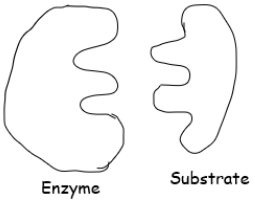
Enzymes

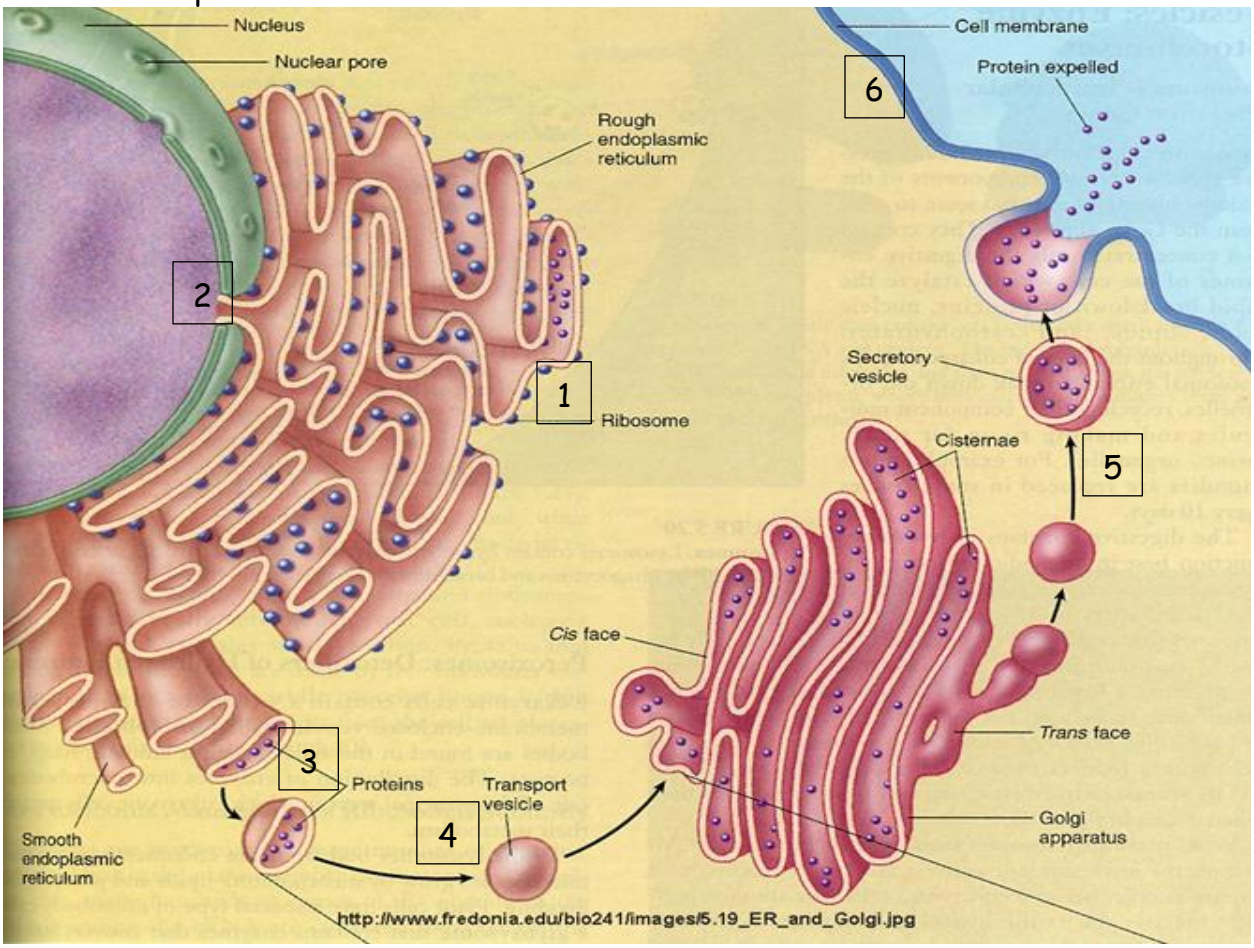


Enzymes: are proteins that function as biological catalysts & are involved in all metabolic reactions. Enzymes are made of genes in the form of DNA in the nucleus, which is needed as the instruction by the ribosome for protein synthesis. Everything a cell depends on is the enzyme it makes. It builds up, synthesizes complex substances. Enzymes are vital to our health and change the rate at which chemical reactions happen. Enzymes are globular proteins (pockets = active sites) with a specific tertiary structure that breaks down food substances in cells to release energy & poisonous substances in cells. Enzymes catalyse metabolic reactions in living organisms. They have active sites that can hold substrates. When all substrates are nestled in a particular enzyme's active sites, the enzyme can cause them to react quickly. Once the process is over the products detach.

Enzyme	Location of Reaction	Substrate	Product
Amylase	Mouth, Pancreas, Ileum	Starch	Maltose
Maltase	Ileum	Maltose	Glucose
Lipase/Steapsin	Pancreas, Ileum	Lipids	Fatty Acids & Glycerol
Catalase	Liver	Hydrogen Peroxide	Oxygen & Water
Pepsin	Stomach	Proteins	Proteoses & Peptones
Trypsin	Stomach, Pancreas, Ileum	Proteoses & Peptones	Polypeptides
Cellulase	Plant Cell Walls	Cellulose	Simple Sugars
Chitinase	Plants, Stems, Flower	Chitin	Dimers
Autolysins	Bacteria	Peptidoglycan	New Peptidoglycan
Lysozyme	Tears, Mouth (Saliva)	Peptidoglycan	-
Protease	Pancreas, Stomach	Proteins	Peptides
Sucrase	Ileum	Sucrose, Fructose	Glucose
Hydrolase	Cells	Allophanate	Ammonium, CO_2
Rennin	Cow Calves	Caseinogen	Casein
Lactase	Ileum	Lactose	Glucose & Galactose
Enterokinase	Ileum	Trypsinogen	Trypsin
Erepsin	Intestinal Juices	Polypeptides	Amino Acids
Hexokinase	Cells	Glucose	-

3.1 Mode of Action of Enzymes

Catalyst: is a substance that increases the rate of a chemical reaction and is not changed by a reaction and can be reused. It is needed in small quantities and may be reused, but the catalyst required varies with every reaction.
Extracellular enzymes: are released from the cells that make them.
Intracellular enzymes: found in the cytoplasm or attached to cell membranes, their action takes place inside the cells.



1. Ribosome synthesis protein.
2. Protein enters the lumen of the Rough Endoplasmic Reticulum & transported through it.
3. Protein reaches at the end pf the Rough Endoplasmic Reticulum, the membrane of Rough Endoplasmic Reticulum buds off & forms the transport vesicle.
4. Transport vesicle that contains protein moves towards Golgi Apparatus & fuses with Golgi Apparatus.
5. In Golgi Apparatus, protein is modified into enzyme & packed into secretory Vesicle. Secretory Vesicle buds off the tip of the Golgi Apparatus.
6. Secretory Vesicle moved towards plasma membrane & fuse with it to secrete out the enzyme.

7. E.g. Lysozyme: is an enzyme found in the secretions (tears) of the lacrimal glands of animals and in nasal mucus, gastric secretions, and egg white. Lysozyme catalyzes the breakdown of certain carbohydrates found in the cell walls of certain bacteria (*e.g.*, cocci). It thus functions, in the case of lacrimal fluid, to protect the cornea of the eye from infection. It is a cell organelle formed by the Golgi body in animal cells)- contain different hydrolytic/ digestive enzymes for protection.

White blood cells called phagocytes take in and digest bacteria using lysosomal enzymes

Some of the white blood cells take in invading microbes like bacteria. The endocytosed vesicle is fused with one of the many lysosomes present and lysomal enzymes digest the bacterium

Intracellular Enzymes	Extracellular Enzymes
Synthesize in a cell to be used inside a cell.	Synthesize in a cell & secreted out to be used outside a cell.
Example: Hexokinase enzyme, used in glycolysis process (breakdown of glucose) during cellular respiration.	Example: Trypsin enzyme, synthesized in pancreatic cells & secreted into the duodenum to break down polypeptides.

Enzyme action:

1. Active site: is a part of the enzyme where the substrate (reactant, key) binds to the enzyme.
2. Substrate: it is reactant (key) that changes during the source of a reaction. It's a molecule an enzyme acts upon.
3. Enzyme-Substrate complex: When the enzyme binds to the desired required/ substrate, it is called enzyme substrate complex. Enzyme binds, lower the Ea. needed to initiate a reaction, allowing products to be made more easily.
4. Products: Desired output. It is made from the substrate and is released.

Specificity of enzymes:

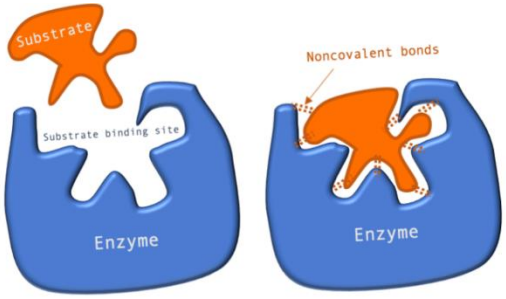
Substrate: Key Active site of Enzyme: Lock

All enzymes are globular proteins → spherical in shape.

Enzymes are specific to one particular substrate is as the active site of an enzyme. Active site is complementary shape to a substrate.

Enzymes are large molecules (100's of amino acids) most of which are involved in maintaining the specific shape of the enzyme. Very few (often fewer than 10) amino acids form the actual active site. Each enzyme has a very specific, individual active site shape, maintained by a very specific overall tertiary structure. Enzymes may also alter their shape in accordance to a substrate. The shape of the enzyme determines how it works.

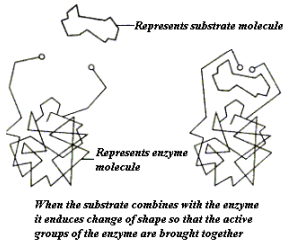
Different enzymes made of different amino acid combinations.



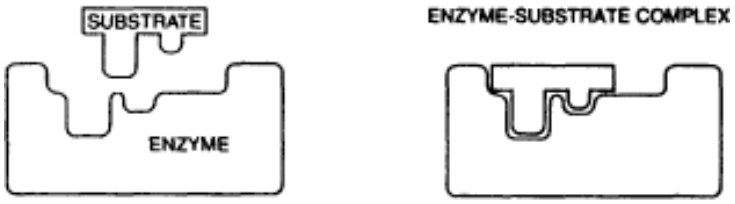
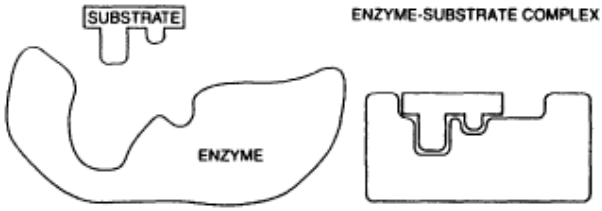
- Active site has a complementary shape to a substrate for it to fit.
- The substrate collides with the active side of an enzyme and gets attached to it like a key, fits into a lock. (Perfect fit.)
- Substrate binds with the active site.
- A catalyst reaction takes place with the enzyme catalyzes; substrate breaks down.
- Product is formed and the unchanged enzyme is free to be reversed.
- The product leaves the active site like a key leaves a lock.

<u>Induced Fit:</u>

- When the enzyme and substrate form a complex, structural changes occur so that the active site fits precisely around the substrate (the substrate induces the active site to change shape).
- The reaction will take place and the product, being a different shape to the substrate, moves away from the active site. The active site then returns to its original shape.
- Substrate binds to the enzyme's active site.
- The shape of the active site changes and moves the substrate closer to the enzyme. Amino acids are moulded into a precise form.
- Enzyme wraps around substrate to distort it
  This lowers the activation energy
  An enzyme-substrate complex forms → fast reaction
  E + S → ES → P + E
- Enzyme is not used up in the reaction (unlike substrates)



Stereospecificity: relationship of substrate (s) to active site.	
Lock and Key Hypothesis	Induced Fit

Emil Fischer's lock & key hypothesis suggested that the active site & the substrate were exactly complementary.	Koshland's more recent work proposes the induced fit hypothesis which suggests that active site & substrate are only fully complementary after the substrate is bound.
It does not easily explain how activation energy is lowered. It does not easily explain the role of competitive inhibitors. It does not easily explain the role of non-competitive inhibitors.	It can explain how the activation energy is lowered, the stretching & distorting of bonds or causing the closer orientation of reactive groups. It explains how non-competitive inhibitors can bind to a region away from the active site & change its shape so that substrate can no longer bind to the active site. Explains how competitive inhibitors can bind to the active site or other molecules with similar shapes to the substrate.
	

3.2 Factors that Effect Enzyme Action

Activation Energy: is the energy required to initiate a reaction.

- Enzymes are catalysts → speed up chemical reactions.
- Reduce activation energy required to start a reaction between molecules.
- Substrates (reactants) are converted into products.
- Reaction may not take place in absence of enzymes (each enzyme has a specific catalytic action).
- Enzymes catalyse a reaction at max. rate at an optimum state.

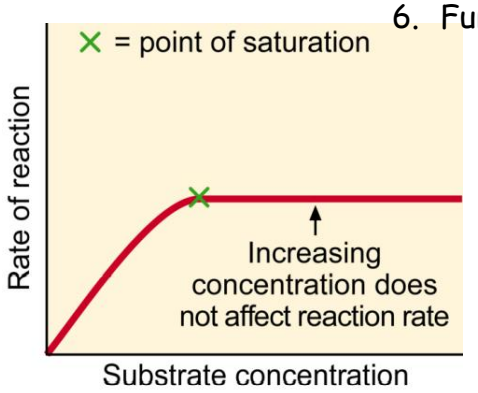
Measuring Reaction Rate:

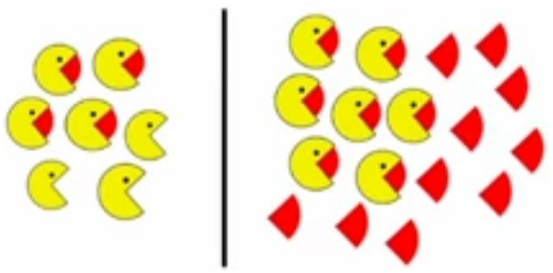
- It is easy to measure the rate of the catalyst hydrogen peroxide reaction, because one of the products is a gas, which is released and can be collected. Unfortunately, it is not always easy to measure the rate of reaction. If for example you want to investigate the rate of which amylase breaks down starch, it would be very difficult to observe the course of the reaction because the substrate (starch) and product (maltose) remain as colourless substances in the reaction mixture.
- The easiest way to measure the rate of reaction is to measure the rate at which starch disappears from the reaction mixture. This can be done by taking samples from the mixture at known times, and adding each sample to some

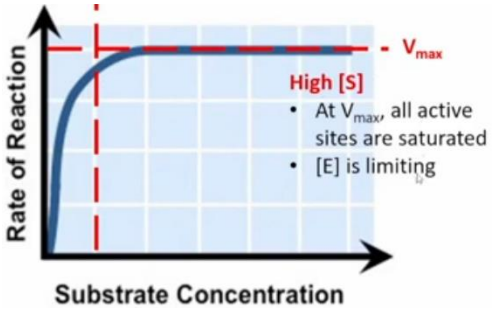
iodine in potassium iodide solution. Starch forms a blue-black colour with this solution. Using a colorimeter, you can measure the intensity of the blue-black colour obtained, and use this as a measure of the amount of starch still remaining. If you do this over a period of time you can still plot a curve of 'amount of starch remaining' against 'time' and then calculate the initial reaction rate.

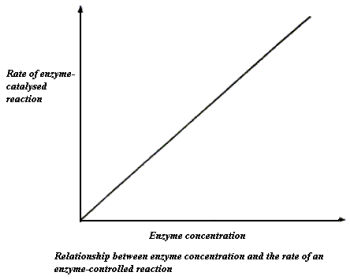
- It is even easier to observe the course of this reaction if you mix starch, iodine in potassium iodide solution and amylase in a tube, and take regular readings of the colour of the mixture in this one tube in a colorimeter. However, this is not ideal, because the iodine interferes with the rate of reaction and slows it down.
- Rate of Reaction can be affected by:
  - <u>Substrate Concentration:</u> As the substrate concentration increases, the rate of reaction increases, because more substrate molecules can collide with enzyme molecules, so ore reactions will take place.
    At higher concentrations, the enzyme molecules become saturated with substrate, so there are few free enzyme molecules. Adding more substrate doesn't make much difference.
    At low (S):
    1. Some active sites used for binding/not all occupied.
    2. Few collisions between enzymes & substrate,
    3. Less Substrate binds with active site.
    4. Few Enzyme-Substrate Complexes formed.
    5. Substrate is the limiting factor.
    6. More active sites occupied as substrate increases. The rate or reactions ins proportional to substrate concentration.

    At high (S):
    1. Rate increases to a plateau V_{Max} is reached.
    2. Maximum rate of enzymatic reaction = V_{Max}.
    3. At V_{Max} all active sites are saturated.
    4. Maximum number of Enzyme-Substrate Complexes formed.
    5. Enzymes are the limiting factor.
    6. Further increase of substrate does not increase rate.



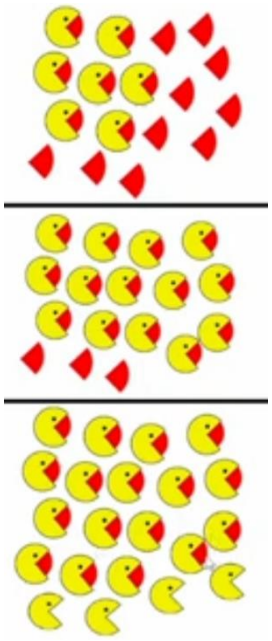






Relationship between enzyme concentration and the rate of an enzyme-controlled reaction

- Enzyme Concentration: As the enzyme concentration increases so will the reaction rate: as there are more enzyme substrate complexes forming. At very high concentrations of enzymes, the rate remains constant as substrate becomes the limiting factor. Adding more enzyme will cause the rate to increase again.



At low (E), as (S) increases:

1. More enzyme is present.
2. More active sites available for substrate to bind.
3. Increase in frequency of collision.
4. More Enzyme-Substrate Complexes form.
5. Rate of reaction increases as enzyme increases.

At high (E):

1. Rate of reaction levels off.
2. Substrate is the limiting factor.
3. Max number of Enzyme-Substrate Complexes formed.

Proportional to rate of reaction until there are more substrates than enzymes present.

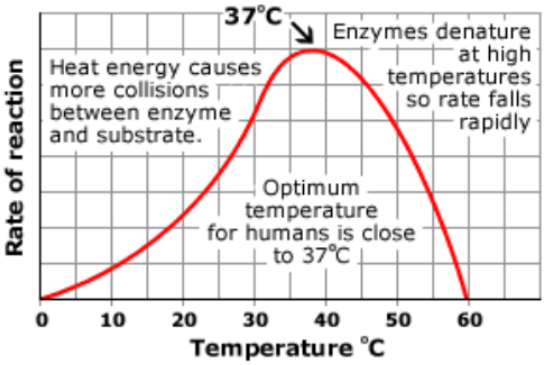
Rate of reaction increases:

1. Substrate binds to active site, but more enzymes are available.
2. Rate increases if more substrate is added.

Eventually, curve becomes constant (no increased rate).

Substrates occupy all active sites (all enzymes). Adding more substrate won't yield more product, as no more active sites are available.

- Temperature: Increase in temperature increases kinetic energy some more enzyme substrate complexes form. High temperatures cause denaturation due to the breaking of bonds holding the tertiary structures together (H Bonds/disulfide bridges/ ionic bonds). Active site altered (changes shape), substrate cannot bind, no enzyme substrate complex is form.



Increased Temperature

Increases speed of molecular movement → chances of molecular collisions → more ES complexes

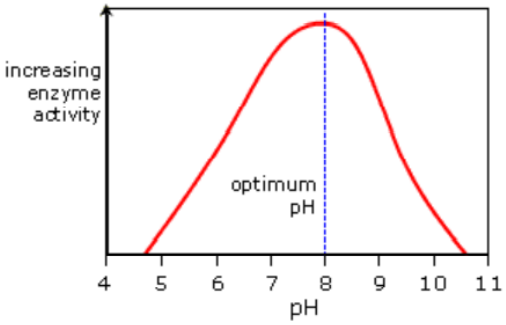
1. At 0-42°C rate of reaction is proportional to temp.
2. Enzymes have optimum temp. for their action (usually 37°C in humans).
3. Above ≈ 42°C, enzyme is denatured due to heavy vibration that breaks -H bonds.
4. Shape is changed → active site can't be used anymore

Decreased Temperature

1. Enzymes become less and less active, due to reductions in speed of molecular movement.
2. Below freezing point

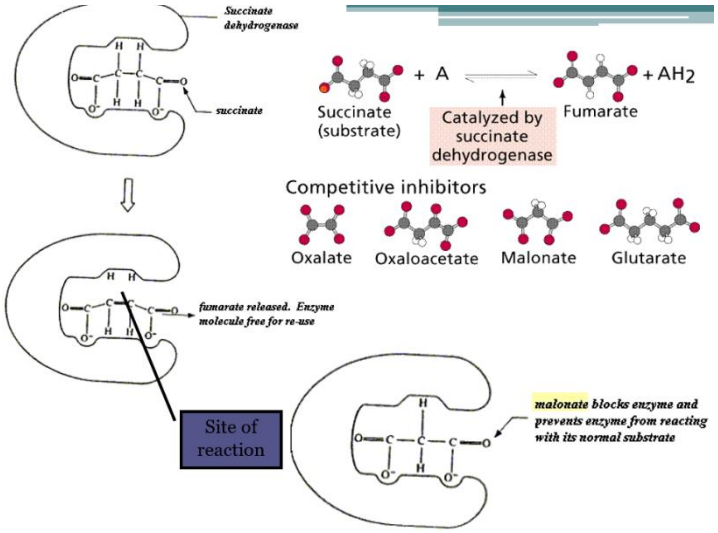
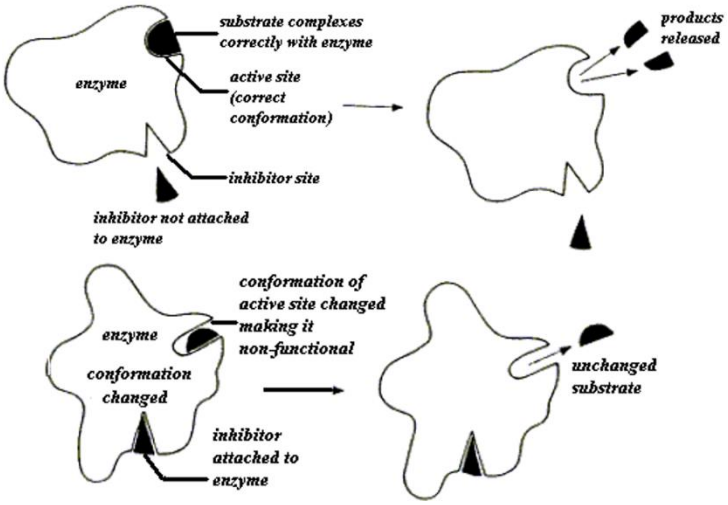
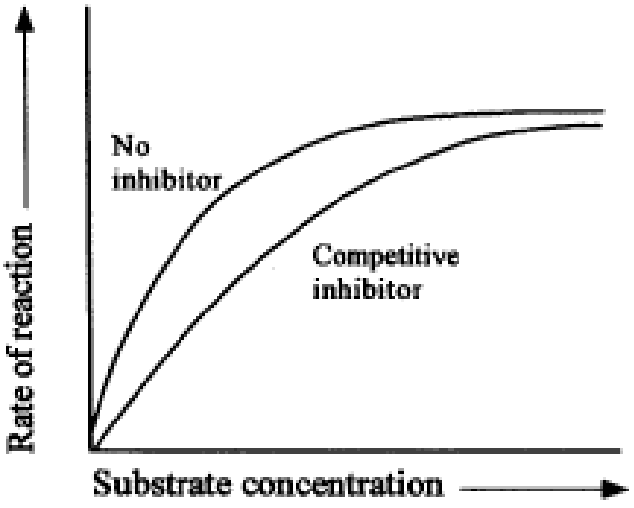
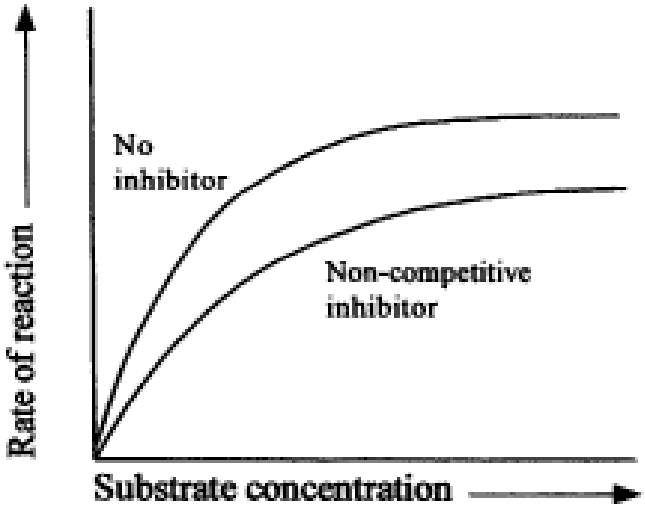
   Inactivated, not denatured: Regain their function when returning to normal temperature.

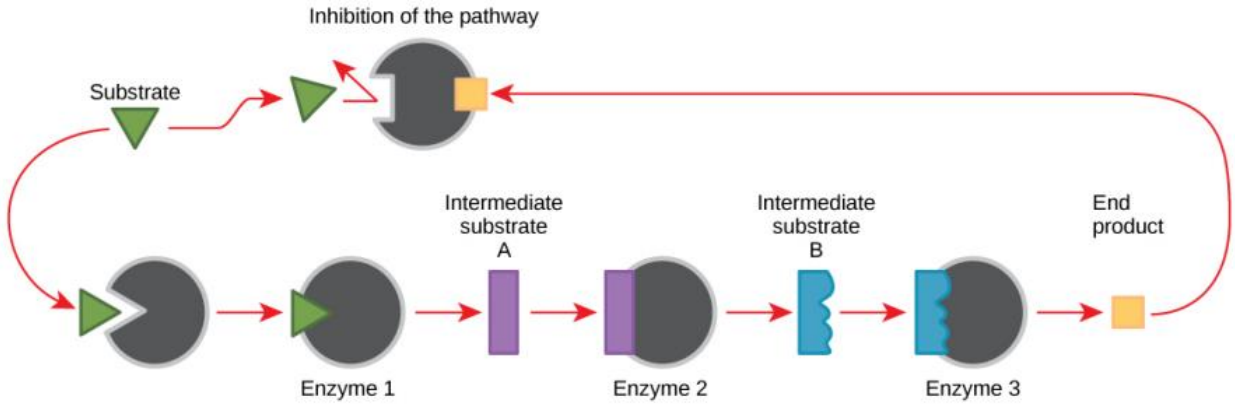
- pH: Deviations from the optimum page caused a decrease in enzyme activity. Small deviations can change the charge at the active site and affect the binding of the substrate. Larger deviations can cause the hydrogen an ionic-bonds holding the tertiary structure together to change and the enzyme denatures, meaning and same substrate complexes can no longer form. Extreme pH levels will produce denaturation. The structure of the enzyme is changed. At pH values slightly different from the enzyme's optimum value, small changes in the charges of the enzyme and its substrate molecules will occur. This change in ionisation will affect the binding of the substrate with the active site.



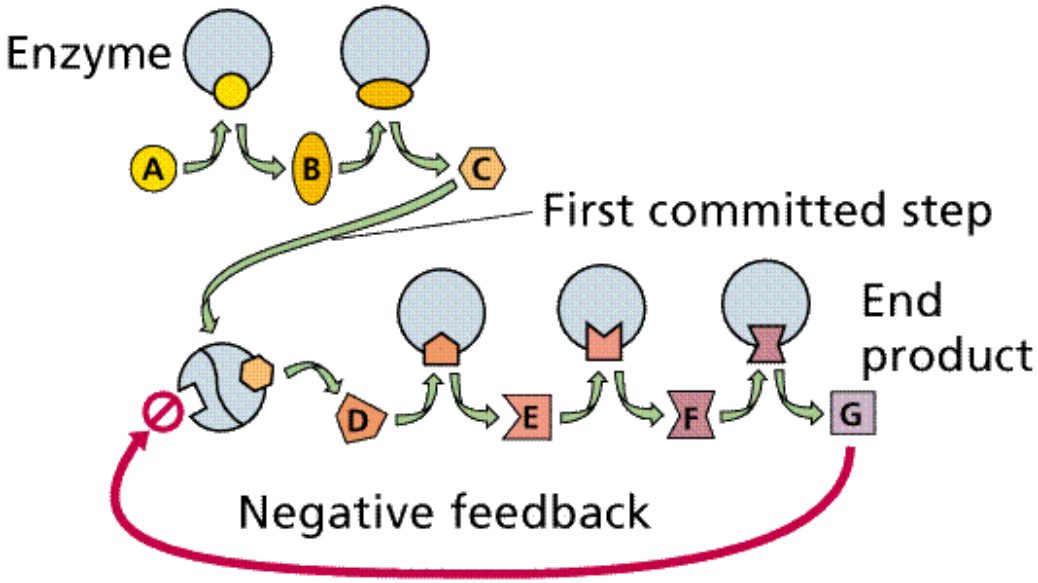
- **Inhibitors:** Enzyme activity can be inhibited. Enzyme activity can be prevented by enzyme inhibitors. These are molecules that bind to the enzyme that they inhibit. Inhibition can be either competitive or non-competitive. Inhibitors prevent the binding of substrate to active site; Therefore, fewer enzyme substrate complexes form, reducing the rate of their reactions. They are found naturally, but are also used artificially as drugs, pesticides and research tools.

Competitive Inhibitors	Non-Competitive Inhibitors
Inhibitor has a structure similar to the substrate. They compete with the substrate molecules to bind to the active site, but no reaction takes place. Blocks the active site so no	Inhibitor has no real structural similarity to the substrate. Binds to site other than the active site. Binding changes the shape of the active site. They bind to the enzyme away from its active site. This

substrate molecules can fit. But cannot react with the enzyme. The level of activity inhibited depends on the relative concentrations of the inhibitor and the substrate. High concentration of inhibitor means it will take up nearly all of the active sites and hardly any substrate when you get to the enzyme. With a lower concentration, the chances of the substrate getting to the inactive side before the inhibitor increases. So, increasing the concentration of a substrate will increase the rate of reaction up to a point. Example: arabinos competes with glucose for the active site on glucose oxidase enzyme.	causes the active site to change the shape so the substrate molecules cannot bind to it. They don't compete with the substrate molecules to bind to the active site because they are different shapes. Increasing the concentration of substrate doesn't affect the reaction rate- enzyme activities still inhibited.
	
	



Metabolic pathways are a series of reactions catalysed by multiple enzymes. Feedback inhibition, where the end product of the pathway inhibits an upstream step, is an important regulatory mechanism in cells.



<u>End Product of Inhibition/ Allosteric effectors:</u> The activity of some enzymes is controlled by certain molecules binding to a specific regulatory or allosteric site on the enzyme, distinct from the active site. Different molecules can inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this and they are often at the start of a long biochemical pathway. They are generally activated by the substrate off the pathway and inhibited by the product of the pathway, thus only turning the pathway on when it is needed.

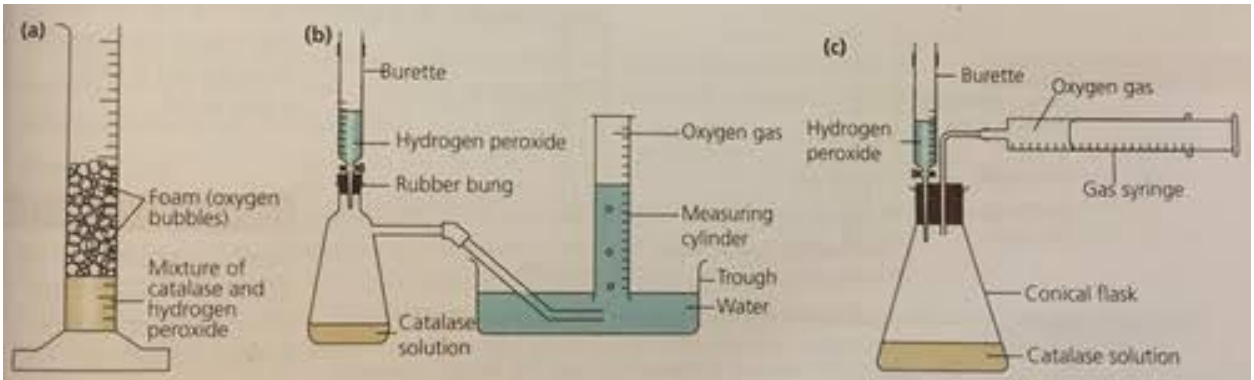


Figure 6 Measuring the amount of oxygen produced (a) as height of foam, (b) oxygen collected by displacing water and (c) oxygen collected in a gas syringe

Coenzymes:

Coenzymes - these are organic compounds, often containing a *vitamin* molecule as part of their structure.

Coenzymes are not permanently bound to the enzyme but may be temporarily and loosely bound for the duration of the reaction and then move away once it is completed.

Coenzymes take part in the reaction and like the substrate are changed in some way. Unlike substrate the coenzymes are recycled back to take part in the reaction again.

	Coenzymes	**Cofactors**	**Activators**
Definition	Small organic molecules that help enzyme function.	Non-proteins molecules required for enzyme function.	Molecules that increase enzyme activity.
Examples	NAD +, FAD, coenzyme A, ATP	Iron, Zinc, Magnesium, Heme, Biotin, Vitamins	cAMP, Calcium, Calmodulin, Protein Kinase Activators
Function	Transfer functional groups in enzyme reactions.	Binds to enzyme to allow foe proper function.	Increase enzyme activity & modifies enzyme shape.

Michaelis Menten Model: when enzymes are working the hardest and can't go any faster – enzyme saturation. This is the V_{Max} – a maximum rate in which an enzyme can work at. The substrate concentration at which an enzyme works at halves its maximum rate ($\frac{1}{2}$ V_{Max}). It is also used as a measure of affinity of enzyme for its substrate or efficiency of an enzyme.

V_{Max}: the theoretical maximum rate that an enzyme can perform. Measured at the point of saturation- every enzyme has a substrate. This is the theoretical maximum rate (velocity) of a reaction an enzyme catalyzes, and at Vmax all the enzymes are occupied by substrate molecules. At high substrate concentrations, the enzyme concentration will become the limiting factor and at this point, the curve will level

off. Measured by increasing substrate consideration while leaving the enzyme concentration constant.

K_M: $V_{Max}/2$ K_M. K_M measures the affinity/ efficiency (the degree of attraction between molecules) of an enzyme- how quickly an enzyme reaches V_{Max}. It only points to when a substrate is already in an enzyme. Similar to acceleration - how quickly it reaches the maximum speed.

High K_m values: When Km is high, affinity will be low. This will result in less effective collisions, as enzymes form fewer enzyme substrate complexes, as the active sites of these enzymes are less good fit for the substrates, so the reaction will take longer to get to V_{Max}. A higher concentration of substrate is required to reach V_{Max}.

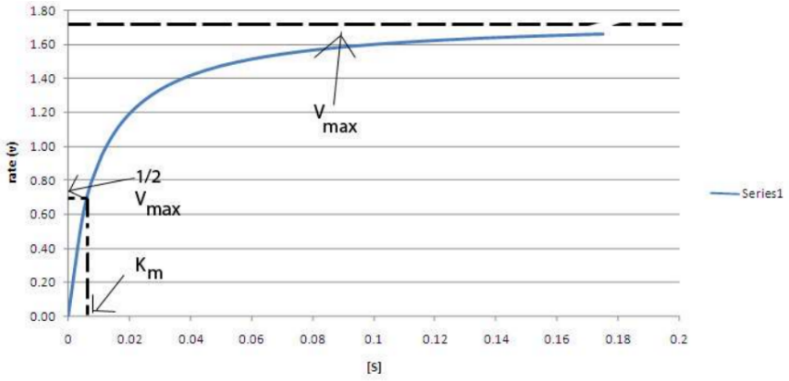
Low K_m value: There will be more effective collision, as enzymes form more enzyme substrate complexes. The active sites of these enzymes are a very good fit for the substrate so the reaction will take lesser time to get to V_{Max}. Less concentration of substrate is required to reach V_{Max}.

Michaelis Menton Constant:

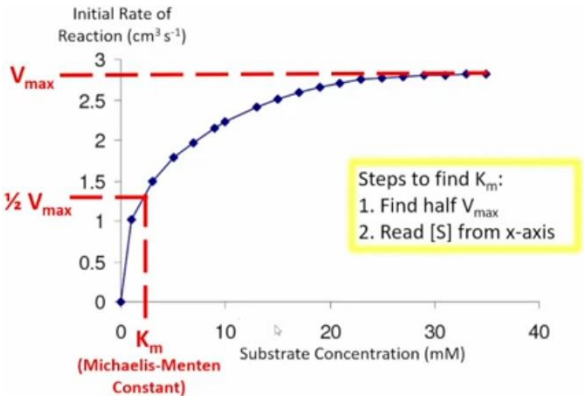
K_m= [S] = $\frac{1}{2}$ V_{max}

It is the strength of association between enzyme and the substrate

↑ K_m = ↓ affinity & ↓ K_m = ↑ affinity



Take half of Vmax.
Reconcile this with substrate concentration on the x axis.



Denaturing Enzymes:

Enzymes exposed to different pH or High Temperature different from the optimum would result in the active site of the enzyme being denatured, for the substrate is no longer able to fit the enzyme. (mismatched)

Effect of temperature and pH:_

The progress of enzyme catalyst reaction can be followed by the measuring the concentrations of the reactants and products.

- Enzyme shape is denatured by the amino acid.
- Enzymes work fastest at an optimum temperature of 37°C in humans.
- Overheating breaks the bonds holding enzymes together, it denatures can no longer bind.
- Increasing temperature from 0°C to optimum increases enzyme activity as more the energetic molecules have greater collisions with the substrate resulting in a faster ROR.
- Low temperature doesn't denature but less K.E, so slow ROR.

Temperature Increase - Successful collisions - enzyme + substrate binds more K.E - more product

- Enzymes work fastest at optimum pH 7.
- Different pH breaks the bonds holding enzymes together because the enzyme denatures and can no longer bind.

Relationship in Retrospect:

- Rate of Reaction at 30 seconds is initial rate of reaction.
- Initial rate of reaction is the velocity in Michaelis-Menten Model which tries to calculate the V_{Max} (a theoretical maximum velocity of a certain enzyme) & how quickly the enzyme can reach that, expressed in terms of K_M.
- K_M = $\frac{1}{2}$ of V_{Max} – calculated by the reciprocal plot or a hyperbolic normal plot.

Comparing Enzyme Affinities:

- There is enormous variation in the speed at which different enzymes work. A typical enzyme molecule can convert around 1000 substrate molecules into product per second this is known as the turnover rate.
- More precise measurements of the rate at which enzymes work are difficult and complex to make but are important for understanding of how enzymes work together to control cell metabolism. When is the key steps towards understanding how well an enzyme performs is to measure the theoretical maximum rate (velocity), Vmax, of the reaction it catalyzes. At Vmax all the enzyme molecules are bound to substrate molecules – the enzyme is saturated with substrate.
- The Michaelis-Menten Constant (K_m) is the substrate concentration at which an enzyme works at half its maximum rate ($\frac{1}{2}$ V_{Max}). At this point half the active sites of the enzyme are occupied by the substrate. The higher the affinity of the enzyme for the substrate, the lower the substrate concentration needed for this to happen. Thus, the Michaelis- Menten Constant is a measure of the affinity of the enzyme for its substrate.

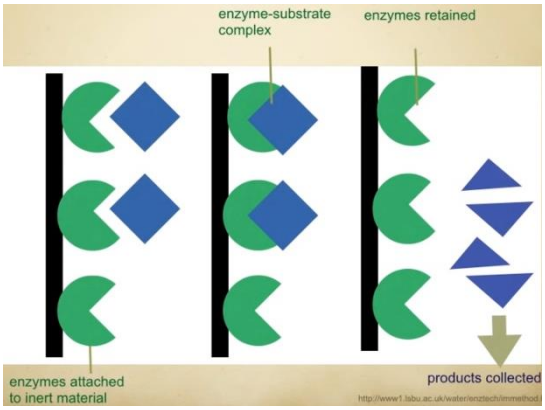
- The Higher the affinity, the lower the Michelis Menten Constant and the quicker the reaction will proceed to its maximum rate, although the maximum rate itself is not affected by the Michaelis-Menten Constant.
- V_{Max} and K_m therefore provide two different ways of comparing the efficiency of different enzymes.

Enzyme Immobilization:

Enzyme immobilization may be defined as the process of confining the enzyme molecules to a solid support over which a substrate is passed and converted to products. An immobilized enzyme is one whose movement in space has been restricted either completely or to a small, limited region.

Enzymes are immobilized because:

- Prediction from degradation and deactivation.
- Reuse of enzymes from many reaction cycles, lowering the total production cost of enzyme mediated reactions.
- Ability to stop the reaction rapidly by removing the enzyme from the reaction solution.
- Enhanced stability.
- Is the separation of the enzyme from the product.
- Product is not contaminated with the enzyme.



An ideal carrier matrix for enzyme immobilization:

- Inert.
- Physically strong and stable.
- Cost effective.
- Regenerable.
- Reduction in production inhibition.

Examples:

- Immobilized enzyme an inert glass or in alginate beads.
- Enzymes linked to other materials.
- Enzymes trapped in a mesh of inert material.
- Enzymes held in semipermeable membrane.

Advantages	Disadvantages
Reuse enzymes.	Immobilization may alter enzyme shape & function.
Good for continuous operation.	More expensive.
Product is free of enzyme.	Enzyme may detach.
Enzyme heat & pH stability is improved.	
Can develop multi-enzyme reaction systems.	
Reduces problems of effluent disposal.	