substrate access or reduce catalysis. The curve depends on the inhibitor and conditions; inhibition need not involve denaturation.

Schematic, not measured data

PLAN THE FACTOR YOU CHANGE

Temperature	Use water baths; equilibrate solutions before mixing.
pH	Use suitable buffers; keep buffer concentration comparable.
Enzyme concentration	Dilute enzyme stock; keep final substrate concentration fixed.
Substrate concentration	Dilute substrate stock; keep final enzyme concentration fixed.
Inhibitor concentration	Vary inhibitor; include a no-inhibitor / solvent control.

MEASURE, REPEAT, COMPARE

Keep total reaction volume, mixing and all non-tested factors constant. Record early product formation or substrate loss; calculate change / time. Repeat each condition and compare mean rates and variability. Follow the teacher's risk assessment for enzymes, inhibitors and reagents.

Mechanism background: Assay Guidance Manual, enzyme inhibition.
Practical example: Practical Biology, trypsin concentration investigation.

Scope: Cambridge Biology 9700 syllabus, section 3.2, outcome 1, p. 20.