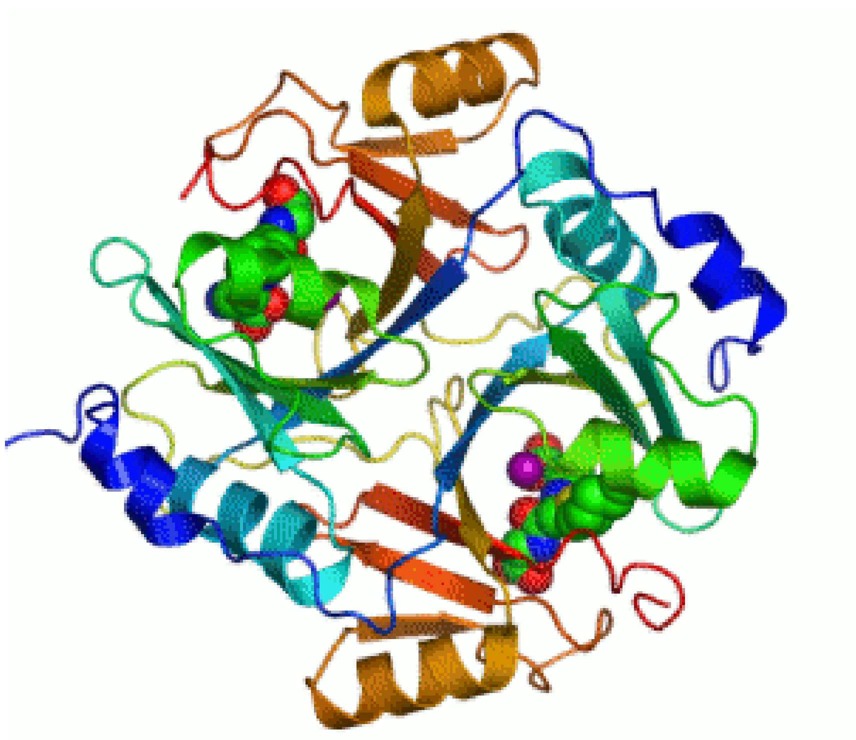


Enzymes

FE 461

Enzymes in Fats and Oil Industry

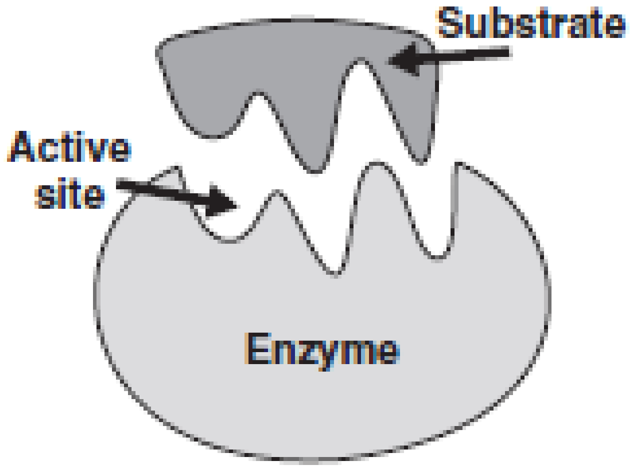
Dr. Hasene KESKİN ÇAVDAR

ENZYMES

- Enzymes are biological catalysts
- They are organic thermolabile catalysts that increase the chemical reaction rate without change.
- They accelerate the rate of chemical reaction without being consumed in the reaction.

Chemistry of Enzymes

- Enzymes are generally globular proteins, having a size range from just 60 to more than 2500 amino acids.
- Most enzymes are much larger than the substrates they act on. It is therefore only a small part of the enzyme is directly involved in catalysis.
- This small section is called the active site and this site usually contains not more than a few (3–4) amino acids.



CHEMISTRY of ENZYMES

- All enzymes are proteins in nature except ribozymes.

- Enzymes are classified into two types:

1. Simple protein enzymes
2. Complex protein enzymes

CHEMISTRY of ENZYMES

1. Simple Protein enzymes: They are formed of protein only.

2. Complex (conjugated) Protein : They are formed of protein part and non protein part.

Complex (conjugated) Proteins

- Complex (conjugated) Protein : enzymes formed of two parts:

1. Protein part: called apoenzyme
2. Non- protein: called cofactor

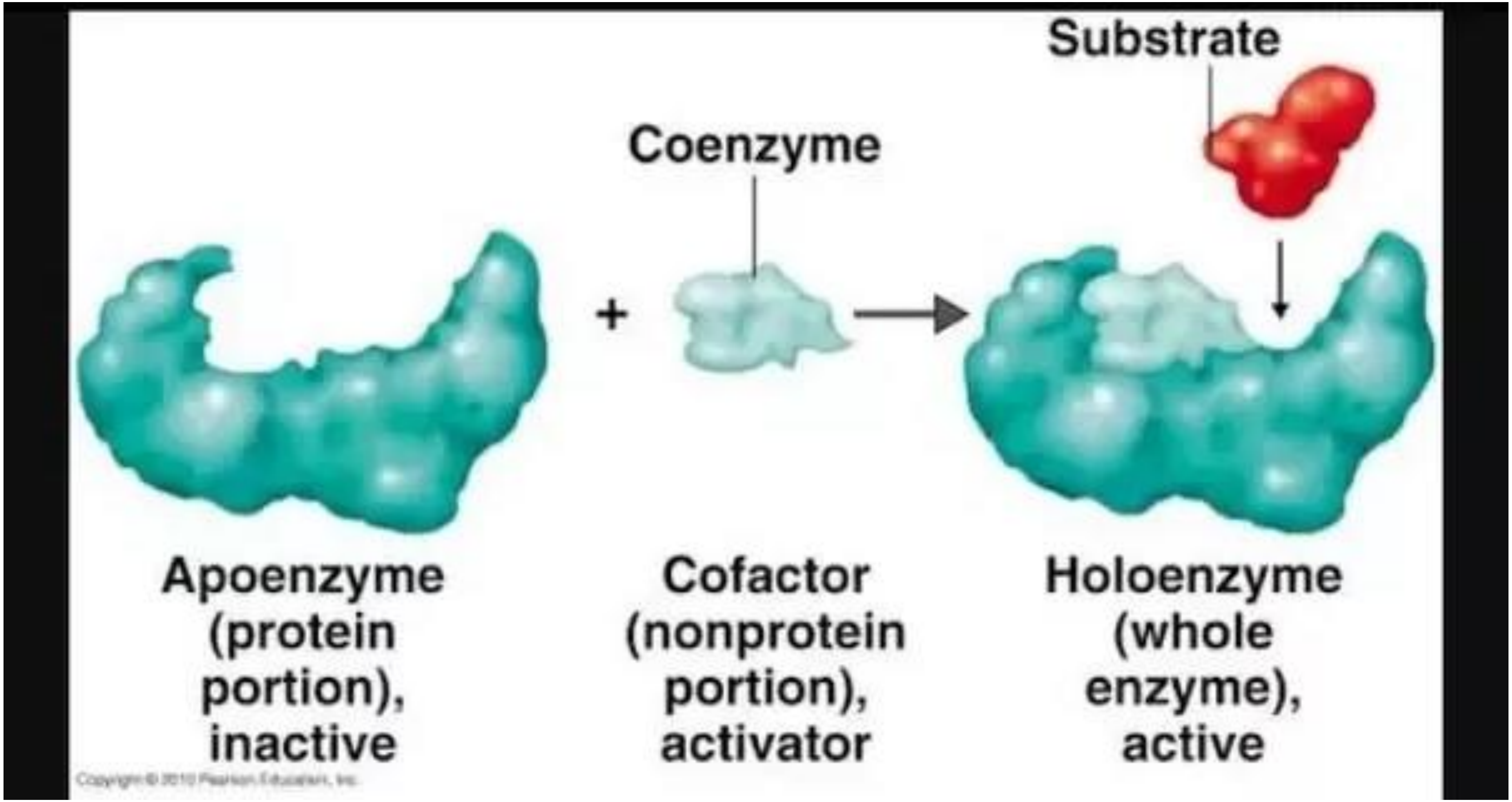
- The whole enzyme is called holoenzyme.

Complex conjugated proteins

The cofactor may be coenzyme or prosthetic group

- Coenzyme: is organic, thermo-labile , loosely attached to enzyme. They are mainly vitamin B derivatives e.g. FAD, NAD.

- Prosthetic group: is inorganic, thermo-stable, firmly attached to enzyme. They are usually metal ions e.g. Ca, Zn


Substrate
Coenzyme
+
Apoenzyme
(protein
portion),
inactive
Cofactor
(nonprotein
portion),
activator
Holoenzyme
(whole
enzyme),
active

ENZYMES

Key definitions:

Enzymes are proteins that accelerate chemical reactions.

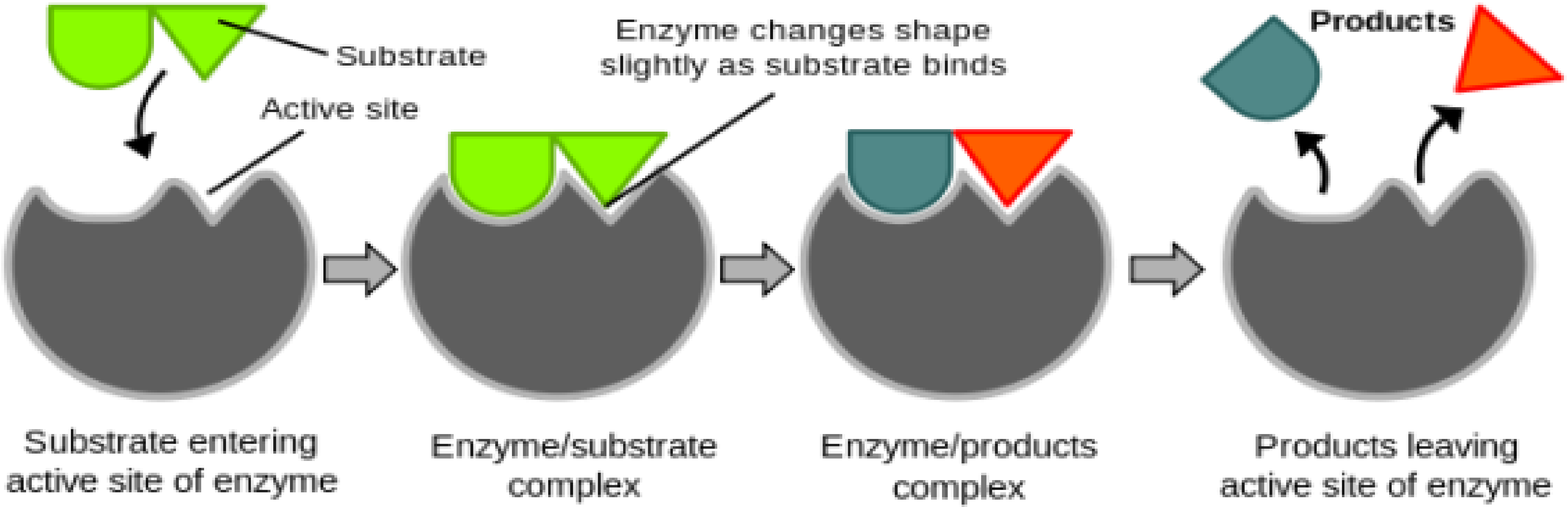
Substrates: The molecules present at the beginning of the reaction are called substrates. Reactant which binds to enzyme

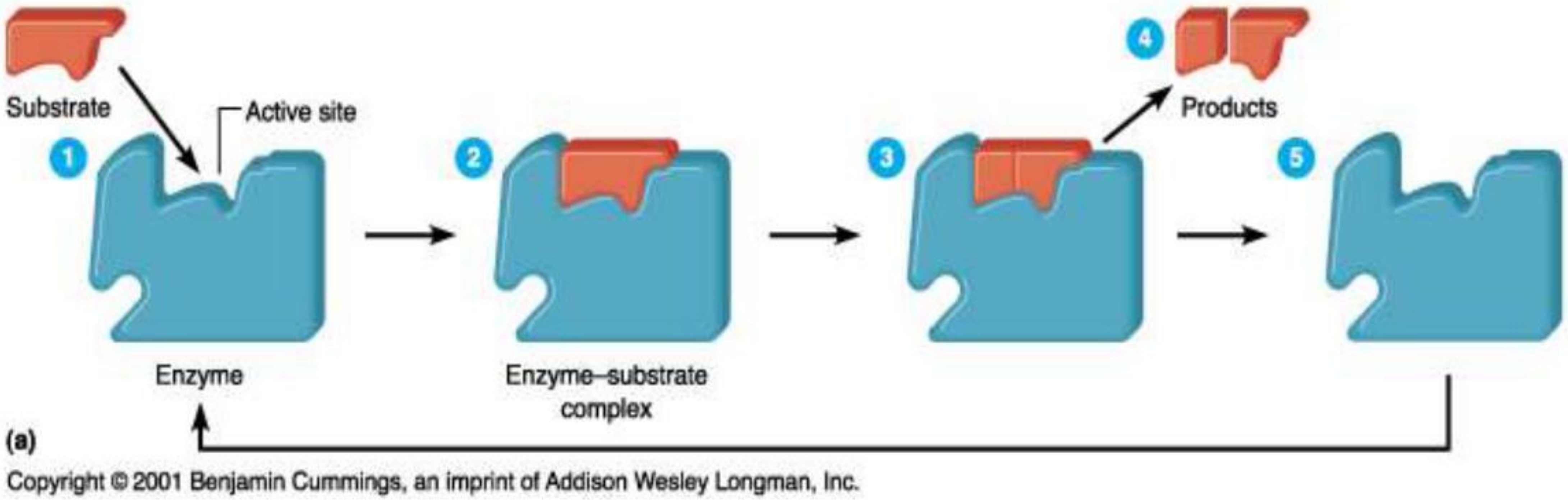
Product: Enzymes convert substrates into different molecule

Active site: A restricted region of an enzyme molecule which binds to the substrate. It is formed from amino acids sequences in the polypeptide chain .

Mechanisms

- Enzymes are very **specific** (fit to certain reaction only). To catalyse a reaction, enzyme molecule and substrate molecule need to meet and joint together by a temporary bond





Mechanism

- 1. Substrate approaches active site
- 2. Enzyme-substrate complex forms
- 3. Substrate transformed into products
- 4. Products released
- 5. Enzyme recycled

- 1- The substrate (S) binds to the enzyme (E) at its active catalytic site to form activated intermediate enzyme substrate complex (ES).

- 2- The activated complex (ES) cleaved to the products (P) and the original enzyme (E)

$$E + S \leftrightarrows ES \rightarrow E + P$$

Theories of enzyme substrate binding

1. **Lock and key theory: “key fits into lock”**

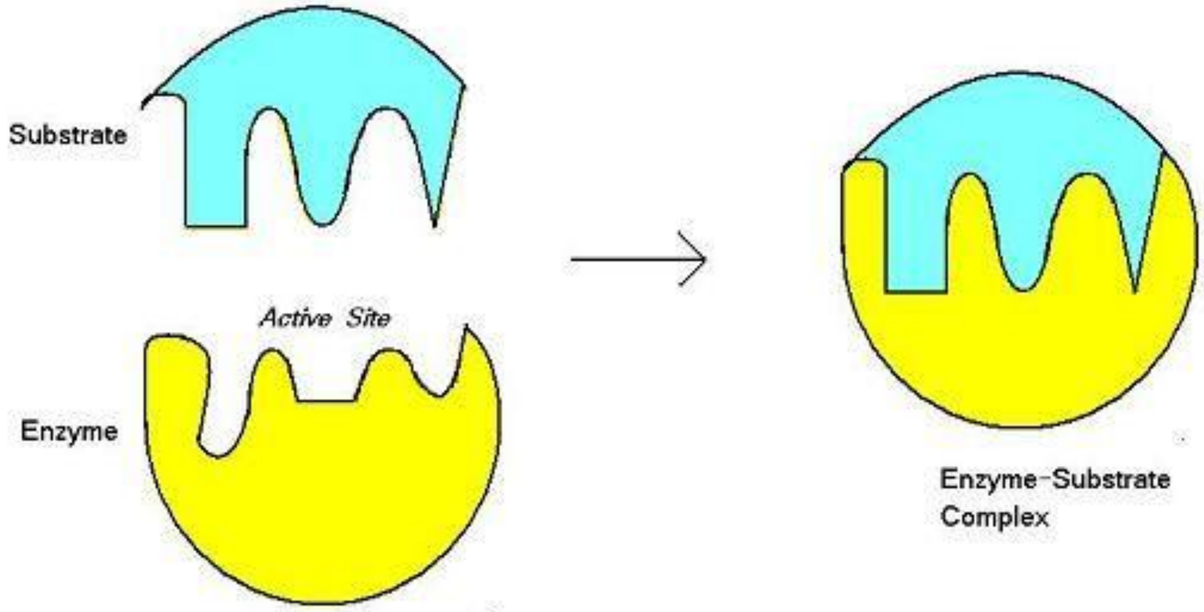
The **catalytic site** of the enzyme has a shape that is **complementary (fit)** to the shape of the **substrate**.

The substrate fits in this catalytic site in a similar way to lock and key. The key will only fits its own lock .

Theories of enzyme substrate binding

2. Induced fit model:

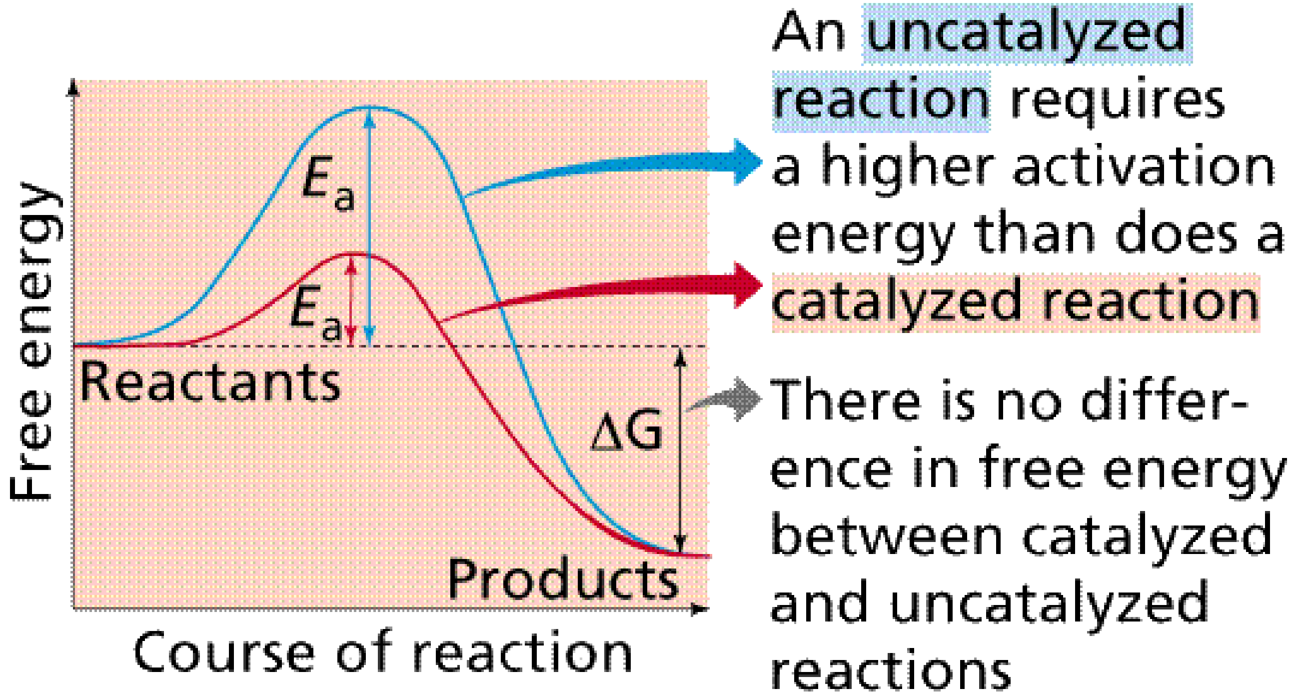
In this model, the enzyme changes shape on substrate binding for best fitting



Induced-fit Model. - The enzyme active site forms a complementary shape to the substrate after binding.

ENZYME ACTION

- Enzymes increase the rate of reaction
- Like all catalysts, enzymes work by lowering the activation energy for a reaction.
- Catalysts, like enzymes, act by lowering the energy difference between the reactants (A, B) and the transition state. This lowers the activation barrier for the reaction, allowing it to proceed more rapidly.



Nomenclature Of Enzymes

- **There are many methods for naming enzymes:**

1. The **old trivial name** as **pepsin** and **trypsin.**

2. The name of **substrate and the suffix – ase**

- Maltose-maltase
- Sucrose-sucrase
- Lactose-lactase
- Protein-protease
- Lipids-lipase

3. Two words, one for the **substrate** and the other for the **type of reaction** e.g. **Succinate dehydrogenase, pyruvate decarboxylase** and **glutamine synthetase**.

Enzyme Code (EC):

- Each enzyme has a numerical code which is formed of **four digits** separated by dots:
- The **first digit** denotes the **class (reaction type) of the enzyme.**
- The **second digit** denotes the **functional group** upon which the enzyme acts.
- The **third digit** denotes the **coenzyme.**
- The **fourth digit** denotes the **substrate.**

Enzyme Classification Based on Reaction Types

Enzymes can be classified by the kind of chemical reaction catalyzed. Officially, six groups of enzymes have been classified:

Class	Type of Reaction Catalyzed	Example
Hydrolase	Hydrolysis (catalyze the hydrolysis of various bonds)	Lipase-breaks down lipids
Isomerase	Rearrangement of atoms within a molecule (neither catabolic nor anabolic)	Glucose-isomerase
Ligase or polymerase	Join two or more chemicals together	Acetyl-CoA synthetase
Lyase	Splitting a chemical into smaller parts without using water	Desmolases
Oxidoreductase	Catalyze oxidation/reduction reactions	Oxidases or dehydrogenases
Transferase	Transfer a functional group from one molecule to another	Hexokinase

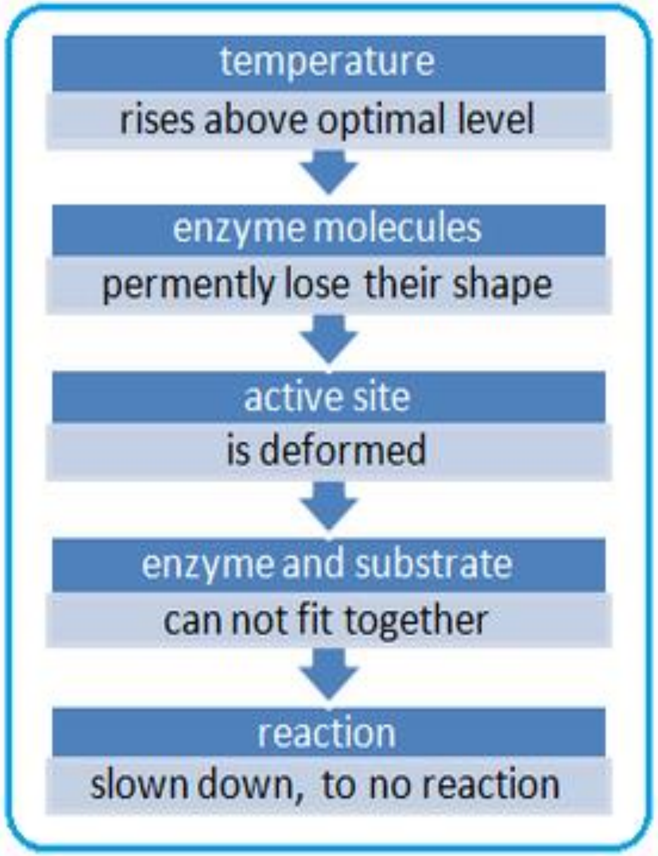
FACTORS AFFECTING ENZYME ACTIVITY

Several factors affect the rate at which reactions proceed, such as

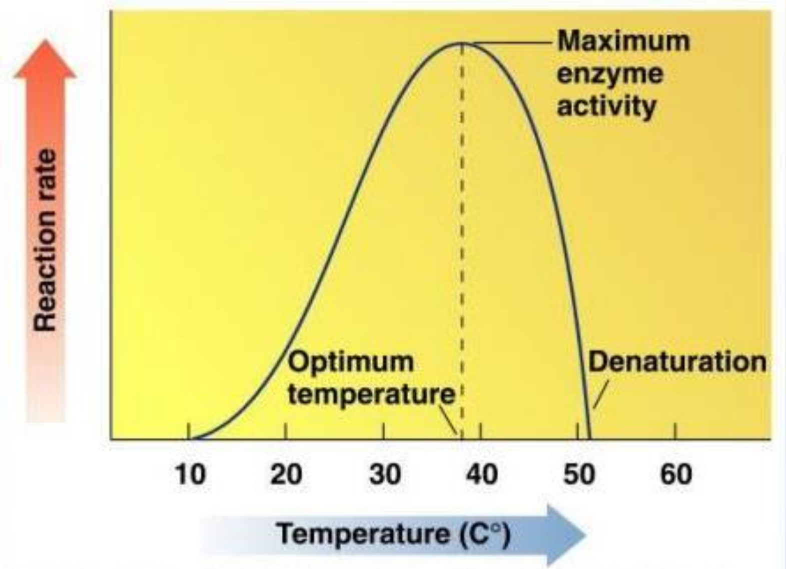
- temperature,
- pH,
- enzyme concentration,
- time,
- substrate concentration and
- the presence of any inhibitors or activators.

FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- *Effect of Temperature*



- The rate of an enzyme-catalyzed reaction increases as the temperature is raised.
- However, very **high** temperatures **denature** enzymes.
- Most animal enzymes rapidly become denatured at temperatures above 40°C.
- Enzymes are most active at on optimum temperature



Timberlake, *General, Organic, and Biological Chemistry.* Copyright © Pearson Education Inc., publishing as Benjamin Cummings

- **The effect of temperature on reaction rate is due to:**

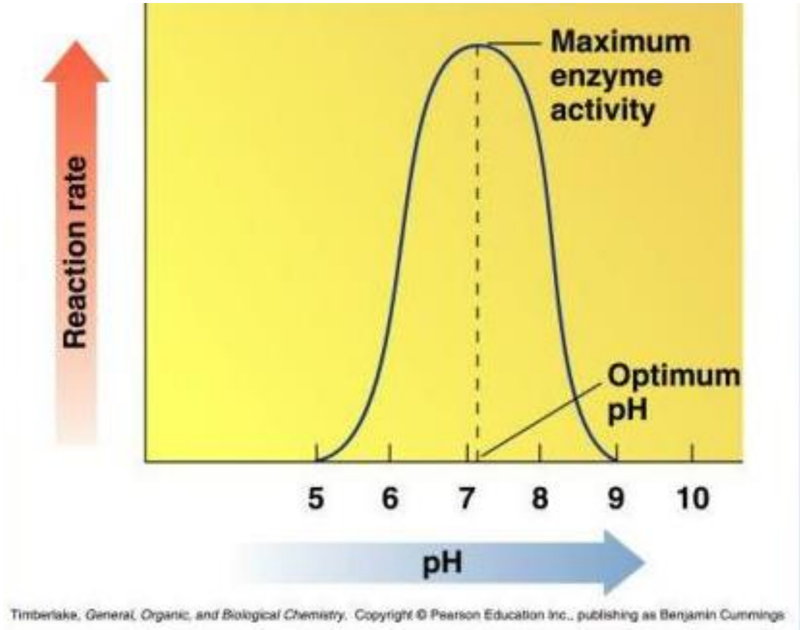
1- Increase of temperature **increase the initial energy** of substrate and thus **decrease the activation energy**

2- **Increase of collision** of molecules: more molecules become in the bond forming or bond breaking distance.

FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- *Effect of pH*

- The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH.
- Extremely high or low pH values generally result in complete loss of activity for most enzymes.
- An extreme pH can **denature** enzymes – the active site is deformed permanently.

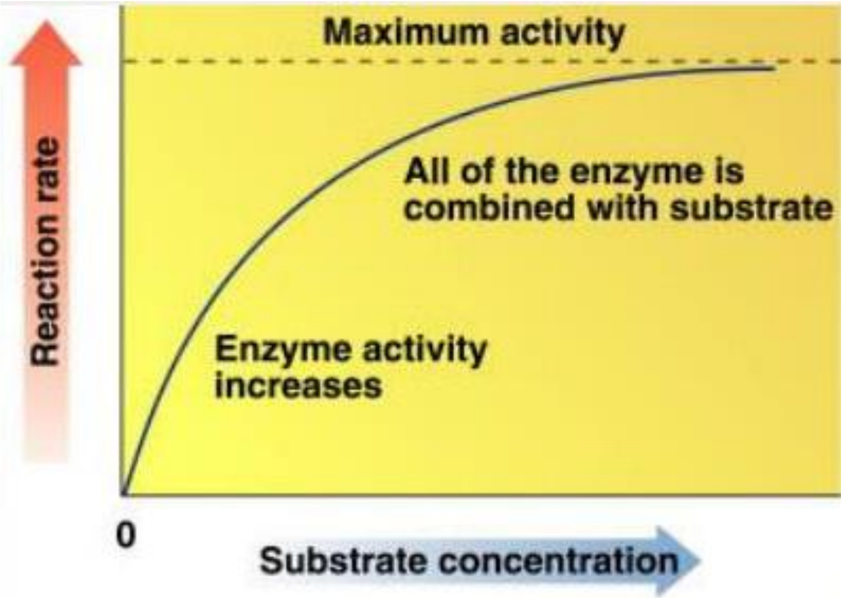


Enzymes	Substrate	End products	Location	pH
Salivary amylase	starch	Maltose Glucose	mouth	6.8
Pancreatic lipase	fat	Fatty acids, Glycerol	duodenum	9.0
Protease	protein	Amino acids	stomach	2.0
			duodenum	9.0

FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- *Effect of Substrate Concentration*

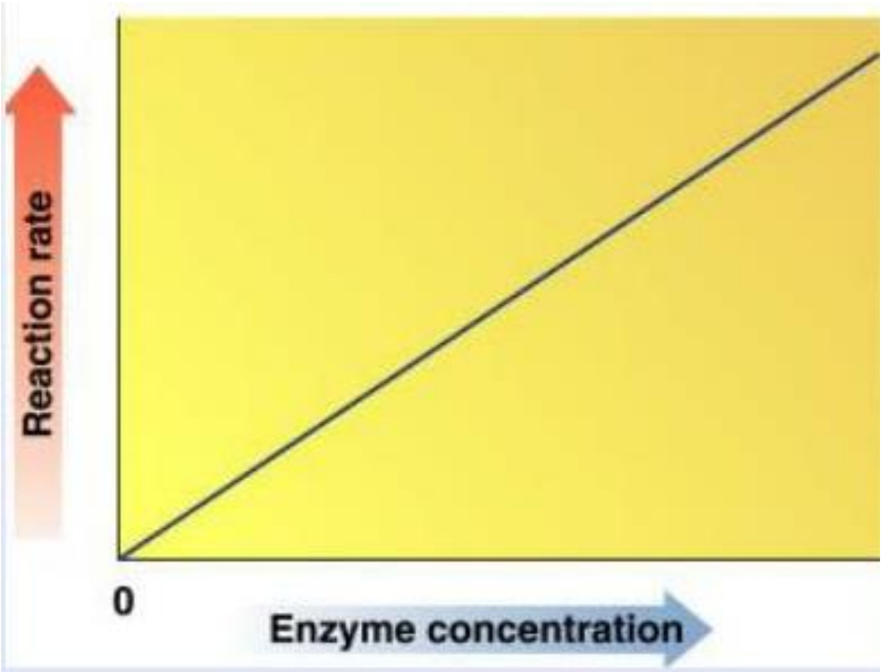
- If the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity



FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- *Enzyme Concentration*

- As the concentration of enzyme increases, rate of reaction will also increase (at constant substrate concentration)



FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- *Effect of time*

In an enzymatic reaction, **the rate of reaction is decreased by time.**

Because of the reasons

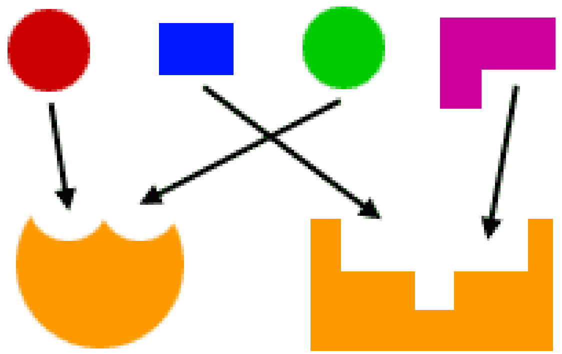
1. The **decrease in substrate** concentration.

2. The accumulation of the **end products**.

3. The **change in PH** than optimum PH.

Specificity of Enzymes

- *What is enzyme specificity?*
    - Ability of an enzyme to choose exact substrate
    - It is a molecular recognition mechanism
    - Recogntion and specificity is based on structural complemetarity



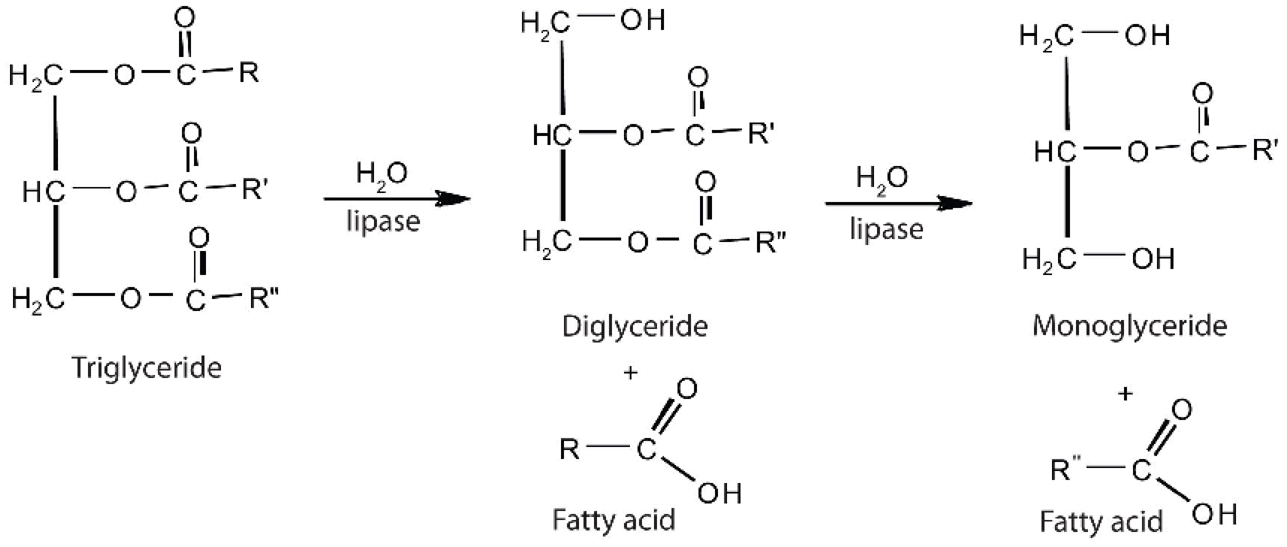
The specificity of enzymes is determined by complementary shape, charge, hydrophilic/ hydrophobic characteristics of the substrates and their three-dimensional organization.

Specificity of enzymes

- The specificity of enzymes is one of their unique properties.
- They are specific not only to the reactions they catalyze, but also to the substrates they utilize.
- Therefore, they may be categorized based on their different exhibited specificities, as *absolute specificity*, *group specificity*, *linkage specificity*, and *stereospecificity.*

Specificity of Enzymes (Continued)

(1) Absolute specificity: Enzymes exhibit absolute specificity act only on one substrate or one reaction

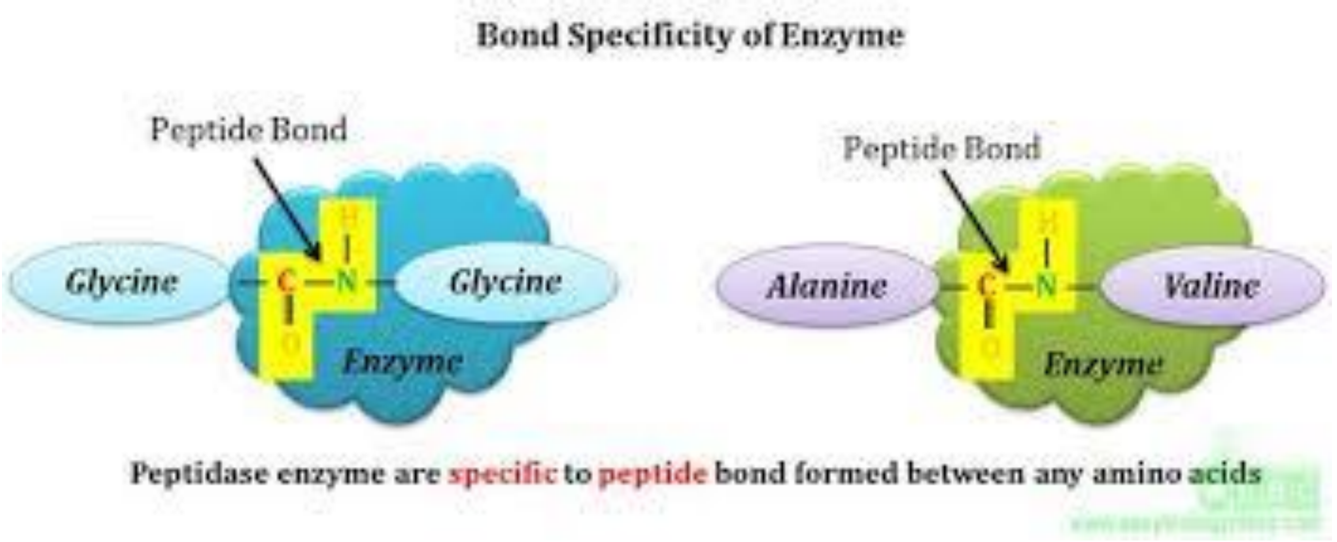


Specificity of Enzymes (Continued)

(2) **Group specificity**: group specific enzymes act only on molecules that have specific functional groups, such as amino, phosphate or methyl groups.

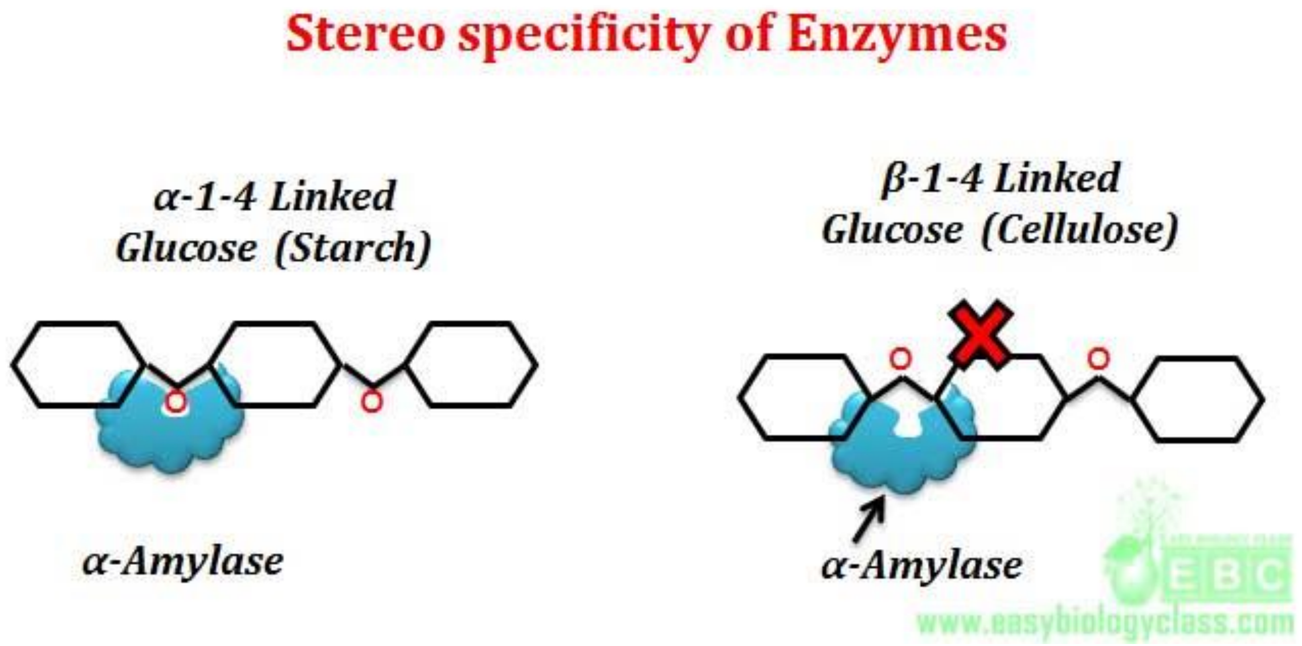
Specificity of Enzymes (Continued)

(3) **Linkage specificity**: such enzymes act on chemical bonds of certain nature, regardless of the rest of the molecular structure.



Specificity of Enzymes (Continued)

(4) **Stereochemical specificity**: stereospecific enzymes act only on a particular steric or optical isomer and not on their isomeric counterparts.



INDUSTRIAL ENZYMES

- Enzymes are highly efficient biocatalysts researched for industrial-scale catalysis because of their several distinct advantages in comparison with chemicals catalysts
  - The ability to work under milder reaction conditions
  - Product selectivity
  - Catalysts
  - Lower environmental and physiological toxicity.
- Industrial enzymes may be derived from a wide variety of **plant, animal or microbial sources**, although most production processes rely on microbial sources. However, over the years, advancements in biotechnology have resulted in newer and more highly efficient varieties of enzymes.

INDUSTRIAL ENZYMES (Continued)

- Microbes are preferred to plants and animal as industrial enzyme source for the following reasons:

1. Higher levels.

2. Higher purity (% enzyme protein vs. % other components).

3. Cheaper production due to the above.

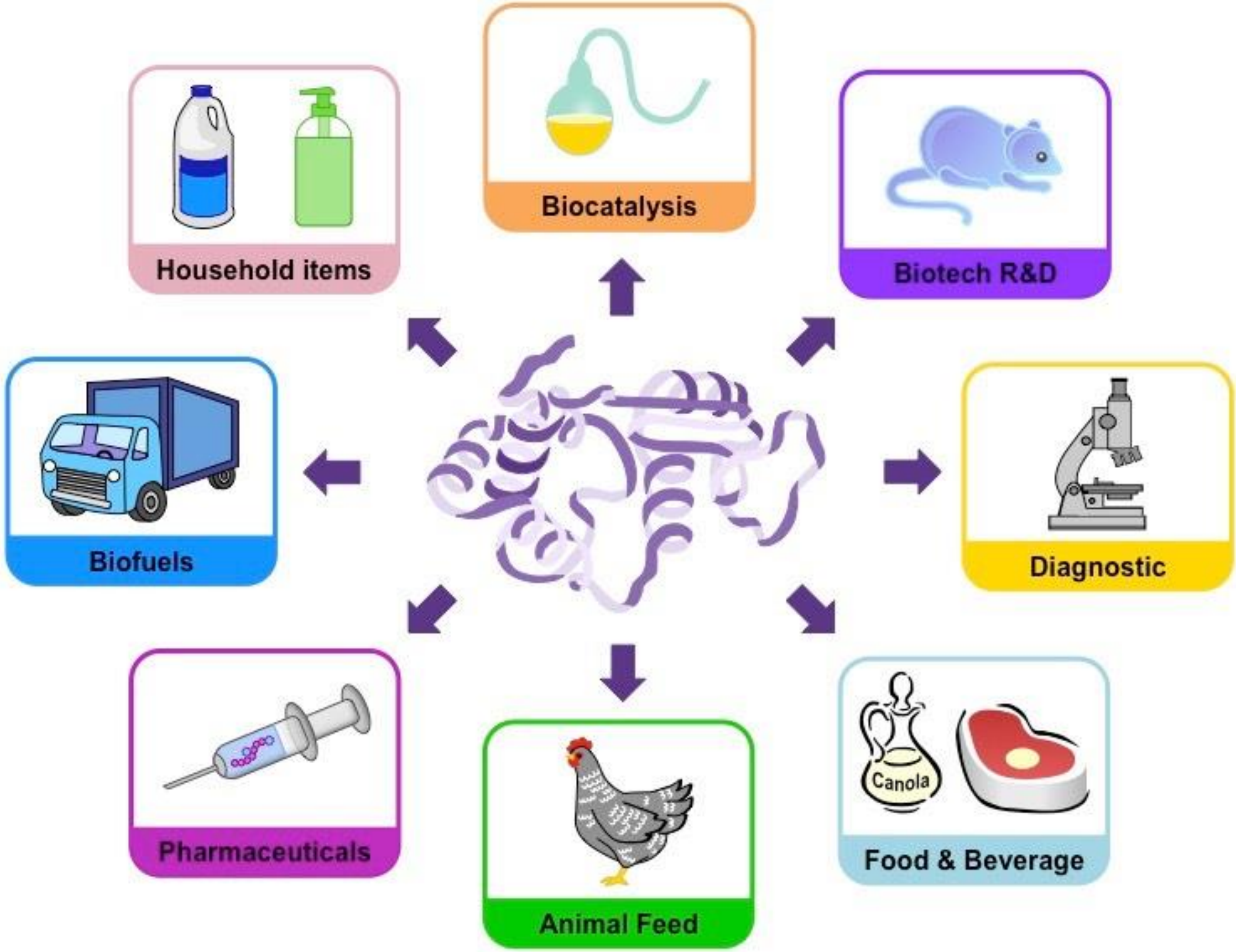
4. Their enzyme contents are more predictable and controllable

5. Plant and animal tissues contain more potentially harmful materials than microbes

Industrial Enzymes

- Commodity enzymes
  - ✓ High volume (tonnes p.a)
  - ✓ Low purity (but not necessarily so)
  - ✓ Low cost (e.g. $5-40 per kg)
  - ✓ Low profit margins
- Speciality enzymes
  - ✓ Low volume (g – kg)
  - ✓ High purity
  - ✓ High cost ($5 – 10,000 per g)
  - ✓ High profit margins

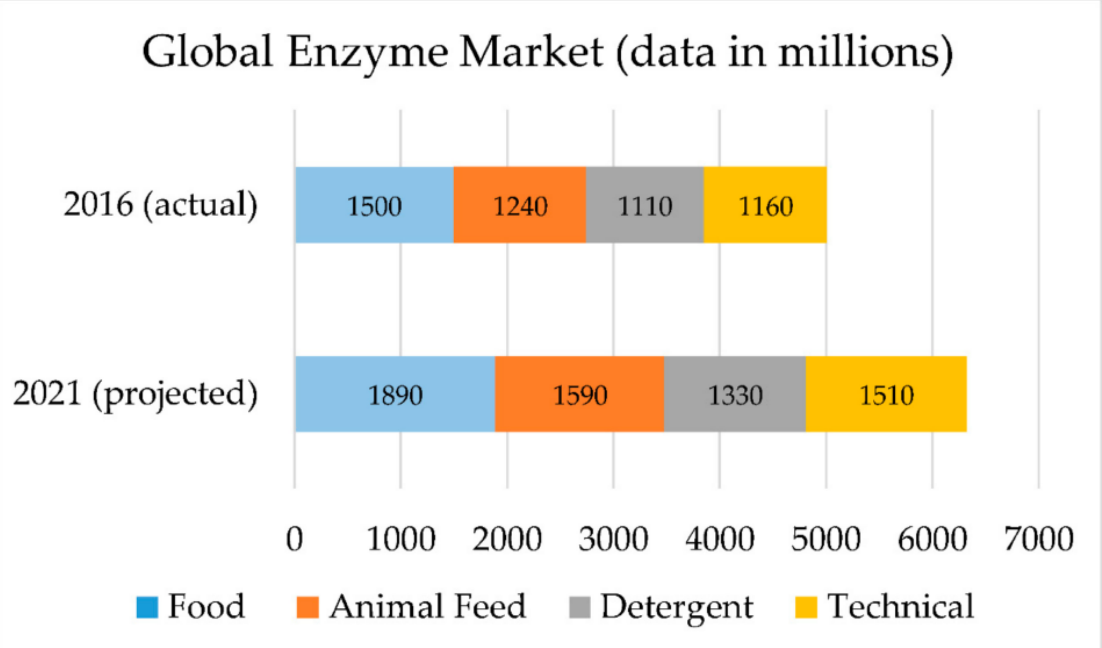
INDUSTRIAL ENZYMES (Continued)



INDUSTRIAL ENZYMES (Continued)

https://www.alliedmarketresearch.com/enzymes-market (27.02.2018)

INDUSTRIAL ENZYMES (Continued)



- According to Business Communication Company (BCC) Research, the global enzyme market is projected to grow from $5.01 billion in 2016 to $6.32 billion in 2021

INDUSTRIAL ENZYMES (Continued)

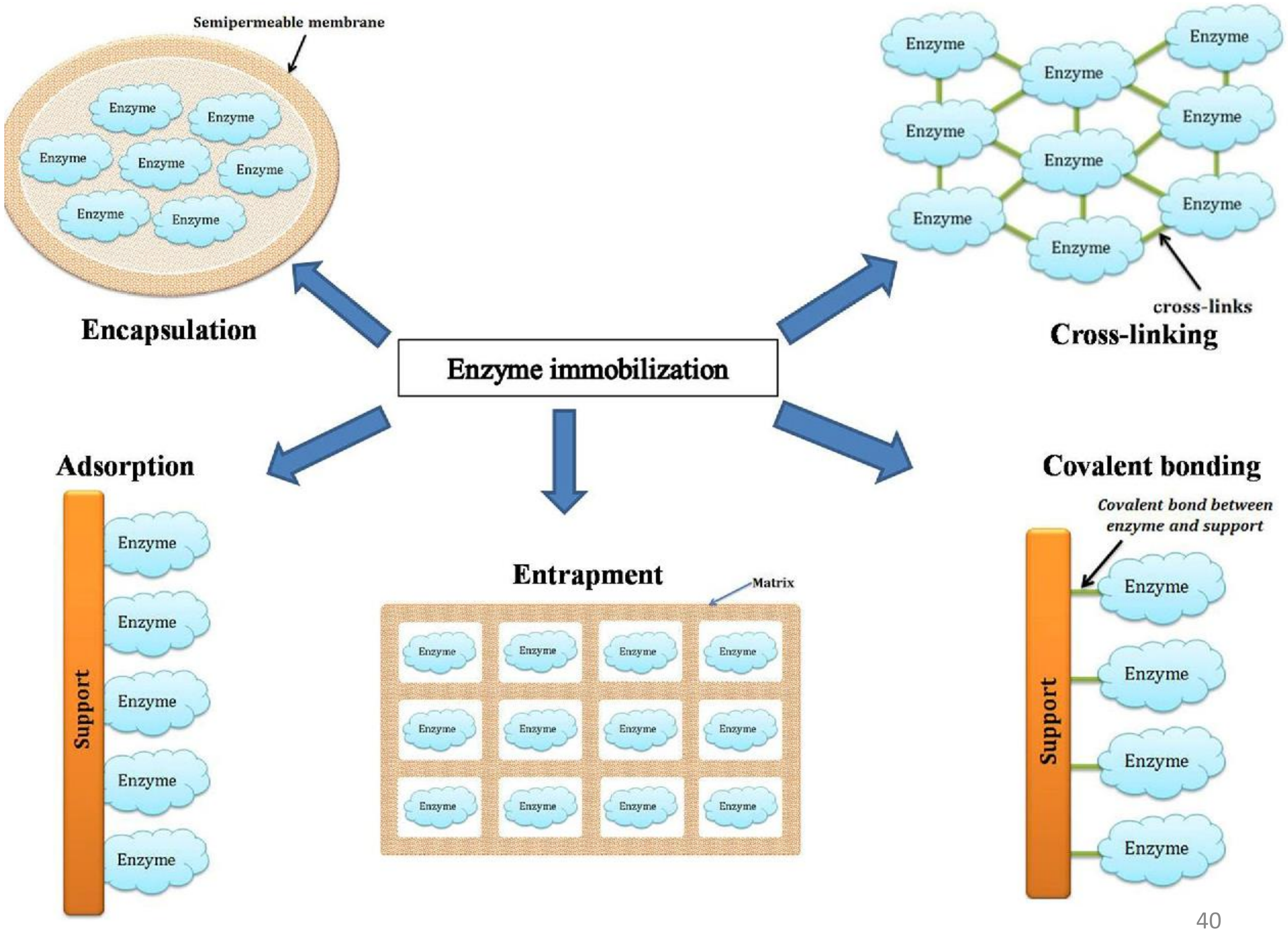
Industrial applications of enzyme catalysis

Sector	Enzymes	Applications
Pharmaceuticals	Nitrile hydratase, transaminase, monoamine oxidase, lipase, penicillin acylase	Synthesis of intermediates for production of active pharmaceutical ingredients
Food Processing	Trypsin, amylase, glucose isomerase, papain, pectinase	Conversion of starch to glucose, production of high fructose corn syrup, production of prebiotics, debittering of fruit juice
Detergent	Protease, lipase, amylase, cellulase	Stain removal, removal of fats and oils, color retention,
Biofuels	Lipase, cellulase, xylanase	Production of fatty acid methyl esters, decomposition of lignocellulotic material for bioethanol production
Paper and Pulp	Lipase, cellulase, xylanase	Removal of lignin for improved bleaching, improvement in fiber properties

INDUSTRIAL ENZYMES (Continued)

- In industry, generally immobilized enzymes are used.
- Immobilized enzyme is either physically entrapped or covalently bonded by chemical means to an inert insoluble matrix or carrier. This matrix or carrier protects enzyme from degradation and deactivation.
- Enzyme immobilization is a process of confining the enzyme to a solid support over which a substrate is passed and converted to products.

INDUSTRIAL ENZYMES (Continued)



INDUSTRIAL ENZYMES (Continued)

- **Advantages of Immobilized Enzymes:**
  - **Attachment to polymers/ matrix causes repetitive use of enzyme**
  - **Immobilized enzymes are usually more stable compared to free enzyme (e.g. pH, temperature..)**
  - **Easy separation of the enzyme from the product**
  - **Ability to stop the reaction rapidly by removing the enzyme from the product**
  - **Product is not contaminated with the enzyme**
  - **Reduces the effluent disposal problems**
  - **Cost effective**

INDUSTRIAL ENZYMES (Continued)

- **Disadvantages of Immobilized Enzymes:**
  - **Additional cost**
  - **Loss or reduction in activity**
  - **Diffusional limitation problems (Diffusion of substrate to enzyme)**

Determining the appropriateness of implementing immobilized biocatalysis on an industrial scale requires a critical evaluation of both technical and economic factors as well as of the process in consideration.

ENZYMES IN FOOD INDUSTRY

- Food processing combines raw food ingredients to produce marketable food products that can be easily prepared and served to consumer.

- Traditional chemical synthesis routes are not viable for food products due to reagent toxicity and complex reaction chemistries that result in unfavorable process economics.

- Their high substrate specificity, rapid reaction rates, and ability to work effectively at relatively low temperatures make them ideal for food processing.

Sources of Enzymes in Food Industry

- Enzyme preparations used in food industries are obtained from animal, plant, or microbial sources.
- They may be available in liquid, semiliquid, dry, or an immobilized form.

Enzyme Applications in Food Industry

- In food production, enzymes have a number of advantages.

(1) Enzymes are alternatives to traditional chemical-based technology. Enzymes can thus replace synthetic chemical-based technology.

(2) Enviromental performance of processes by lowering energy consumption

(3) Enzymatic processes have fewer side reactions and by-products (waste-products) since they are more specific.

The result is higher quality products and less pollution.

(4) Enzymes can catalyze reactions under very mild conditions, allowing mild processing conditions which do not destroy valuable attributes of foods and food components.

Enzyme Applications in Food Industry (Continued)

- The use of enzymes in industrial processes is often associated with reduced consumption of energy, chemical inputs, and waste streams.

For example, using phospholipase to degum vegetable oil led to a decrease of 44 tons of equivalent CO2 generation per 1000 tons of oil produced, due to improvement in oil yield and a subsequent decrease in feedstock requirements.

Enzyme Applications in Food Industry (Continued)

- ENZYMES USED IN FRUIT JUICE EXTRACTION

- To increase the juice yield
- To clarify the juice
- To break down all insoluble carbohydrates (pectins, hemicelluloses and starch)

Enzymes	Benefits
Pectinase used for maceration of fruits/vegetables prior to extraction Carbohydrase used in the maceration prior to extraction	Increases yield, Faster extraction, Increases sugar extraction, Increasinfg process capacity
Pectinase	Produces clear juice and concentrate

Enzyme Applications in Food Industry (Continued)

- ENZYMES USED IN STARCH INDUSTRY

- Production of high fructose corn syrup and sweeteners

FEEDSTOCK	PRODUCT	ENZYME(S)
STARCH	CORN SYRUPS	α-AMYLASES,PULLULANASE
	GLUCOSE	α-AMYLASE,GLUCOAMYLASE
	FRUCTOSE	α- AMYLASE,GLUCOAMYLASE, GLUCOISOMERASE

Enzyme Applications in Food Industry (Continued)

- ENZYMES IN MILK AND DAIRY PRODUCTS

- Milk is processed and converted into yoghurt, buttermilk, cheese, butter, cream via fermentation.

ENZYMES	FUNCTIONS
Chymosin	Milk coagulation
Proteases	Flavour improvement, decrease ripening time of cheeses
Lipases	Flavour improvement, decrease ripening time of cheeses
Sulphydryl oxidase	Remove cooked flavour
β-Galactosidase	Lactose removal
Proteases	Soyabean milk coagulation

Enzyme Applications in Food Industry (Continued)

- ENZYMES IN BAKING

- They improve the dough and bread quality, leading to improved dough flexibility, stability, loaf volume and crumb structure

	PURPOSE
Amylase	Maximise fermentation purpose;prevent staling
Protease	Improve handling and rheological properties
Glutamyl transferase	Improve dough elasticity;retention of bread softness on storage

Enzyme Applications in Food Industry (Continued)

- ENZYMES IN BREWING

- Employs the fermantation process to produce the wine, beer and sake

ENZYME	PURPOSE
amylase(α and β)	Convert starch to maltose and dextrins
Proteases	Hydrolyze proteins to amino acids;used by yeast to grow
Papain	Chill proofing beer

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