DP IB Biology: HL

Your notes

2.4 Enzymes

Contents

2.4.1 Enzymes


Your notes

Structure of Enzymes

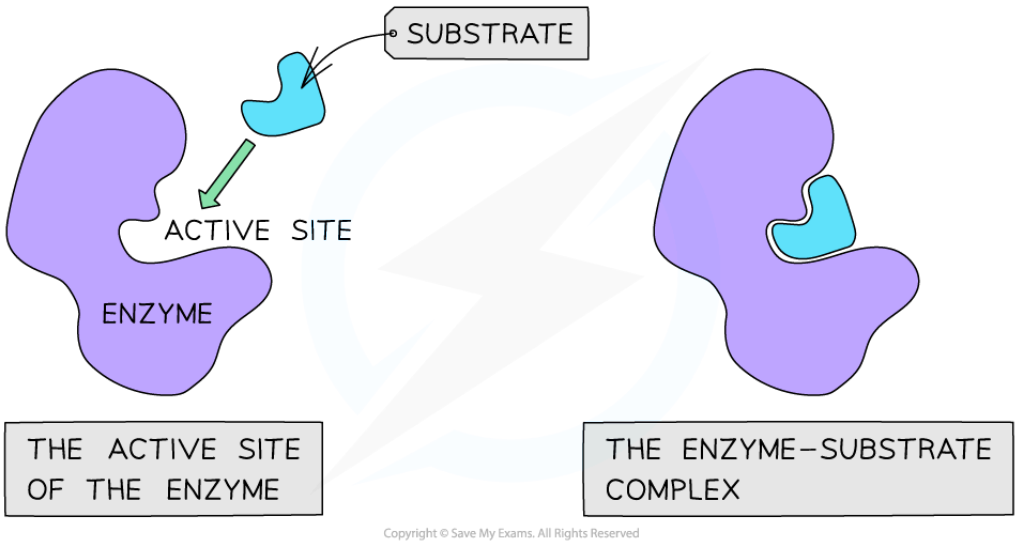
- Enzymes are **biological catalysts**
  - 'Biological' because they function in **living systems**
  - 'Catalysts' because they **speed up the rate of chemical reactions** without being used up or changed themselves
  - Enzymes have an active site to which specific substrates bind
- Enzymes are also **globular proteins**
- Critical to the enzyme's function is the **active site** where the **substrate** binds
- Enzymes are **specific** to the substrate
  - The shapes of the enzyme and substrate and their **chemical properties** are **complementary**, to allow the substrate to fit into the active site, like two jigsaw pieces fitting together
  - This is called **enzyme-substrate specificity**
- Due to this specificity, thousands of enzymes are needed throughout an organism, to carry out **individual chemical reactions**

Enzyme Activity

Your notes

- Enzyme catalysis involves molecular motion and the collision of substrates with the active site
- For an enzyme-catalysed reaction to take place, substrates **collide at random** with the enzyme's active site
- This must happen at the correct **orientation** and **speed** in order for a reaction to occur
  - **Unsuccessful collisions** can occur when the molecules are not correctly aligned with each other at the moment of collision
  - The molecules 'bounce' off each other and **no reaction** takes place

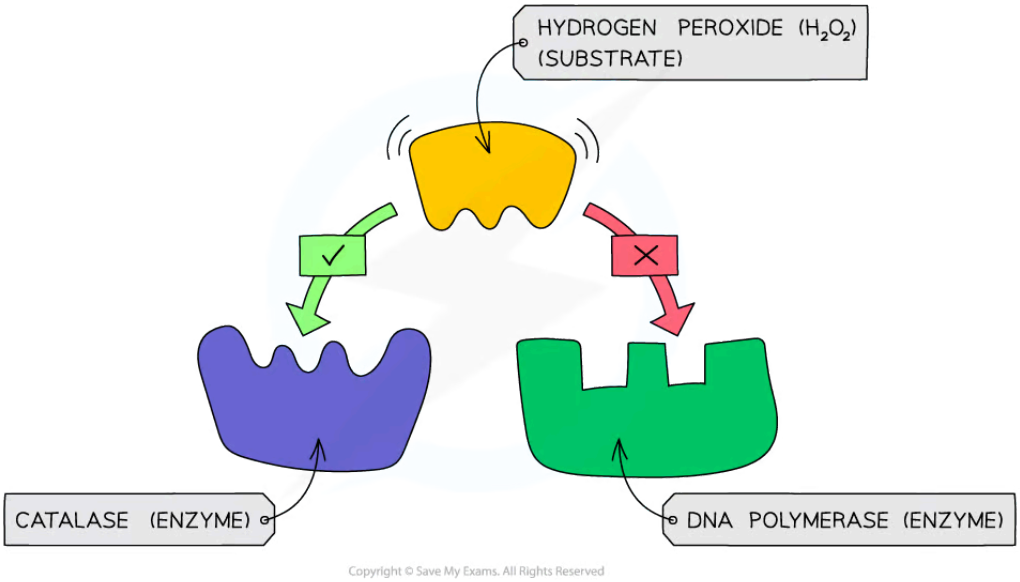
- Some enzymes have **two substrates** that must each collide with a separate active site **at the same time**
- Substrates bind to enzymes, forming a temporary **enzyme-substrate complex**
- The **active site** of an enzyme has a **specific shape** and **chemical properties** to bind with a specific substrate
- The reaction occurs within the enzyme-substrate complex which leads to changes in the **chemical structure of the substrate**
- **Products** are formed, which **detach** and **move away** from the active site, which can be re-used



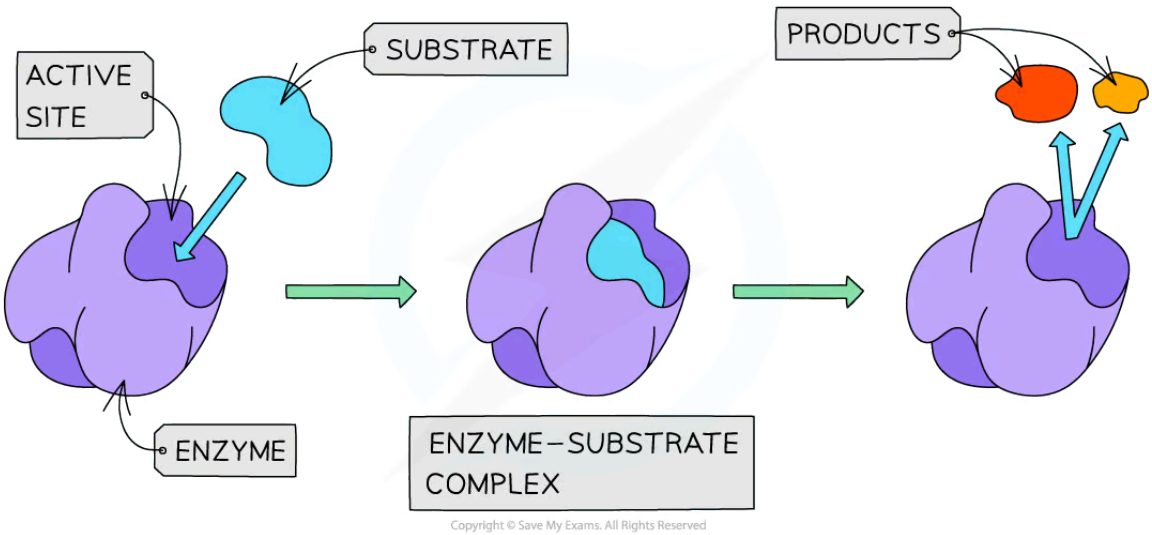
The active site of an enzyme has a specific shape to fit a specific substrate (when the substrate binds an enzyme-substrate complex is formed)

- The **specificity** of an enzyme is a result of the **complementary nature** between the shape of the active site on the enzyme and its substrate(s)
- The **shape** of the active site (and therefore the specificity of the enzyme) is determined by the **complex 3-D shape** of the protein that makes up the enzyme
  - Proteins are formed from **chains of amino acids** held together by peptide bonds
  - The order of amino acids in this chain determines the shape of an enzyme
  - If the order is altered, the resulting three-dimensional shape changes

Your notes



An example of enzyme specificity – the enzyme catalase can bind to its substrate hydrogen peroxide as they are complementary in shape, whereas DNA polymerase is not

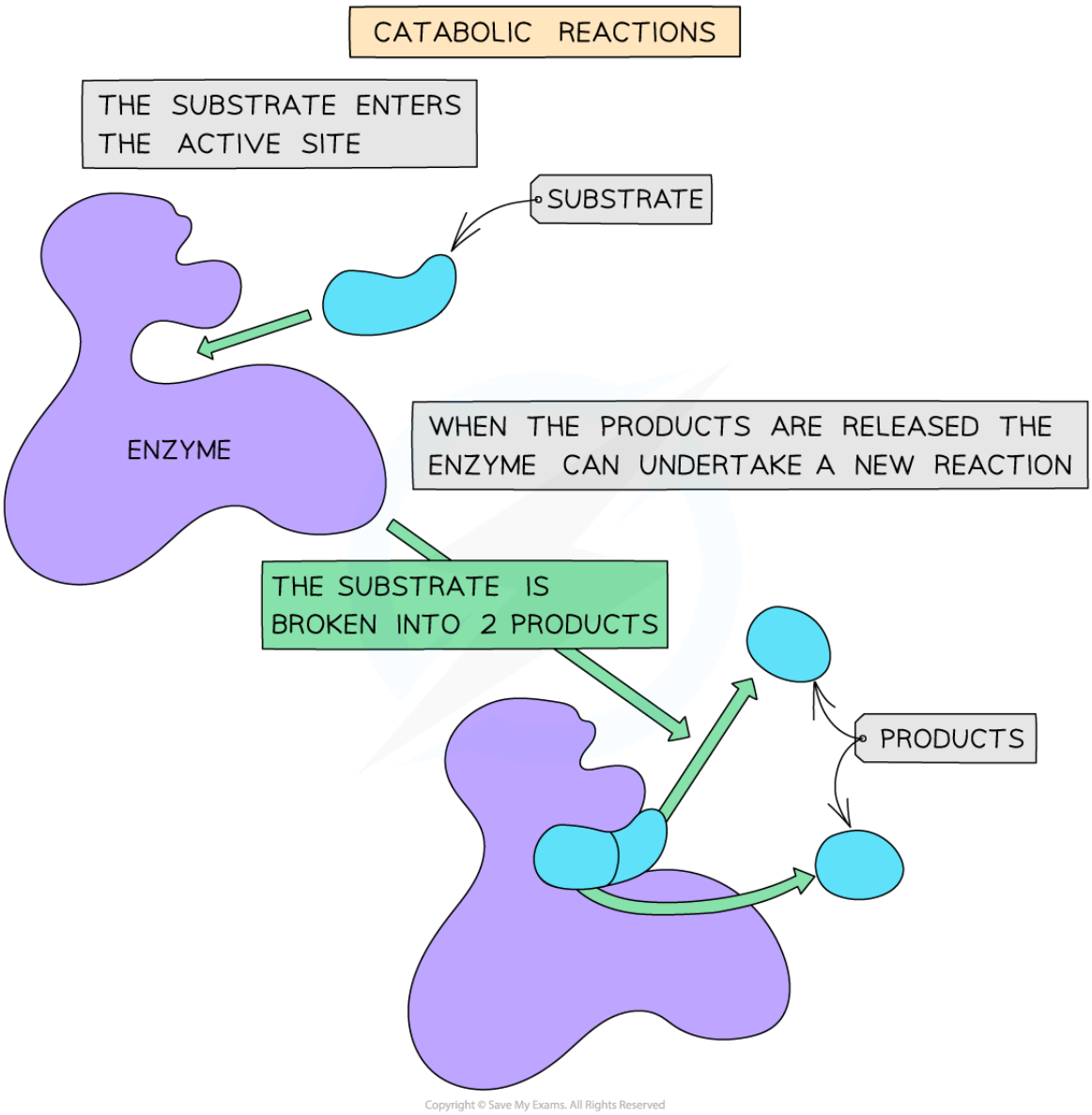


The temporary formation of an enzyme-substrate complex

- Enzyme reactions can either be **catabolic** or **anabolic**
- **Catabolic** reactions involve the **breakdown** of complex molecules into simpler products, which happens when a single substrate is drawn into the active site and broken apart into two or more distinct molecules

- Examples of catabolic reactions include **cellular respiration** and **hydrolysis** reactions

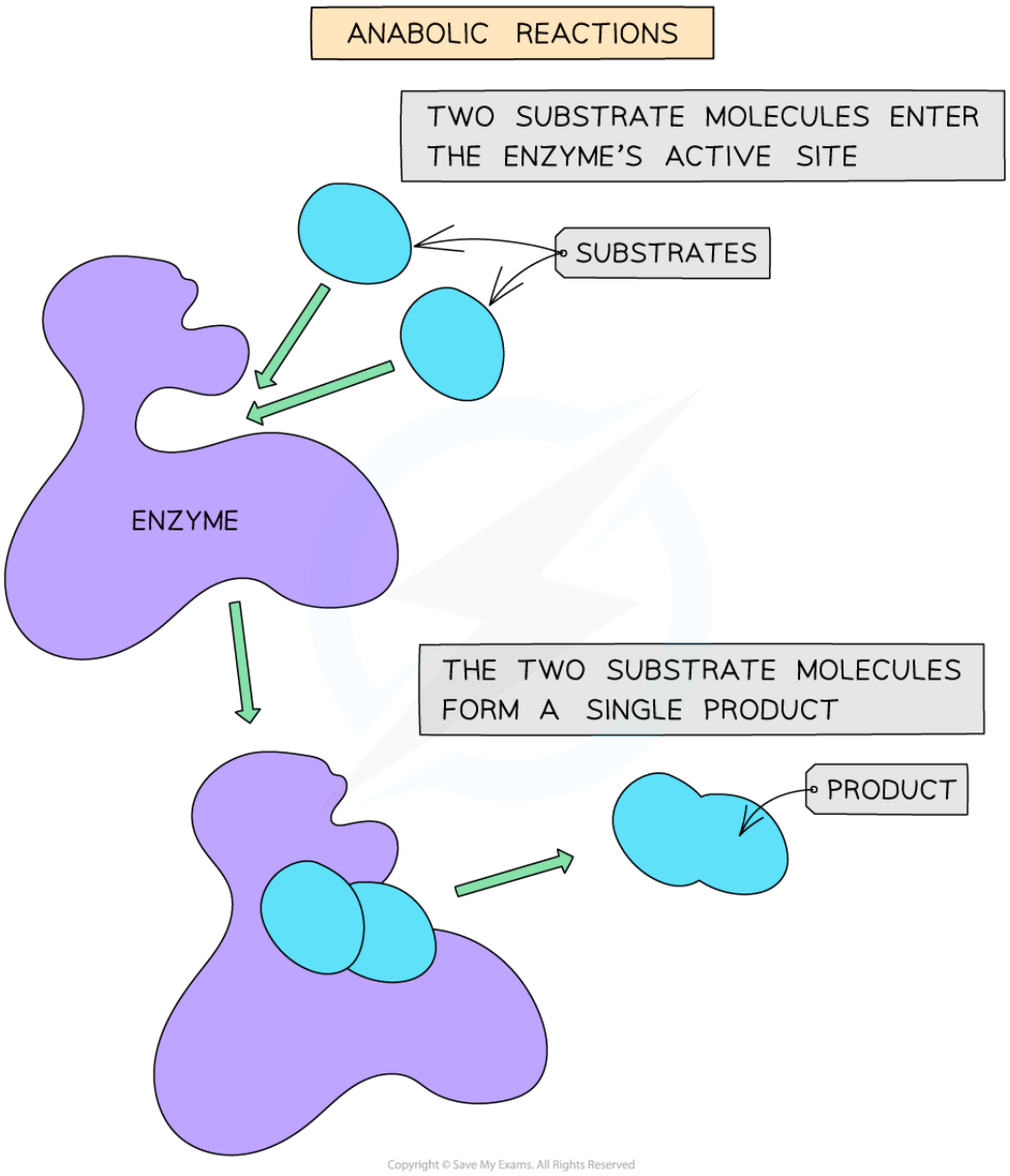
Your notes



A catabolic reaction

- **Anabolic** reactions involve the **building** of more complex molecules from simpler ones when two or more substrates are **held in the active site**, forming bonds between them and releasing a **single product**
- Examples of anabolic reactions include **protein synthesis** and **photosynthesis**

Your notes



An anabolic reaction

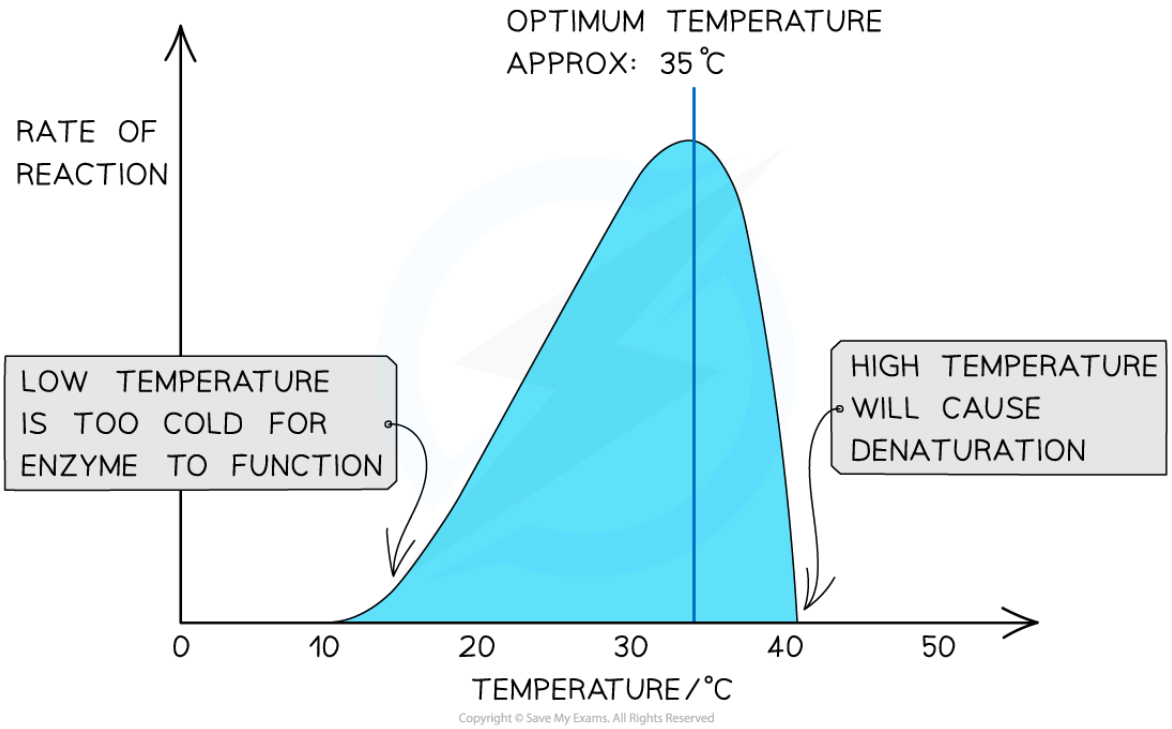
Examiner Tip

Don't forget that both enzymes and their substrates are highly specific to each other – this is known as enzyme-substrate specificity.

Factors Affecting Enzyme Activity

Your notes

- Temperature, pH and substrate concentration affect the rate of activity of enzymes
- Enzymes have a **specific optimum temperature** – the temperature at which they catalyse a reaction at the **maximum rate**
- **Lower temperatures** either **prevent** reactions from proceeding or **slow them down**:
  - Molecules move relatively **slowly**
  - **Lower frequency of successful collisions** between a substrate molecule and the active site of enzyme
  - Less frequent enzyme-substrate complex formation
  - Substrate and enzyme collide with **less energy**, making it less likely for bonds to be formed or broken (stopping the reaction from occurring)
- **Higher temperatures speed up reactions**:
  - Molecules move more **quickly**
  - **Higher frequency successful collisions** between a substrate molecule and the active site of enzyme
  - More frequent enzyme-substrate complex formation
  - Substrate and enzyme collide with **more energy**, making it more likely for bonds to be formed or broken (allowing the reaction to occur)
- However, as temperatures continue to increase, the rate at which an enzyme catalyses a reaction **drops sharply**, as the enzyme begins to **denature**

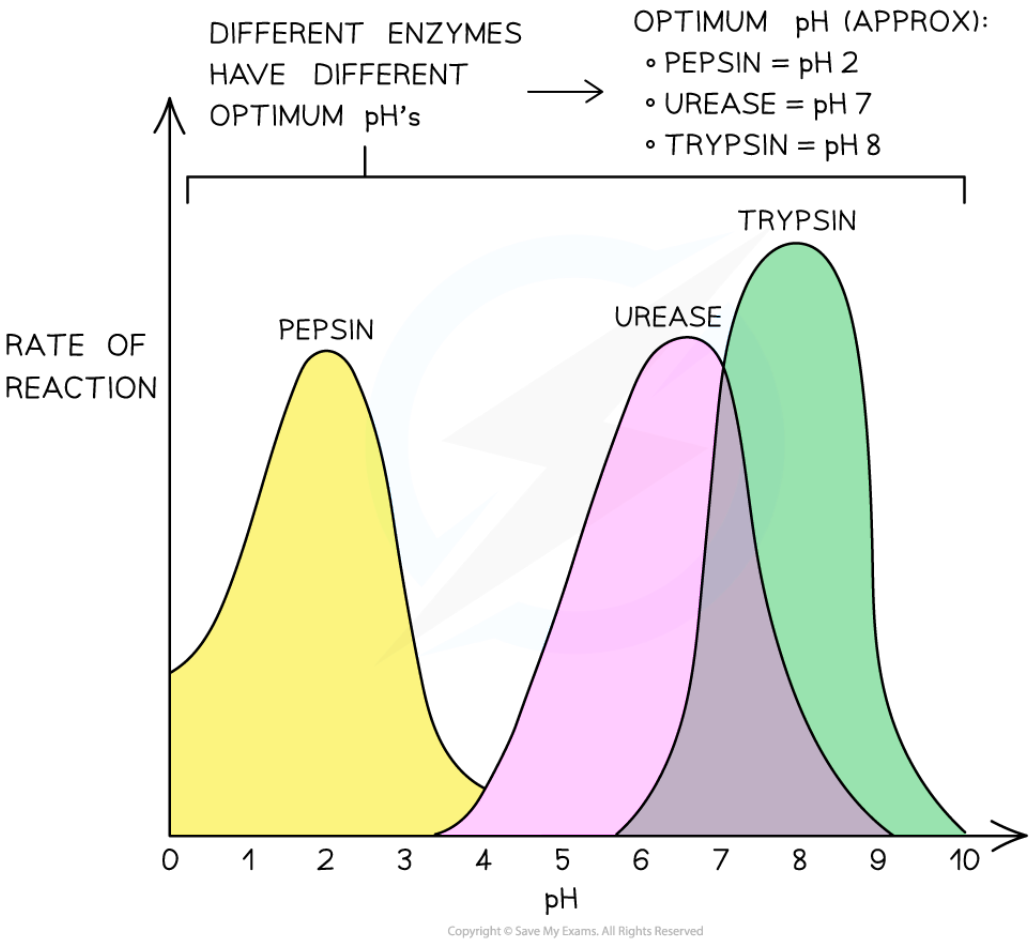


The effect of temperature on the rate of an enzyme-catalysed reaction

Your notes

Changes in pH

- pH is a result of the **hydrogen ion concentration** in a solution
- A **low pH** is **acid** and has a **high** hydrogen ion concentration
- A **high pH** is **alkaline** and has a **low** hydrogen ion concentration
- A **10× increase** in hydrogen ion concentration lowers the pH by **1 unit**
  - pH is therefore measured on a **logarithmic scale** of hydrogen ion concentration, **not a linear scale**
- Water has a pH of 7, regarded as **neutral**
- **Extremes of pH** can also alter hydrogen bonding within an enzyme's structure and cause irreversible **denaturation**
- Each enzyme has an **optimum pH**
- Not all enzymes have an optimum pH near to neutral. For example
  - The **stomach enzyme** pepsin is adapted to work best at **pH 2**
  - Certain bacterial enzymes work at **pH 9-10**, in line with the pH of the bacteria's main habitat

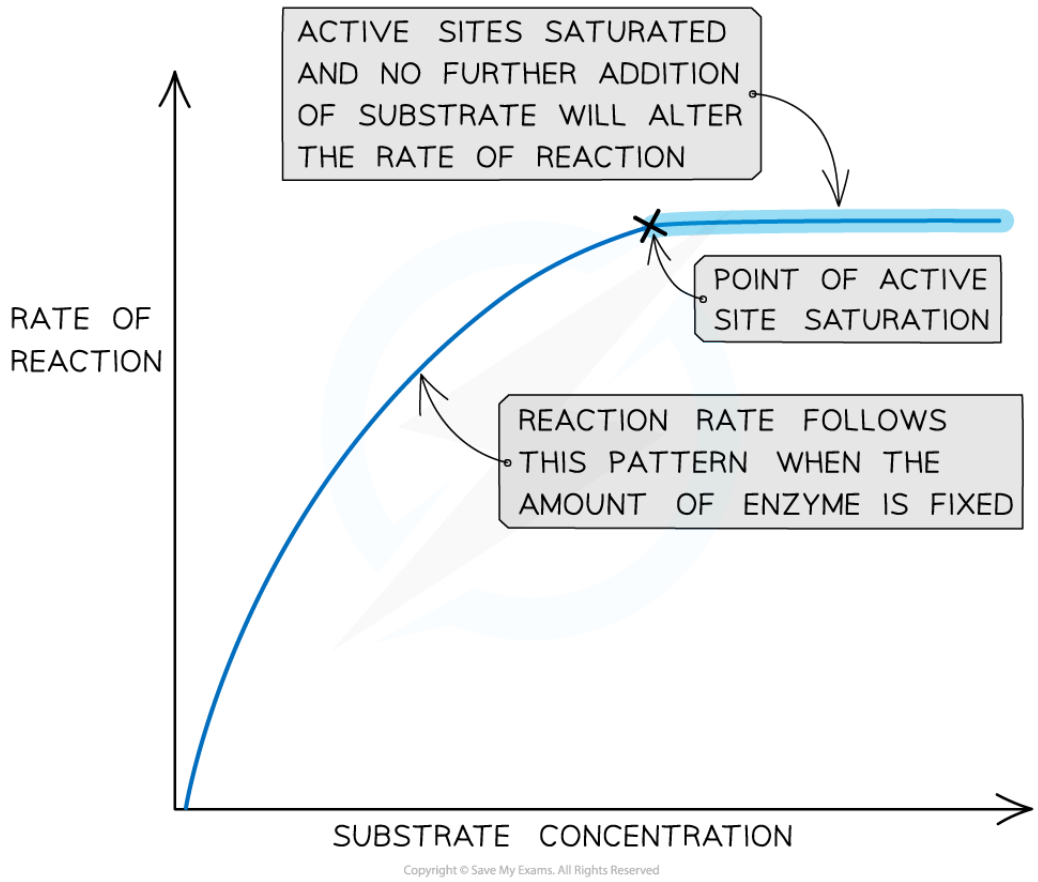


The effect of pH on three enzymes' rates of reaction

Your notes

Changes in substrate concentration

- The more substrate molecules are present in a solution, this **increases the frequency of collisions** with the enzyme's active site
- Active sites are **occupied** or 'blocked' by substrates whilst the reaction is taking place
- The more active sites are occupied, **the fewer are available to catalyse other substrate** molecules
- As substrate concentration rises, the slower the rise in the rate of the enzyme-catalysed reaction
- The active sites have become **saturated**
- At the **point of active site saturation**, increasing the substrate concentration will cause **no further increase** in the rate of reaction
- At the point of active site saturation, a method of increasing the rate of reaction would be to make more active sites available by **increasing the enzyme concentration**



The effect of substrate concentration on enzyme activity

Examiner Tip

When answering questions about reaction rates for enzyme-catalysed reactions, make sure to explain how the temperature affects the speed at which the molecules (enzymes and substrates) are moving and how this, in turn, affects the number of **successful collisions**.You should memorise the sketch graphs of temperature, pH and substrate concentration and be able to sketch new curves for changed conditions.

Your notes

Denaturation: Enzymes

- Enzymes can be denatured
- **High temperatures** and **extremes of pH** cause denaturation
- **Bonds** (eg. hydrogen bonds) holding the enzyme molecule in its precise 3D shape start to **break**
- This causes the **3-dimensional shape** of the protein (ie. the enzyme) to **change**
- This permanently **damages** the **active site**, preventing the **substrate** from **binding**
- **Denaturation** has occurred if the **substrate can no longer bind**
- The reaction that was previously catalysed **now no longer takes place**
- Denaturation often causes the enzyme to **become insoluble** and form a **precipitate**
- Very few human enzymes can function at temperatures **above 50°C**
  - This is because humans maintain a body temperature of about 37°C, therefore even temperatures exceeding 40°C will cause the denaturation of enzymes
  - High temperatures cause increased vibrations in the bonds and the **hydrogen bonds between amino acids** start to break, changing the conformation of the enzyme

Examiner Tip

Don't forget that enzymes are always proteins and so anything that could denature a protein, rendering it non-operational (extremes of heat, temperature, pH etc.) would also denature an enzyme.Avoid using the term 'destroyed' when describing the disruption to enzyme structure; the more accurate term is 'denatured'.

2.4.2 Immobilised Enzymes

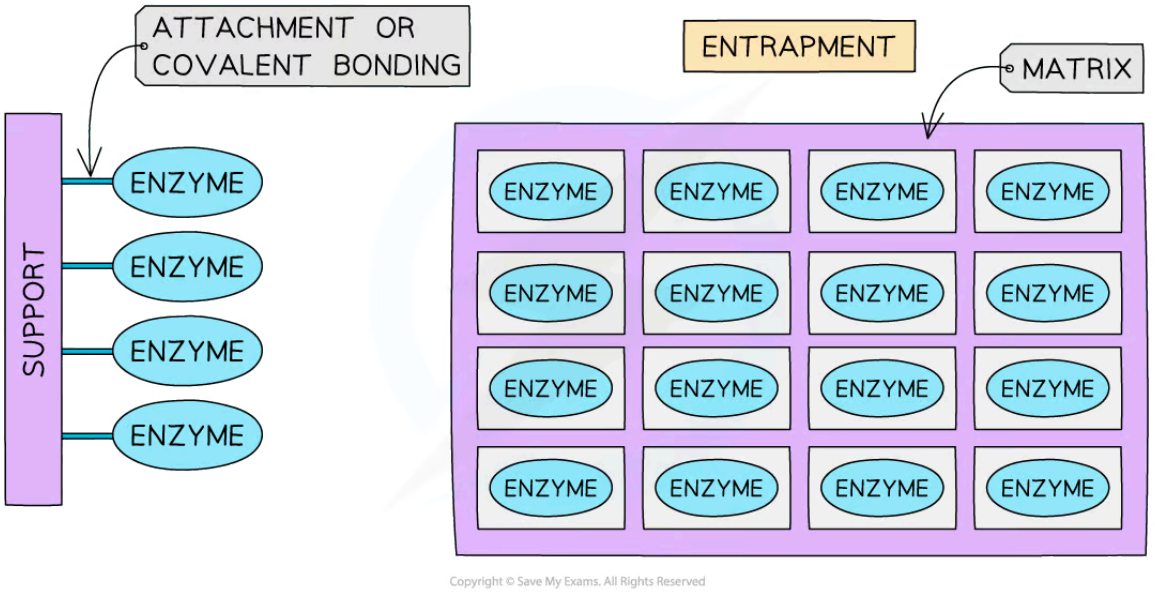
Your notes

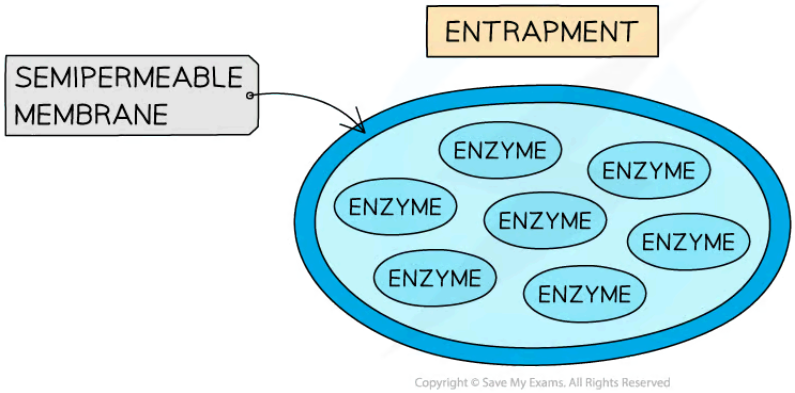
Immobilised Enzymes

Immobilised enzymes are widely used in industry

- Just before 1900, it was discovered that enzymes could be used to catalyse production of alcohol in the absence of yeast cell
- Since then, hundreds of enzymes have been developed outside of living cells for **commercial purposes**
- Uses of enzymes in industrial processes can be **expensive,** so we need ways to **reuse** them in order to be cost-effective
- An **immobilised enzyme** is an enzyme that is attached to an **insoluble material** to prevent mixing with the product and through this method, the enzyme can be **reused** in subsequent reactions
- Immobilised enzymes are used in the following commercial processes
  - Agriculture
  - Biosensors (diagnosis, analysis eg. for impurities)
  - Manufacturing processes
  - Energy generation
  - Environmental management
  - Food/drinks industry
  - Medicines
- Methods by which enzymes can be immobilised include:
  - Attachment to an **inert substance** eg. glass
  - Entrapment within a **matrix** e.g. alginate gel
  - Entrapment within a **partially permeable membrane**

Your notes

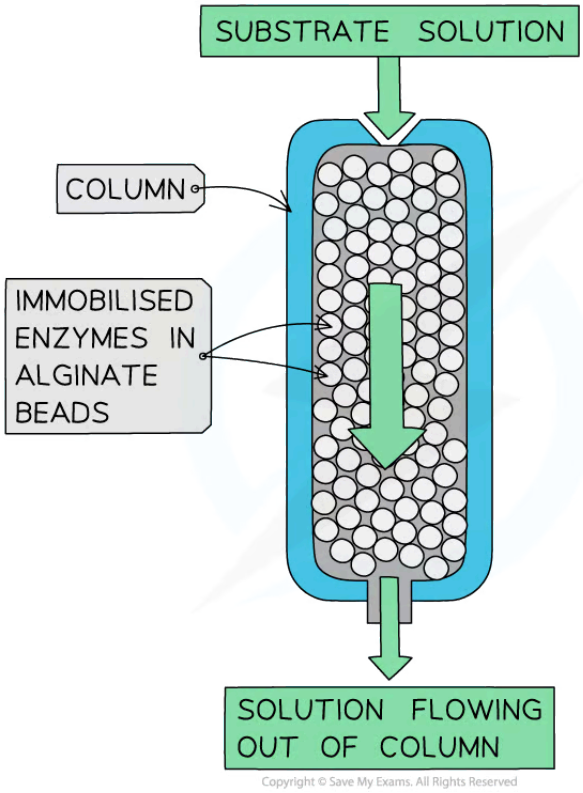




Different ways in which enzymes can be immobilised

- The mechanism of immobilised enzyme use works as follows:
  - The immobilised enzymes are contained within a **column**
  - The substrate is **filtered through** this column in **solution**
  - As the substrate runs through the column, **enzyme-substrate complexes** are formed and products are produced
  - These products then **flow out** of the column, **leaving the enzymes behind** to catalyse the reaction again

Your notes



The immobilised enzymes are contained within a column

Advantages of immobilised enzymes

- There is **no enzyme in the product** (the product is **uncontaminated**) and therefore there is no need to further process or filter the end product
- The immobilised enzyme can be **reused multiple times** which is both efficient and **cost-effective** (many enzymes are **expensive**)
  - Reusing the enzyme also **avoids the need to separate** the enzyme from the product in downstream processing
- Immobilised enzymes have a **greater tolerance of temperature and pH changes** (immobilisation often makes enzymes more **stable**)
- Substrates can be exposed to **higher enzyme concentrations** than when using enzymes in solution, increasing the rate of throughput
- Conditions can be **controlled carefully**, allowing immobilised enzymes to function **close to their optimum conditions** and be **more stable**

Disadvantages of immobilised enzymes

- Specialist **expensive equipment** is required
- Immobilised enzymes are **more costly to buy**, so are unlikely to be financially worthwhile for **smaller industries**

- The **rate of reaction is sometimes lower** when using immobilised enzymes as the enzymes cannot mix freely with the substrate


Your notes

Examples of immobilised enzymes in industry

- There are many **industrial** and **medical** applications of immobilised enzymes, including production of the following:
  - **Lactose-free** dairy products such as milk
    - Enzyme: Lactase
    - Converts lactose to glucose and galactose
  - **Semi-synthetic penicillin** which overcomes issues of penicillin resistance
    - Enzyme: Penicillin acylase
    - Converts the original form of penicillin into one which is effective against penicillin-resistant organisms
  - **Glucose** products used to sweeten and thicken foods
    - Enzyme: Glucoamylase
    - Converts starch and other dextrins into glucose
  - **Fructose** for sweetening of foods where a lower quantity of sugar is necessary
    - Enzyme: Glucose isomerase
    - Converts glucose into the sweeter sugar, fructose
  - Purified samples of **L-amino acids** used in food production
    - Enzyme: Aminoacylase
    - Separates out L-amino acids from D-amino acids
  - **Acrylamide** required in disposable nappy/diaper production
    - Enzyme: Nitrilase
    - Converts acrylonitrile into acrylamide

Examiner Tip

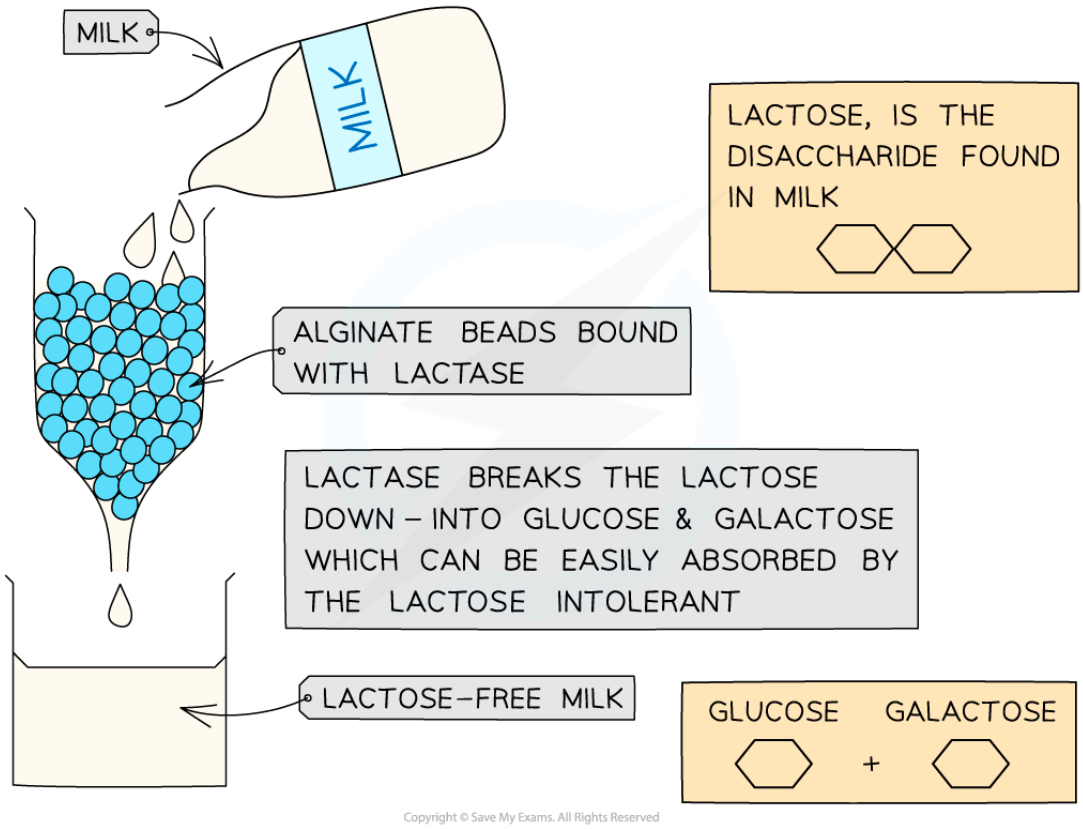
You will not necessarily be asked about these specific examples of industrial uses of immobilised enzymes (except for lactose modification), but it is useful to know of some uses in order to be able to apply your knowledge accurately in the exam.When discussing the advantages and disadvantages of immobilised enzymes, try to be specific about the cost implications as there are various considerations when it comes to the economical value of immobilising the enzymes.

Lactose-free Milk Production

Your notes

A closer look at lactose-free milk production

- **Milk** is a valuable source of nutrients containing protein, fat and the carbohydrate **lactose**
- 5–10% of the UK population are **lactose intolerant**
  - They can't digest lactose and **suffer** from bloating, digestive problems, discomfort and pain
- Lactose is a **disaccharide** that is hydrolysed into **glucose** and **galactose**
- The yeast *Kluyveromyces lactis* grows naturally in milk and can be cultured as a source of the enzyme **lactase**
- Glucose and galactose are **sweeter than lactose**, so less sugar needs to be added to foods with modified lactose **to achieve the same sweetness**
- **Ice cream** and **yoghurt** production also benefit from having more glucose and galactose than lactose in the milk they are made from



Using the immobilised enzyme lactase to modify milk

2.4.3 Skills: Enzyme Experiments


Your notes

Practical 3: Enzyme Experiments

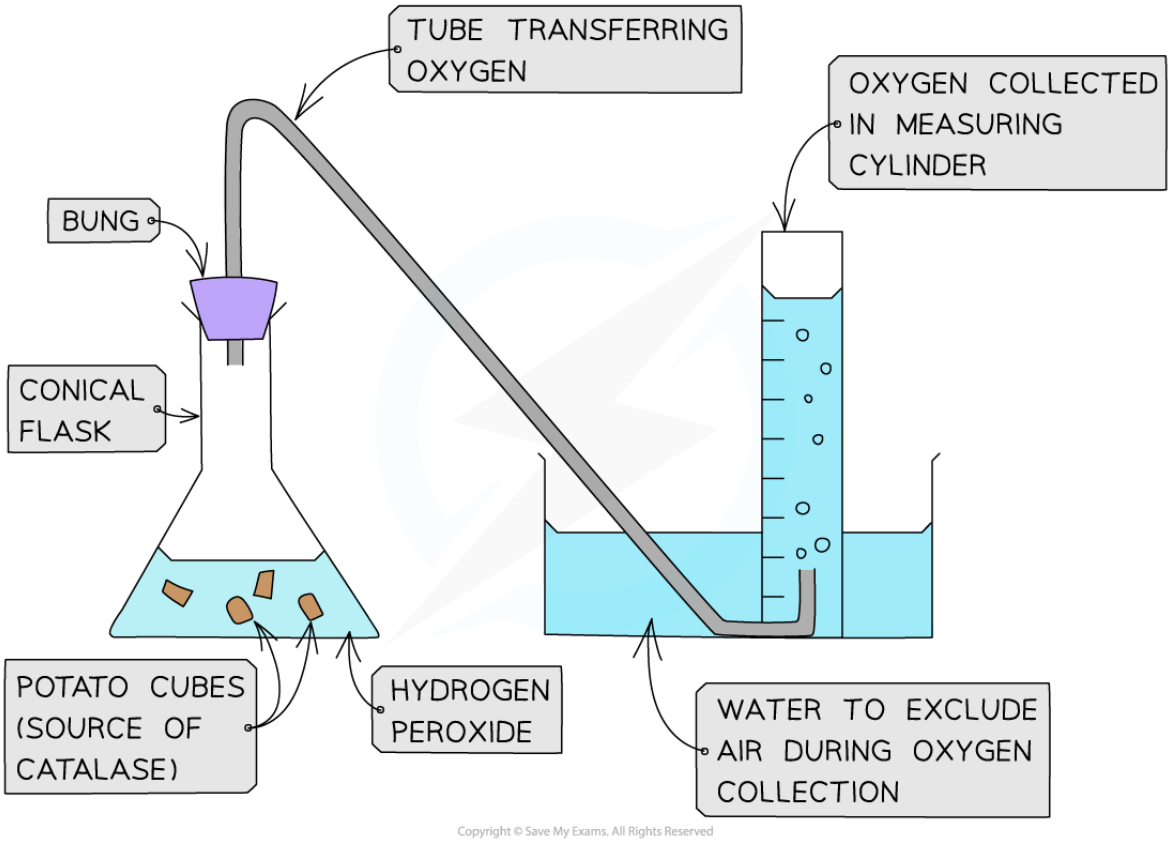
Design of experiments to test the effect of temperature, pH and substrate concentration on the activity of enzymes

- Three different **independent variables** can be tested
  - Temperature
  - pH
  - Substrate concentration
- You should plan how the **dependent variable is going to be measured**
  - With appropriate units
- Also, what **intervals of the independent variable** are going to be chosen
- These factors dictate the **choice of apparatus** and other equipment required for the experiment
- The **control variables** need to be identified and monitored eg. temperature when measuring the effect of pH

Investigating the effects of temperature or pH on catalase activity

- The **progress** of **enzyme-catalysed reactions** can be investigated by:
  - Measuring the **rate of formation of a product**
  - Measuring the **rate of disappearance of a substrate**
- In this investigation, the rate of **product formation** is used to measure the rate of an enzyme-controlled reaction:
  - **Hydrogen peroxide** is a common but **toxic** by-product of metabolism
  - This means it must be **broken down** quickly
  - **Catalase** is an enzyme found in the cells of most organisms that **breaks down hydrogen peroxide into water and oxygen**
  - Hydrogen peroxide and catalase are combined and the **volume of oxygen generated** is measured in a set time
  - The **rate of reaction** can then be calculated

Your notes



Experimental set-up for investigating the rate of formation of a product using catalase

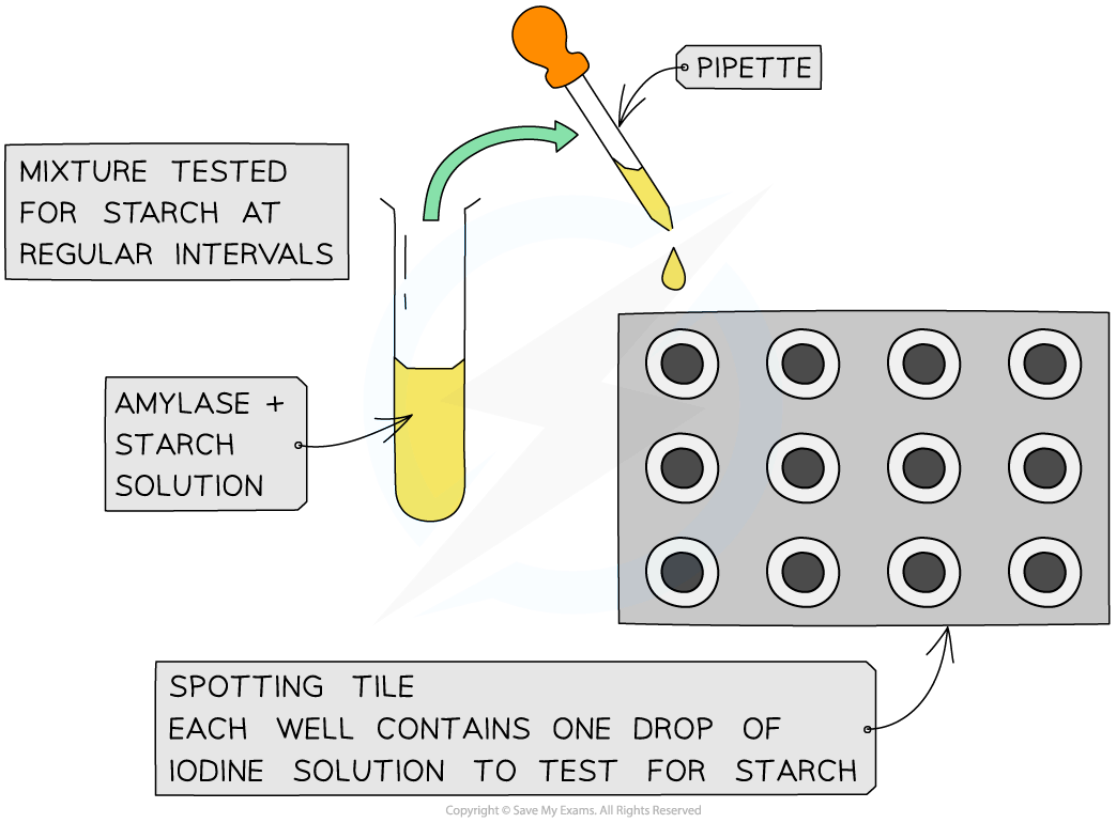
- If measuring the effect of temperature on enzyme activity, the conical flask containing potato pieces can be held in a water bath at the required temperature
  - The water level in the water bath must be higher than the level of H_2O_2 in the conical flask, to ensure even heating
  - The conical flask can also be swirled gently to mix the contents and maintain an even temperature

Investigating the effect of substrate concentration on amylase activity using iodine

- In this investigation, the rate of **substrate disappearance** is used to compare rates of reaction under different conditions
- **Amylase** is a digestive enzyme that **hydrolyses starch into maltose and glucose**
- Amylase functions best at pH 7 and 37°C (all enzymes operate best under specific conditions)
- **Amylase and starch are combined** and this reaction mixture is then **tested for starch** at regular time intervals
- This can be done by taking samples from the reaction mixture at each time interval and adding each sample to some **iodine in potassium iodide solution**
  - Starch forms a **blue-black** colour with this solution
  - If no starch is present, the iodine solution remains yellow-brown

- In this way, the time taken for starch to be broken down can be measured
- The investigation can be **repeated under different starch concentrations** and the **reaction rates can then be compared**
  - This experiment also can be adapted to measure the effects of altering pH, temperature or enzyme concentration

Your notes



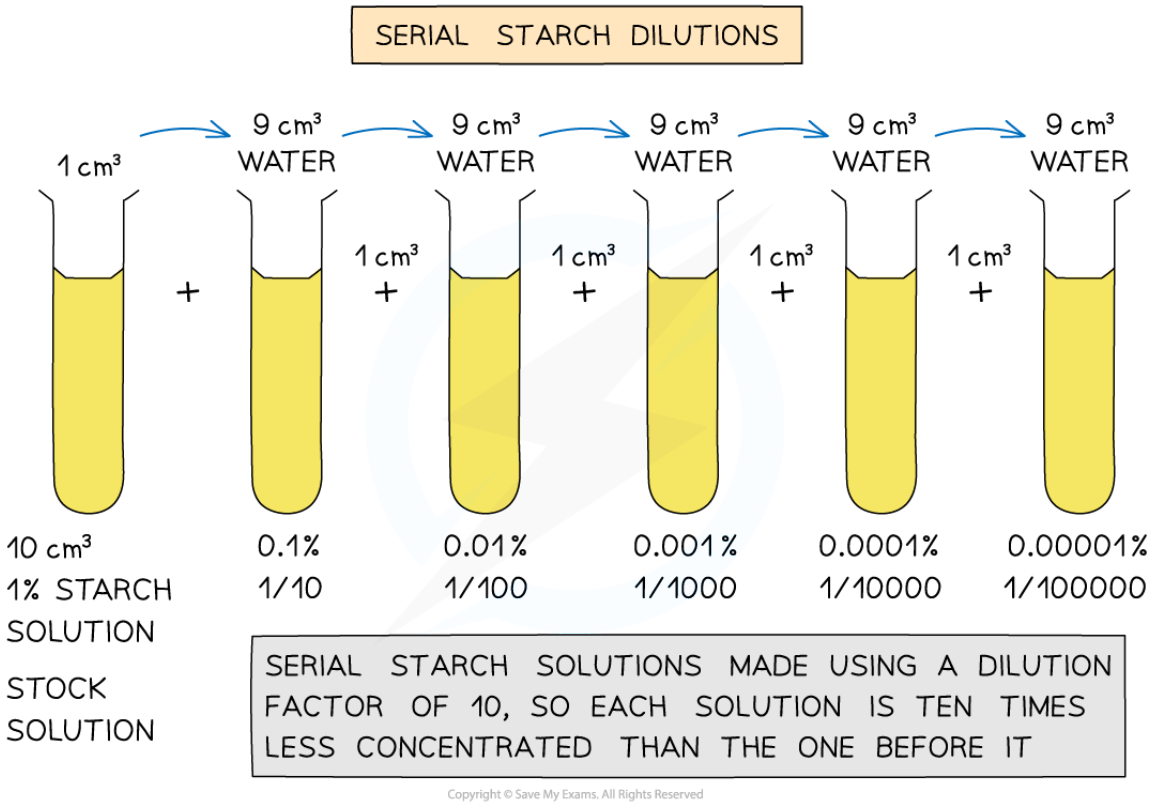
Experimental set-up for investigating the rate of disappearance of a substrate using amylase

Investigating the effect of starch concentration on amylase activity using colorimetry

- A **colorimeter** is able to measure **light absorbance** (how much light is absorbed) or **light transmission** (how much light passes through) a substance
- Colorimetry can be used in any enzyme-catalysed reaction that involves a **colour change**
- As the colour breaks down the **transmission increases** or **light absorption decreases** and this can be used to **measure the rate of the reaction**
- For example, a colorimeter can be used to follow the progress of a **starch-amylase catalysed reaction** as the amylase breaks the starch down into maltose
- This can be carried out as follows:
  - **Colorimeter calibration**: this is an important step in a colorimetric investigation and in this case, a weak iodine solution can be used to calibrate the colorimeter as the endpoint (or 100% transmission)

Your notes

- Preparation of a starch solution of **known concentration** (stock solution), from which a range of concentrations are made using **serial dilutions** (method outlined in diagram below)
- Following calibration and switching on the red filter (to maximise the percentage transmission or absorbance), the colorimeter is used to **measure the percentage absorbance or percentage transmission values**
- Sometimes a reagent or indicator is used to produce the colours detected by the colorimeter and sometimes the solutions themselves absorb light waves
- A **calibration graph** is then plotted of starch concentration (*x*-axis) vs percentage absorbance or percentage transmission (*y*-axis)



Serial dilution of starch to make a range of concentrations

NOS: Experimental design; accurate, quantitative measurements in enzyme experiments require replicates to ensure reliability

- **Accurate** measurements mean data that are close to the true value
- **Quantitative** measurements must be made
  - A **qualitative** measurement might state that, "the enzyme worked at a faster rate at the higher temperature", whereas
  - A **quantitative** measurement for the same experiment might state that, "the enzyme worked at a rate of 2.3 mmol product minute^{-1} at 40°C, versus 1.6 mmol product minute^{-1} at 25°C"
  - **Quantities**, using **numbers and appropriate units**, are quoted in the experimental results

- Reliable data are generated from repeated experiments
  - **Anomalies** can be identified and eliminated
  - A **reliable mean** can be calculated from the data that remain

Your notes

Examiner Tip

RE-member: **RE**-peats bring **RE**-liability to experimental data.