ENZYMES IN FOOD

Presented by : GROUP 1

KEY TOPICS

01. Nomenclature and classification of enzymes

02. Properties and functions of enzymes

03. Enzyme kinetics and reactions

04. Factors affecting enzyme activity

05. Enzyme immobilization

06. Application of enzymes in food processing

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

WHAT ARE ENZYMES AND WHY DO WE CLASSIFY THEM?

Enzymes are biological catalysts, primarily proteins, that accelerate biochemical reactions without being consumed in the process. They are vital for virtually every metabolic function, from digestion to DNA replication. Classifying enzymes provides a universal language for scientists, ensuring clear communication and understanding across various fields of study. It helps in organizing the vast diversity of enzymes, predicting their functions, and facilitating research.

EARLY NAMING CONVENTIONS: HISTORICAL CONTEXT AND INCONSISTENCIES

Historically, enzymes were often named based on their substrate, the reaction they catalyzed, or their discoverer. This led to a plethora of inconsistent and ambiguous names.

01. **Substrate-Based Naming**

Many enzymes were named simply by adding the suffix "-ase" to the name of the substrate they acted upon (e.g., urease acts on urea).

02. **Arbitrary Names**

Older enzymes sometimes had trivial names that offered little information about their function (e.g., pepsin, trypsin). This created confusion as different enzymes might have similar names, or the same enzyme might have multiple names.

03. **Reaction-Based Naming**

Some enzymes were named after the type of reaction they catalyzed (e.g., dehydrogenases remove hydrogen).

THE NEED FOR A SYSTEMATIC APPROACH: TOWARDS A UNIFIED SYSTEM

Eliminate Ambiguity

- Ensure each enzyme has a unique identifier.

Facilitate Communication

- Enable scientists worldwide to understand and discuss enzymes without confusion.

Organize Knowledge

- Create a logical framework for classifying new and existing enzymes.

Predict Function

- Allow for educated guesses about an enzyme's role based on its classification.

EC CLASSES: THE SIX PILLARS OF ENZYME FUNCTION

The EC system categorizes enzymes into six main classes, each representing a broad type of biochemical reaction. This hierarchical classification provides a clear overview of an enzyme's primary role.

EC 1: Oxidoreductases Catalyze oxidation-reduction reactions (transfer of electrons).	**EC 2: Transferases** Transfer functional groups (e.g., methyl, glycosyl, phosphate) from one molecule to another.
EC 3: Hydrolases Catalyze the hydrolysis of various bonds (adding water to break a bond).	**EC 4: Lyases** Cleave various bonds by means other than hydrolysis or oxidation, often forming double bonds.
EC 5: Isomerases Catalyze the rearrangement of atoms within a molecule, resulting in an isomer.	**EC 6: Ligases** Catalyze the joining of two large molecules by forming a new chemical bond, usually coupled with ATP hydrolysis.

EXAMPLES OF ENZYME NAMING

COMMON NAME	EC NUMBER	DESCRIPTION
Alcohol Dehydrogenase	EC 1.1.1.1	Oxidoreductase acting on the CH-OH group of donors with NAD+ or NADP+ as acceptor.
Hexokinase	EC 2.7.1.1	Transferase transferring phosphorus-containing groups, with an alcohol group as acceptor.
Amylase	EC 3.2.1.1	Hydrolase acting on glycosyl bonds, specifically alpha-1,4-glucan glucanohydrolase.

SUBCLASSES AND SUB-SUBCLASSES FOR SPECIFICITY

Beyond the six main classes, the EC number system extends to provide increasingly specific details about an enzyme's function through subclasses and sub-subclasses. This four-part number (e.g., EC X.X.X.X) precisely pinpoints the enzyme's activity.

01. **Fourth Number**
Serial number of the enzyme within its sub-subclass, indicating its absolute specificity.

02. **Third Number**
Indicates the nature of the chemical group involved in the reaction.

03. **Second Number**
Defines the type of bond acted upon or the group transferred.

04. **First Number**
The main EC class (1-6) indicating the general type of reaction.

THE IMPORTANCE OF ENZYME CLASSIFICATION

Foundation for Research

- Essential for genetic engineering and synthetic biology.

Medical Innovation

- Drives drug discovery and diagnostic developments.

Industrial Applications

- Crucial for food processing, biofuels, and environmental remediation.

KEY PROPERTIES AND FUNCTIONS OF ENZYMES

WHAT ARE ENZYMES?

- Enzymes are biological catalysts
- Mostly proteins (some are RNA)
- Speed up chemical reactions without being consumed

- enzymes are not consumed of permanently altered during the reaction.

KEY PROPERTIES OF ENZYMES

- **Highly specific to their substrate**

Ex. lactase enzyme only breaks down lactose (milk sugar) and no other sugar.

- **Reusable and not permanently changed**

- enzymes remain chemically unaltered after catalyzing a reaction and can continue to speed up new reactions with fresh substrate molecules.

- **Efficient even in small amounts**

- a single enzyme can process thousands to millions of substrate molecules per minute.

Ex. Catalase, which breaks down harmful hydrogen peroxide can convert over 40 million substrate molecules per second.

SPECIFICITY

- **Each enzyme acts on a specific substrate**

- a specific enzyme only works with a specific substrate

sucrase - sucrose

lactase - lactose

maltase - maltose

- **Active site matches the substrate shape**

- **Explains lock-and-key and induced-fit models**

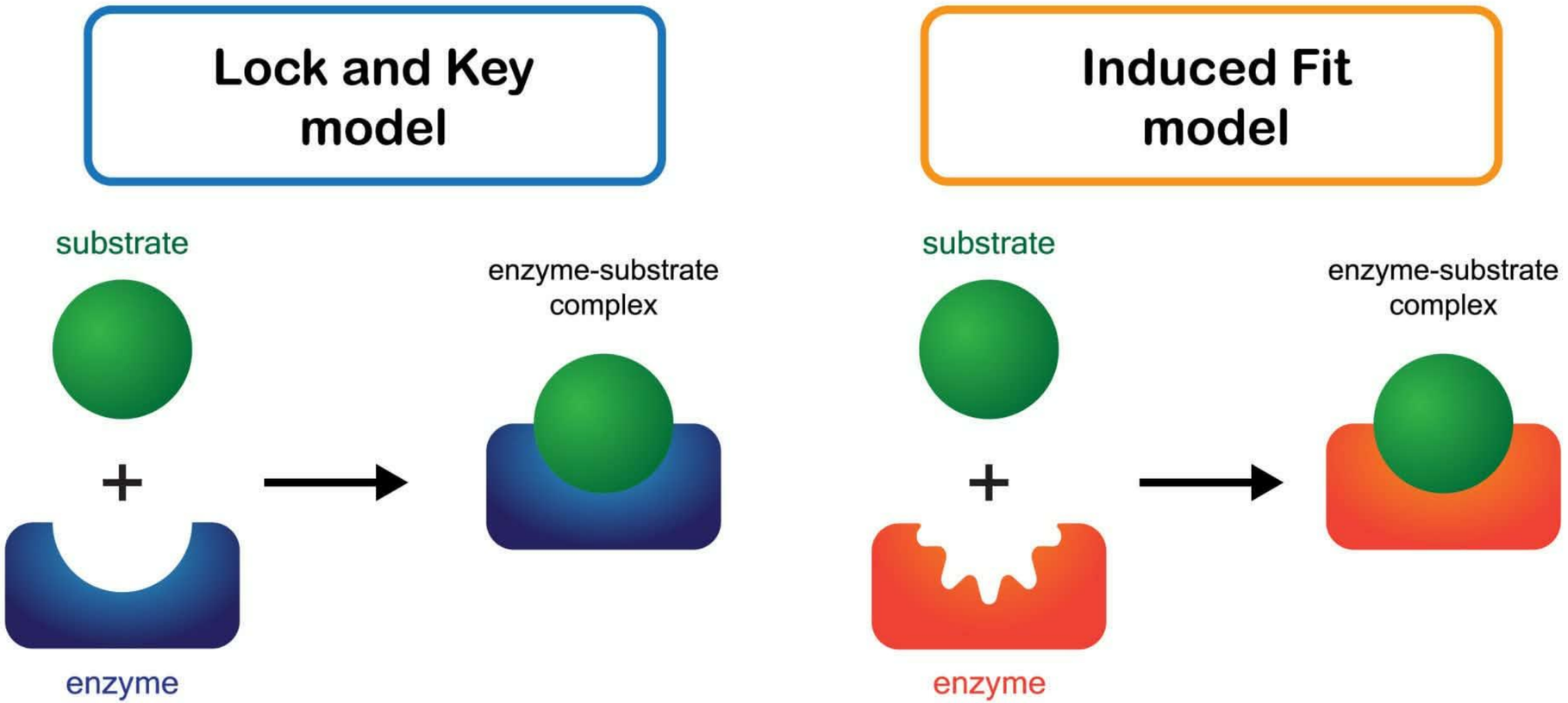
The Models:

Lock and Key Model: The substrate fits perfectly into the active site like a key into a lock

Induced Fit Model: The active site slightly changes its shape according to the substrate molecules

ENZYME

Mechanism of enzyme-substrate interaction



EFFECT OF TEMPERATURE AND PH

- **Temperature**: Each enzymes has an **Optimum Temperature** (usually ~37C in humans). Excessive heat causes **Denaturation** (enzyme loses its shape).

- **pH Levels**: Most enzymes works best at a neutral pH (7), though some, like stomach pepsin, require a highly acidic pH (~2.0).

EFFICIENCY AND REVERSIBILITY

- **High Efficiency:** A very small amount of enzyme can process thousands of substrate molecules per second.
- **Reversibility**: Most enzymatic reactions are reversible. The enzyme can facilitate both the forward and reverse reaction depending on the cell's needs.
- **Colloidal Nature**: Being large proteins, enzymes behave as colloids, providing a large surface area for reactions.

KEY FUNCTIONS OF ENZYMES

- Catalyze metabolic reactions
- Aid in digestion of food
- Support cellular processes

ROLE IN DIGESTION

- Break down carbohydrates, proteins, and fats
- Examples: amylase, protease, lipase
- Allow nutrients to be absorbed

ROLE IN METABOLISM

- **Control biochemical pathways**

 - Direct step-by-step reaction chains (e.g., glycolysis) keeps processes orderly and adjustable.

- **Regulate energy production**

 - Store energy (e.g., ATP synthase makes ATP; photosynthesis enzymes create food energy) – matches supply to demand.

- **Enable synthesis of essential molecules**

 - Build key compounds (DNA polymerase makes DNA; ribosomes build proteins) – for cell structure and function.

ENZYME KINETICS AND REACTIONS

ENZYME KINETICS

- is the study of enzyme reaction rates and the conditions that affect them.
- is an expression of chemical reactions in mathematical terms.
- it is an oldest approach to understand enzyme mechanism.

History

- First began in 1902, **Adrian John Brown** demonstrated the hydrolysis of sucrose by invertase.
- Leonor Michaelis and Maud Menten in 1913, hypothesized the existence of intermediate complex.

REACTION KINETICS

When an enzyme is added to a substrate, the reaction that follows occurs in **three stages** with distinct kinetics:

- **Pre-steady state**
- **Steady-state** (equilibrium)
- **Post-steady state**

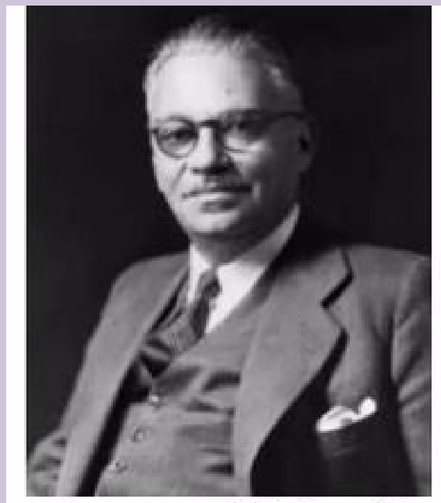
REACTION KINETICS

PHASE	CONCENTRATION OF ES (ENZYME-SUBSTRATE)	RATE OF PRODUCT FORMATION
Pre-steady state	**Rapid burst** of ES complexes form	Initially **slow**, waiting for ES to form, then speeds up
Steady-state (equilibrium)	ES concentration remains **constant** as it is being formed as quickly as it breaks down	**Constant** rate of formation, faster than the pre-steady state
Post-steady state	Substrate **depletes** so fewer ES complexes form	**Slow** as there are fewer ES complexes; slows down as substrate runs out

REACTION KINETICS

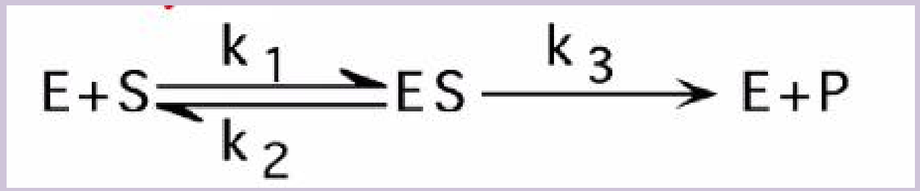
Michaelis-Menten Kinetics

- a model of enzyme kinetics which explains how the rate of an enzyme-catalysed reaction depends on the concentration of the enzyme and its substrate.
- Based on the observation of sucrose.



Leonor Michaelis

Maud Menten



CLASSIFICATION OF CHEMICAL REACTIONS

Order of Reaction – the sum of power of concentrations gives it.

1) **Zero (0) Order Reaction** – does not depend on the concentration of the substrate.
2) **First (1st) Order Reaction** – depends on substrate concentration, and the sum of powers should be 1.
3) **Second Order Reaction** – when the sum of powers of concentration is 2.

MICHAELIS – MENTEN CURVE OF ENZYME KINETICS

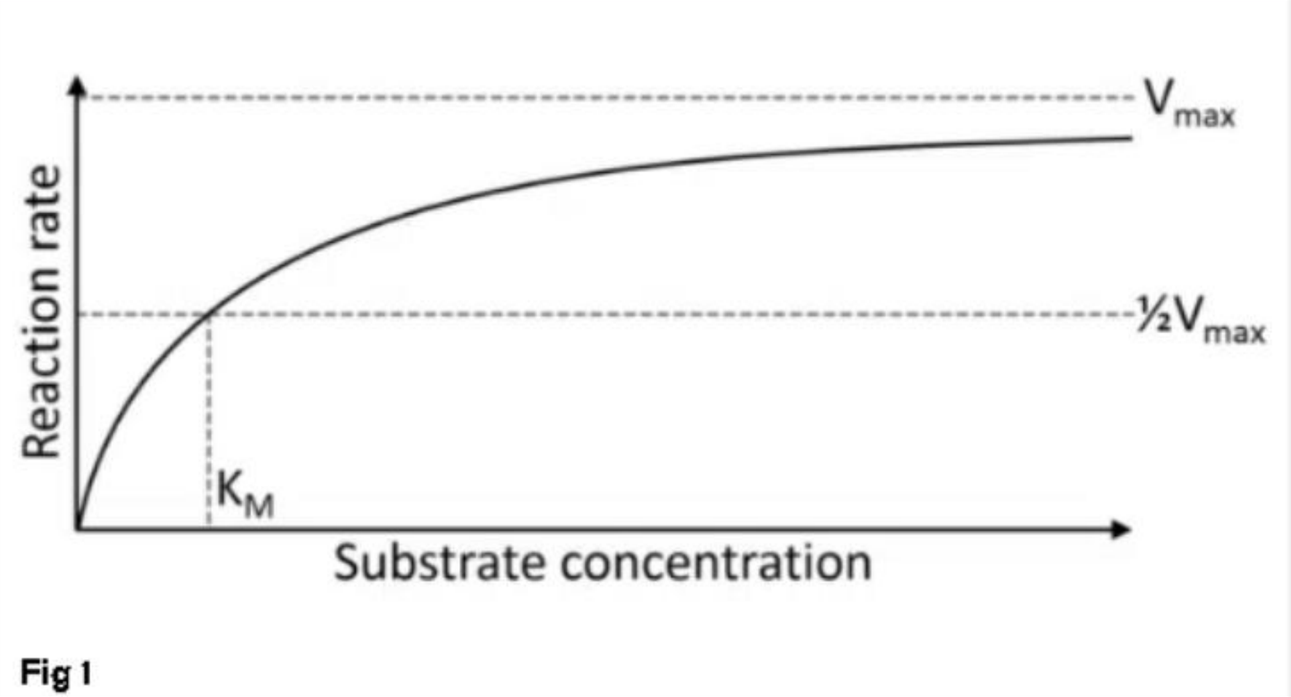


Fig 1

- **A hyperbolic curve**

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Michaelis – Menten Equation

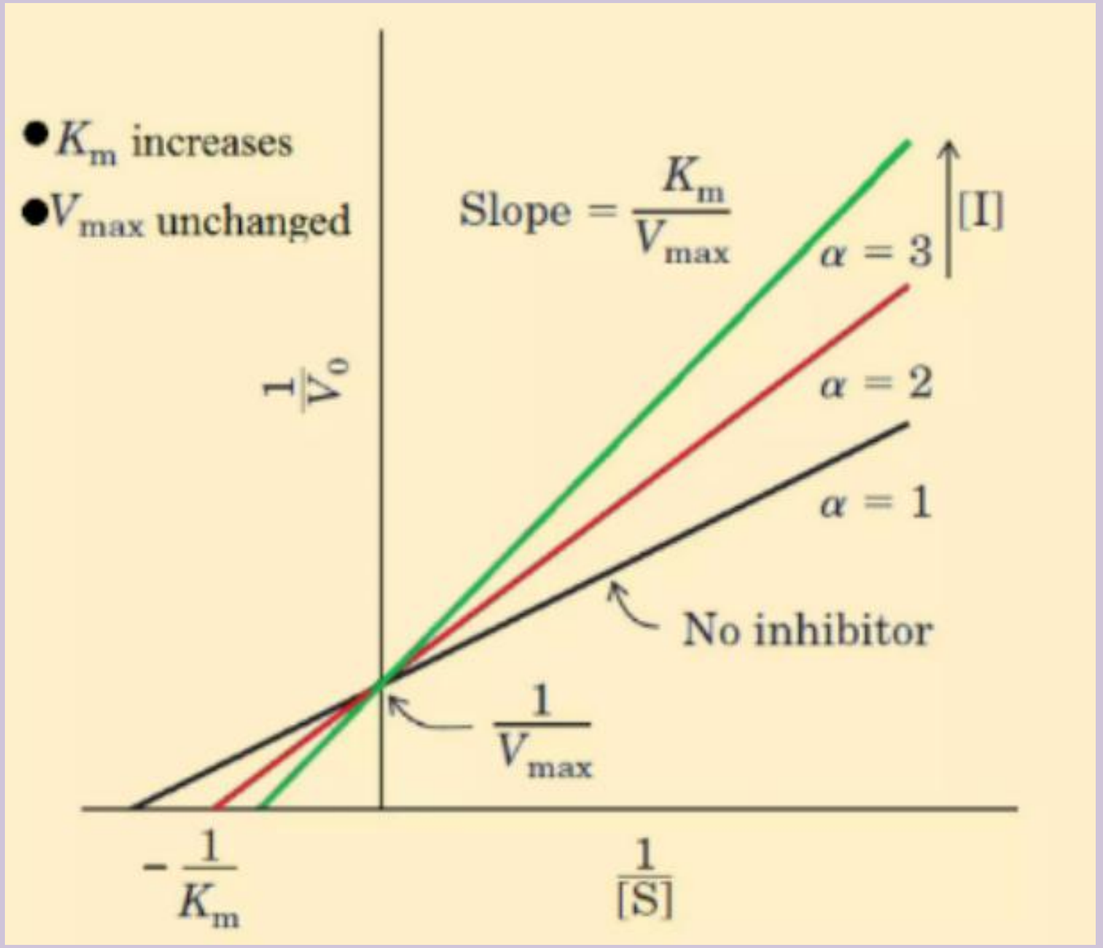
LINEWEAVER-BURK PLOT

- a graph, and a more useful representation of Michaelis–Menten kinetics.
- this produces a straight line, allowing for the easier interpretation of various quantities and values from the graph.
- also useful when determining the type of **enzyme inhibition** present by comparing its effect on Km and Vmax.

Inhibitor – any agent that decreases the velocity of an enzyme-catalyzed reaction. It may be of reversible or non-reversible type.

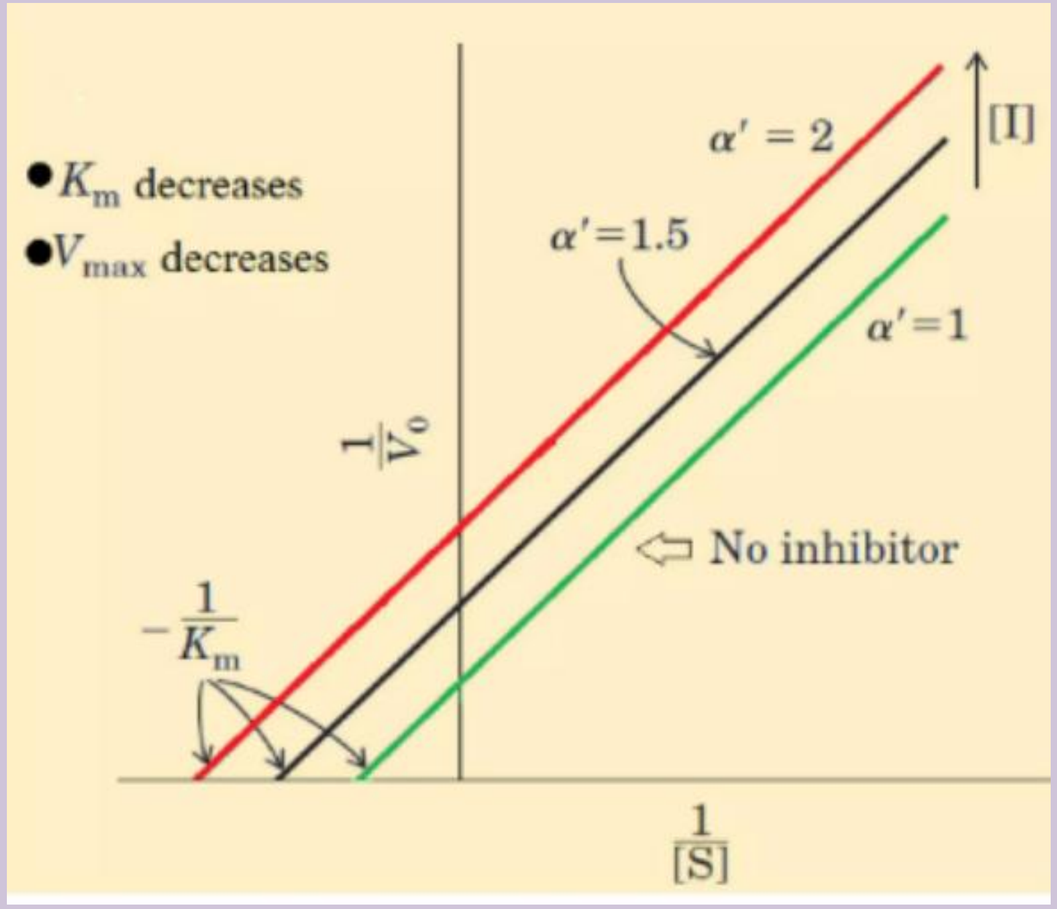
INHIBITORS

Competitive Inhibitors



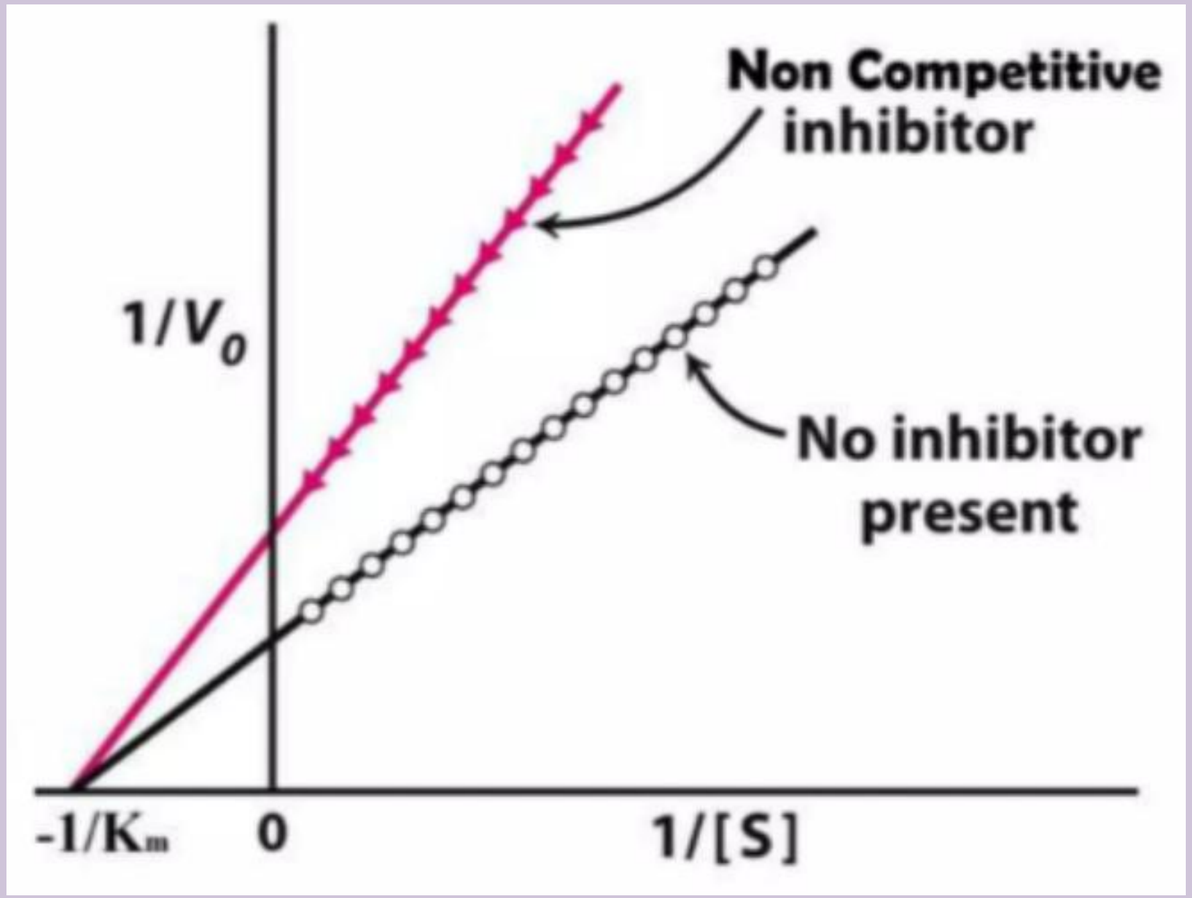
INHIBITORS

Uncompetitive Inhibitors



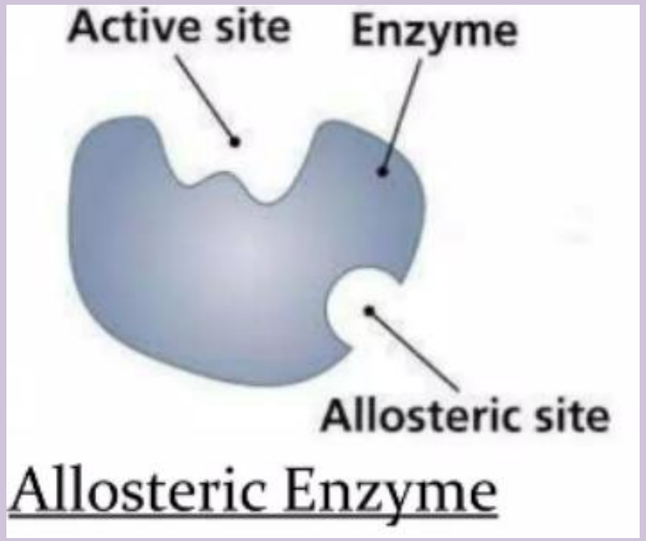
INHIBITORS

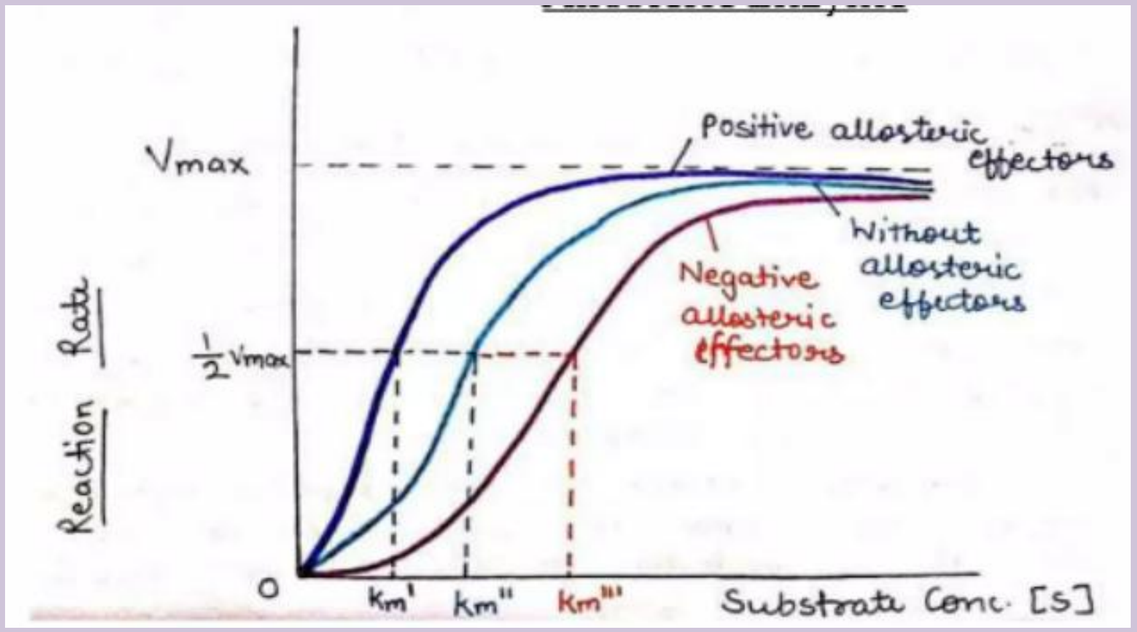
Non-competitive (Mixed) Inhibitors

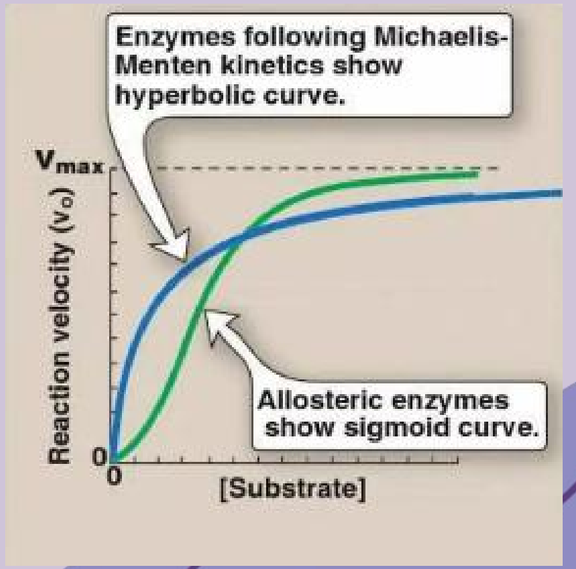


KINETICS OF ALLOSTERIC ENZYMES

- The allosteric enzymes have another site called **allosteric site.**
- It can act either as an activator or an inhibitor.
- They do not follow Michaelis–Menten Kinetics.
- Instead of a hyperbolic curve, it shows a **sigmoidal curve** because a small change in concentration will bring a large change in reaction rate.







FACTORS AFFECTING ENZYME ACTIVITY

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01. Temperature

02. pH

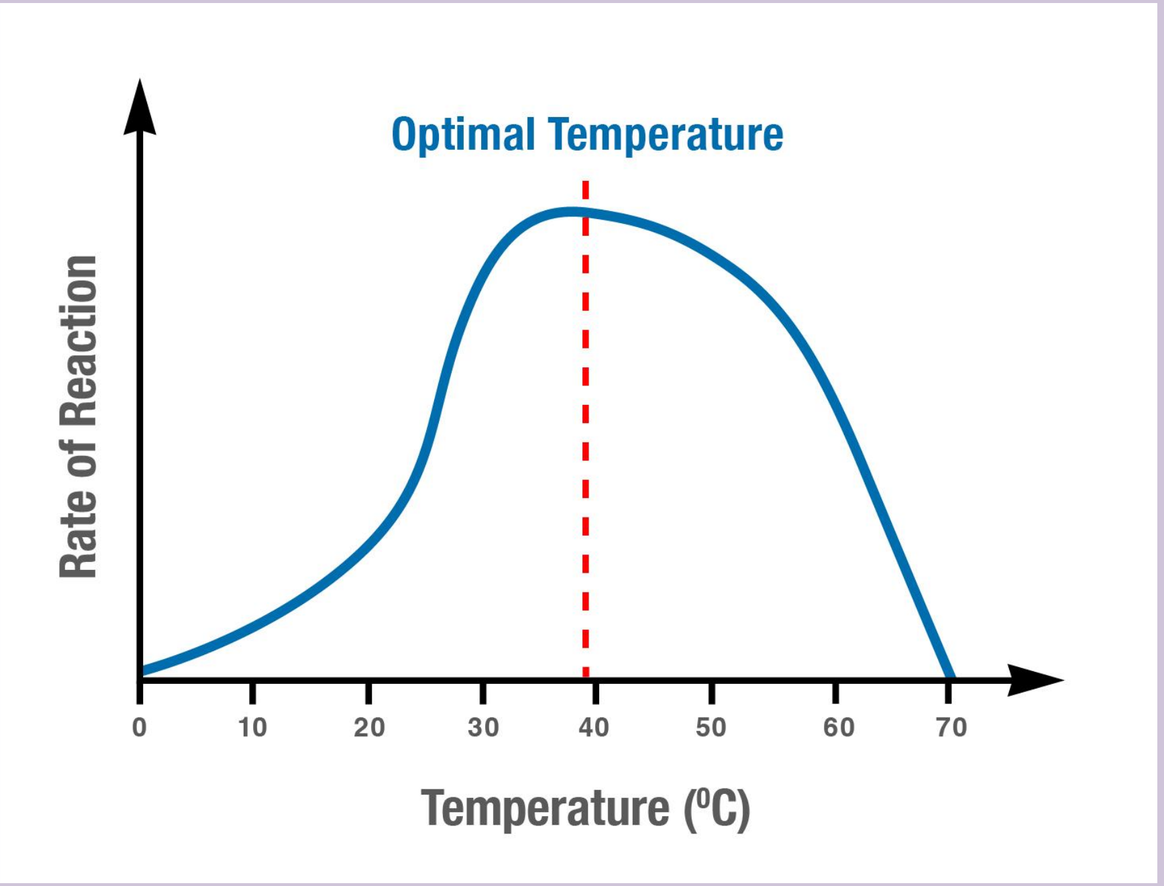
03. Enzyme & Substrate Concentration

04. Presence of Inhibitors and Activators

FACTORS AFFECTING ENZYME ACITVITY

Temperature

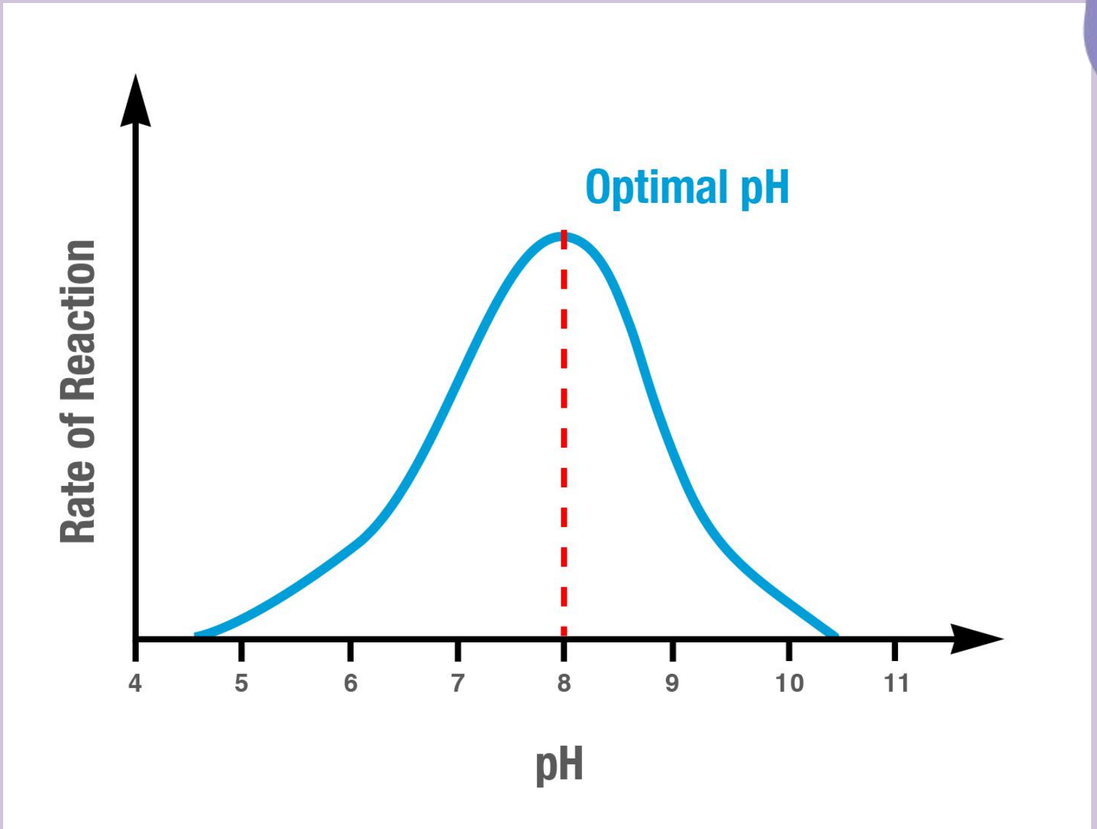
- Each enzyme has an optimum temperature at which it works best. Increasing temperature generally increases the reaction rate until an optimal point is reached; beyond this, heat denatures the enzyme, decreasing activity



FACTORS AFFECTING ENZYME ACITVITY

pH

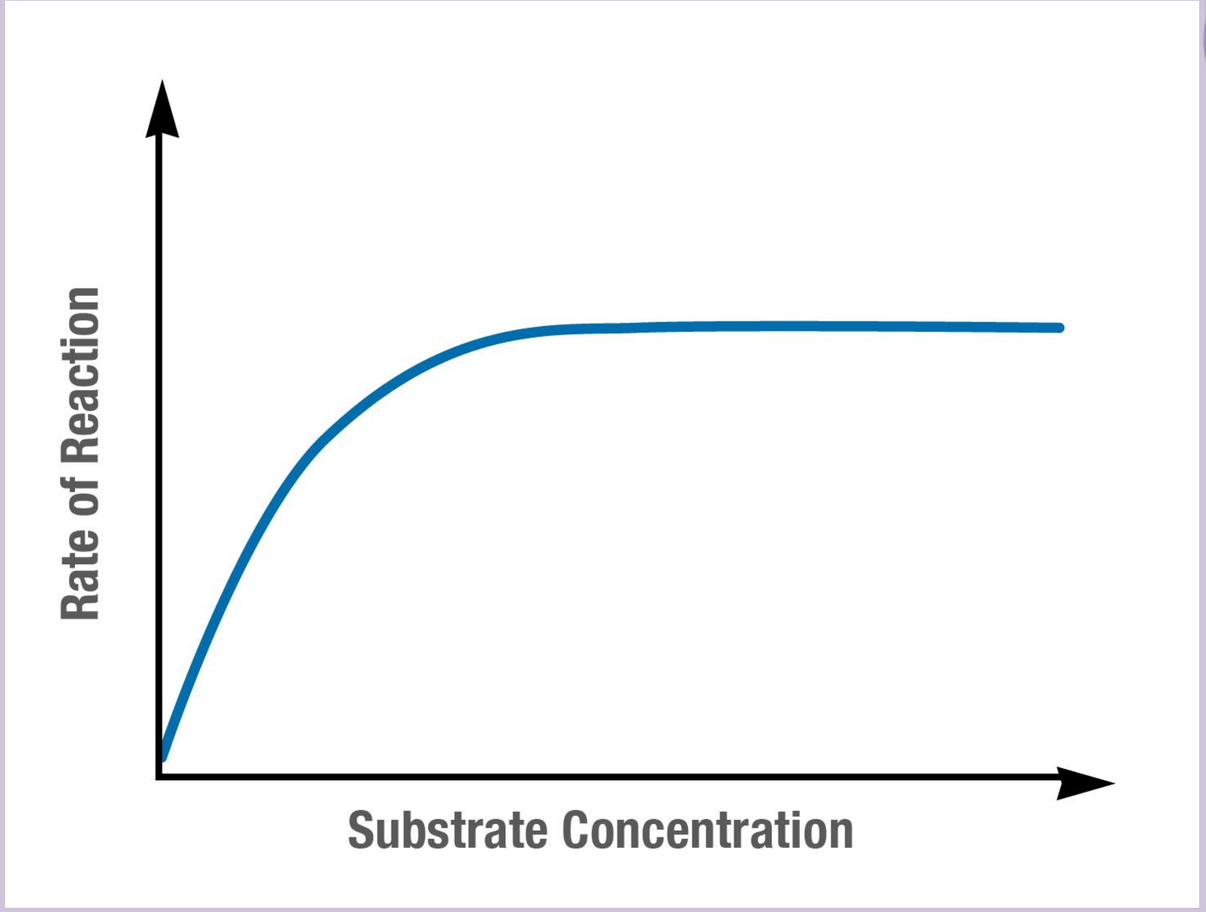
- Each enzyme has an optimal pH that helps maintain its three-dimensional shape. Changes in pH may denature enzymes by altering the enzyme's charge. This alters the ionic bonds of the enzyme that contribute to its functional shape.



FACTORS AFFECTING ENZYME ACITVITY

Substrate Concentration

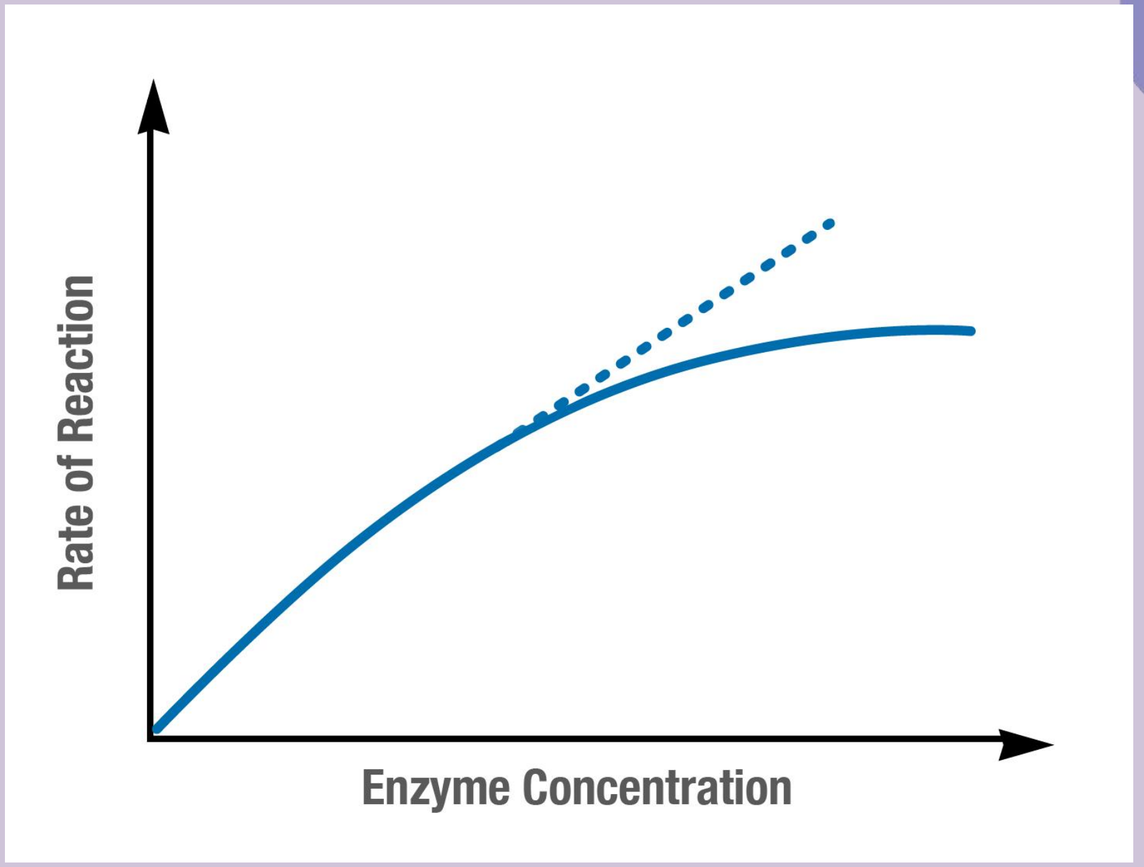
- As the substrate concentration increases, the rate increases because more substrate molecules can collide with enzyme molecules, so more reactions will take place



FACTORS AFFECTING ENZYME ACITVITY

Enzyme Concentration

- As the enzyme concentration increases the rate of the reaction increases linearly
- At very high enzyme concentration, the substrate concentration may become rate-limiting



FACTORS AFFECTING ENZYME ACITVITY

Presence of Inhibitors and Activators

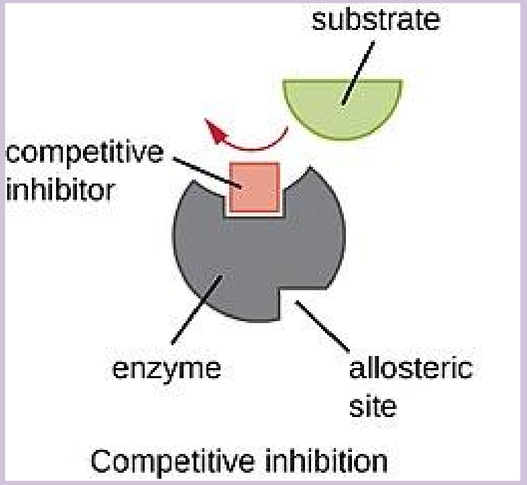
Activators **increase** enzyme activity by promoting substrate binding or reaction rates, acting like a “green light,” while inhibitors **decrease** activity by blocking the active site or changing the enzyme’s shape, acting as a “red light,” controlling how fast biological processes occur. Activators and inhibitors bind to enzymes (either at the active site or an allosteric site) to regulate catalysis, preventing permanent changes and allowing for fine-tuned cellular control.

FACTORS AFFECTING ENZYME ACITVITY

Inhibitor can be either:

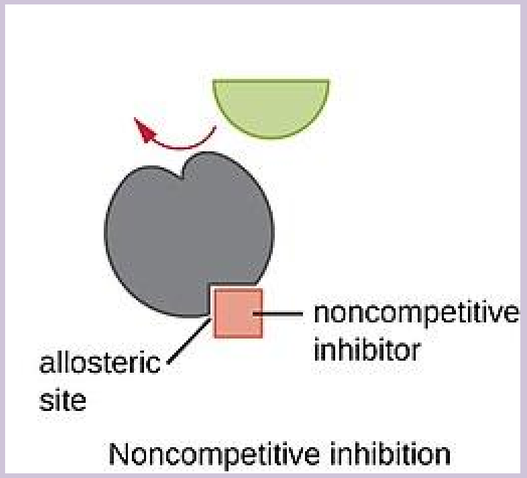
Competitive Inhibitor

- Competitive inhibitor is the interruption of an enzyme's ability to bind to a substrate due to a different molecule binding to the active site.



Non Competitive Inhibitor

- Non competitive inhibitors are not similar to the substrate and they do not bind to the active site of the enzyme. They change the conformation of the active site.



ENZYME IMMOBILIZATION

ENZYME IMMOBILIZATION

Enzyme immobilization is the process of attaching enzymes to an insoluble, food-grade support to control enzyme activity, improve stability, allow easy separation from food products, and enable repeated use during food processing.

IMPORTANCE IN FOOD CHEMISTRY

- **Reusability and Cost Reduction**: Immobilized enzymes such as lactase in lactose-free milk production can be reused, lowering processing costs.
- **Enhanced Stability**: Immobilized enzymes are more resistant to temperature and pH changes during food processing, such as in juice clarification and cheese making.
- **Easy Separation**: Enzymes can be removed from food products easily, ensuring product purity and safety.
- **Continuous Processing**: Enables continuous production systems used in large-scale food industries.

IMMOBILIZATION TECHNIQUES USED IN FOOD PROCESSING

Adsorption

- Enzymes attach to food-grade supports through weak ionic or hydrophobic forces; commonly used with pectinase in fruit juice clarification.

Entrapment/Encapsulation

- Enzymes are trapped in gel matrices like alginate beads; used for lactase in lactose-free milk production.

Covalent Bonding

- Strong bonds provide high stability for enzymes such as invertase used in sugar processing, though activity may slightly decrease.

Cross-linking

- Enzymes are linked to each other or to a support to increase durability during repeated food processing cycles, such as in baking and fermentation industries.

APPLICATION OF ENZYMES IN FOOD PROCESSING

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The application of enzymes in food processing uses natural biocatalysts (like amylases, proteases, lipases) to speed up specific, desirable chemical reactions, improving food quality, texture, flavor, and nutritional value while reducing costs, energy, and waste

KEY ENZYME APPLICATIONS

01. Baking

- **Amylases**: Improve dough handling, increase bread volume, and enhance crust color by converting starch to sugars.
- **Xylanases/Laccases**: Strengthen dough, reduce stickiness, and improve crumb softness.

KEY ENZYME APPLICATIONS

02. Dairy

- **Rennet (Chymosin)/Proteases**: Coagulate milk for cheese making.
- **Lactase**: Hydrolyzes lactose, making dairy products suitable for lactose-intolerant individuals.
- **Lipases**: Develop distinct flavors in cheeses like Parmesan and Romano.

KEY ENZYME APPLICATIONS

03. Brewing & Wine:

- **Amylases**: Convert starches to fermentable sugars for alcohol production.
- **Pectinases**: Clarify fruit juices and wines by breaking down pectin, preventing haze.
- **Beta- Glucanases**: Reduce viscosity in brewing.

KEY ENZYME APPLICATIONS

04. Meat & Fish Processing

- **Proteases** (e.g., Papain, Bromelain): Tenderize tough meat cuts by breaking down muscle fibers.
- **Transglutaminase**: Binds meat proteins, improving texture and yield in processed meats.

BENEFITS OF USING ENZYMES

Energy saving - Enzymes work best to moderate temperature and neutral pH level.

Faster processing - they catalyze reactions much faster than traditional chemical methods, allowing for higher production rates.

Eco-friendly - since enzymes are derived from plants, animals, or microbes, they are biodegradable and generally recognized as safe (GRAS), making them much cleaner than harsh industrial chemicals.

THANK YOU!