Unit -1Enzymes

Introduction

Life is an intricate meshwork involving a perfect coordination of a vast majority of chemical reactions. Some of these reactions result in synthesizing large molecules, others in cleaving large molecules and all of them either utilize energy or liberate energy. All these reactions occur very slowly at the low temperatures and the atmospheric pressures—the conditions under which living cells carry on their life processes. Yet in the living cells these reactions proceed at extremely high rates. This is due to the presence of some catalysts produced and synthesized inside the body of the organisms.

The term ‘enzyme’ was coined in 1878 by Friedrich Wilhelm Kuhne to designate these **‘biological catalysts**’ that had previously been called 'ferments'. As they quicken most of the chemical reactions occurring in the body,

The name Enzyme *(en* = in; *zyme* = yeast) literally means ‘in yeast’. This was referred to denote one of the most noteworthy reactions wherein the production of ethyl alcohol and carbon dioxide through the agency of an enzyme, the *zymase*, present in yeast takes place. This reaction is most popularly known as *alcoholic fermentation.*

Definition of enzyme

- Sumner and Myrback (1950) have beautifully defined the enzymes as "simple or combined proteins acting as specific catalysts. They are soluble, colloidal molecules which are produced by living cells. All enzymes are globular proteins with a complex 3D structure, capable of binding substrate molecules to a part of their surface.
- The enzyme may be defined as a thermo labile organic catalyst, synthesized by a living cell, but capable of functioning in the absence of living cell. The enzyme being a catalyst increases the rate of bio-chemical reactions without becoming a part of the product or not consumed in the reactions.
- Enzymes are biological catalysts, which accelerate the rate of biochemical reactions without changing themselves.

Co-enzyme, Apoenzyme and Holoenzyme

Enzymes are proteins or proteins combined with other chemical groups. The low molecular weight organic molecule associated with proteins in the enzyme is called **co-enzyme.** The protein portion is called **apoenzyme.** Apoenzyme and coenzyme together form complete enzyme called **holoenzyme.**

Apoenzyme	+	Co-enzyme	=	Holoenzyme
(Protein part)		(Organic part)		(Complete Enzyme)
(Inactive)		(Inactive)		(Active)

Endoenyzmes and Exoenyzmes.

Most of the enzymes usually act within the cells in which they are produced and hence are called intracellular enzymes or **endoenzymes**, *e.g.* most of the plant enzymes. As these enzymes catalyze the metabolic reactions of the cell, they are also referred to as *metabolic enzymes.*

On the other hand, certain enzymes which are liberated by living cells catalyze useful reactions outside the cell in its environment and hence are known as extracellular enzymes or **exoenzymes**, *e.g.,* enzymes found in bacteria, and fungi. They act chiefly as digestive enzymes, catalyzing the breakdown of complex substances to simpler ones which can readily be absorbed by the cell.

❖ Important characters of enzymes

- ❖ All enzymes except 'ribozyme' are protein or protein combined with other chemical group.
- ❖ As being an organic catalyst, it increases the rate of cellular reactions.
- ❖ Each enzyme has a unique characteristic shape, specificity and function.
- ❖ It provides a reaction site for substrate.
- ❖ It combines with substrate forming an enzyme substrate complex (Es complex) as an intermediate but does not convert or does not integrate in the product formed.
- ❖ As it is protein in nature, the activity is affected by different environmental factors like pH, temperature, etc.

- ❖ They have high catalytic efficiency and require mild reaction conditions.
- ❖ They cannot pass through semi permeable membrane.
- ❖ Enzymes are bulky, molecular weights range from 10,000 to million.
- ❖ The enzyme activity can be regulated, may be by feedback inhibition or at genetic level.

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- ❖ **Properties of enzymes**

1. Colloidal Nature.

Enzyme molecules are of giant size. Their molecular weights range from 12,000 to over 1 million. They are, therefore, very large compared with the substrates or functional group they act upon.On account of their large size, the enzyme molecules possess extremely low rates of diffusion and form colloidal systems in water. Being colloidal in nature, the enzymes are nondialyzable although some contain dialyzable or dissociable component in the form of coenzyme.

2. Catalytic Nature or Effectiveness.

An universal feature of all enzymatic reactions is the virtual absence of any side products, therefore, just as hemoglobin is precisely tailored to transport oxygen, an enzyme is precisely adapted to catalyze a particular reaction. They act catalytically and accelerate the rate of chemical reactions occurring in plant and animal tissues. They do not normally participate in these reactions or if they do so, at the end of the reaction, they are recovered as such without undergoing any qualitative or quantitative change. This is the reason why they, in very small amounts, are capable of catalyzing the transformation of a large quantity of substrate. Thus, *the catalyse potency of enzymes is exceedingly great.*

3. **Specificity of Enzyme action**

With few exceptions, the enzymes are specific in their action. Their specificity lies in the fact that they may act *(a)* on one specific type of substrate molecule or *(b)* on a group of structurally-related compounds or *(c)* on only one of the two optical isomers of a compound or *(d)* on only one of the two

geometrical isomers. Accordingly, four patterns of enzyme specificity have been recognized:

A. **Absolute specificity**: Some enzymes are capable of acting on only one substrate. For example, *urease* acts only on urea to produce ammonia and carbon dioxide.

$$NH_2CO\,NH_2 + H_2O \longrightarrow 2NH_3 + CO_2$$

Similarly, *carbonic anhydrase* brings about the union of carbon dioxide with water to form carbonic acid

$$H_2O + CO_2 \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

B. **Group specificity:** Some other enzymes are capable of catalyzing the reaction of a structurally-related group of compounds. For example, *lactic dehydrogenase* (LDH) catalyzes the interconversion of pyruvic and lactic acids and also of a number of other structurally-related compounds.

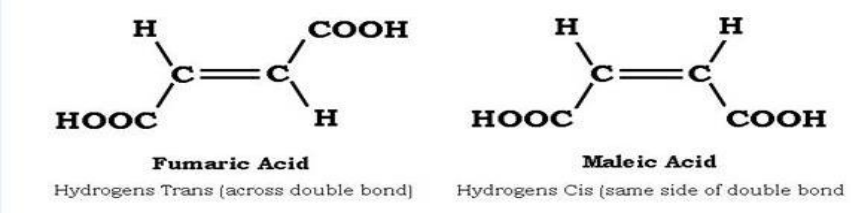
$$\underset{\text{Pyruvic acid}}{CH_3.CO.COOH} + NADH + H \xrightarrow{\text{Lactic dehydrogenase}} \underset{\text{Lactic acid}}{CH_3.CHOH.COOH} + NAD^+$$

C. **Optical specificity:** The most striking aspect of specificity of enzymes is that a particular enzyme will react with only one of the two optical isomers. For example, *arginase* acts only on L-arginine and not on its D-isomer. Similarly, *D-amino acid oxidase* oxidizes the D-amino acids only to the corresponding keto acids.

Although, the enzymes exhibit optical specificity, some enzymes, however, interconvert the two optical isomers of a compound. For example, *alanine racemase* catalyzes the interconversion between L- and D-alanine.

$$\text{L-alanine} \xrightleftharpoons{\text{Alanine racemase}} \text{D-alanine}$$

D. **Geometrical specificity:** Some enzymes exhibit specificity towards the *cis* and *trans* forms. As an example, *fumarase* catalyzes the interconversion of fumaric and malic acids :



It does not react with maleic acid which is the *cis* isomer of fumaric acid or with D-malic acid. The degree of specificity of the enzymes for substrate is usually high and sometimes virtually absolute.

4. Thermolability (Heat sensitivity).

Being proteinaceous in nature, the enzymes are very sensitive to heat. The rate of an enzyme action increases with rise in temperature, the rate being frequently increased 2 to 3 times for a rise in temperature of 10°C. But at higher temperatures, the value of coefficient does not remain constant and decreases rapidly. Above 60°C, the enzymes coagulate and thus become inactivated, because there occurs an irreversible change in their chemical structure. The enzymes of dry tissues like seeds and spores, however, can endure still higher temperatures of about 100° to 120°C.

5. Reversibility of a Reaction.

The enzymes are capable of bringing about reversion in a chemical reaction. The digestive enzymes catalyze the hydrolytic reactions which are reversible. For instance, *lipase*, which catalyzes the synthesis of fat from glycerol and fatty acid, can also hydrolyze them into their component units.

$$\text{Glycerol + Fatty acids} \xrightarrow{\textit{lipase}} \text{Fat} \xrightarrow{\textit{lipase}} \text{Glycerol + Fatty acids}$$

It does not, however, necessarily follow that the same enzyme invariably catalyzes both the synthesis and degradation of a given kind of molecule. For instance, urea is synthesized from arginine by the action of the enzyme,

arginase but is hydrolyzed by action of another enzyme, *urease* to produce ammonia and carbon dioxide.

$$\text{Arginine} \xrightarrow{\textit{arginase}} \text{Urea} \qquad \text{Urea} \xrightarrow{\textit{urease}} NH_3 + CO_2$$

6. pH Sensitivity.

The pH value or the H^+ ion concentration of the medium controls the activity of an enzyme to a great extent. This is mainly related to the degree of dissociation, to the electric charge of the enzyme and, through this, to the formation of the enzyme-substrate complex. Each enzyme, thus, acts best in a certain pH value which is specific to it and its activity slows down with any appreciable change (increase or decrease) in the H^+ ion concentration. In fact, the pH will affect the efficiency of an enzyme and usually there will be a pH at which the activity is at a maximum. The activity will fall off on either side of this value.

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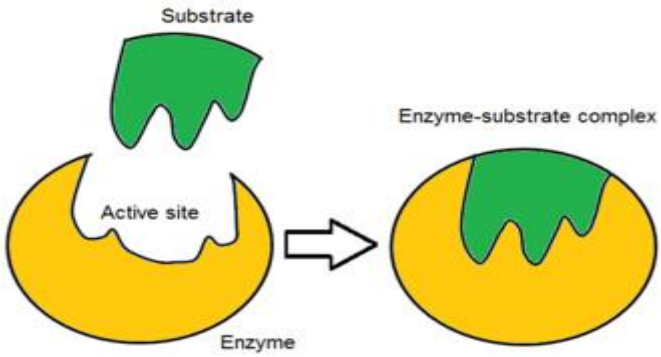
- **ACTIVE SITE**

As the substrate molecules are comparatively much smaller than the enzyme molecules, there should be some specific regions or sites on the enzyme for binding with the substrate. Such sites of attachment are variously called as **'active sites' or 'catalytic sites' or 'substrate sites'.**

Although the enzymes differ widely in their properties, the active site present in their molecule possesses some common features. These are listed below:

1. The active site occupies a relatively small portion of the enzyme molecule.
2. The active site is neither a point nor a line or even a plane but is a 3-dimensional entity. It is made up of groups that come from different parts of the linear amino acid sequence. For example, *lysozyme* has 6 subsites in the active site. The amino acid residues located at the active site are 35, 52, 59, 62, 63 and 107. in case of ribonuclease enzyme, histidine residues 12 and 119 are only required for action while in case of chymotrypsin, histidine residue 57 aspartic acid residue 102, serine residue 195 are playing important role in catalytic function.

3. Usually the arrangement of atoms in the active site is well defined, resulting in a marked specificity of the enzymes. Although cases are known where the active site changes its configuration in order to bind a substance which is only slightly different in structure from its own substrate.
4. The active site binds the substrate molecule by relatively weak forces.
5. The active sites in the enzyme molecules are grooves or crevices from which water is largely excluded. It contains amino acids such as aspaitic acid, glutamic acid, lysine serine etc. The side chain groups like —COOH, —NH_2, —CH_2OH etc., serve as catalytic groups in the active site. Besides, the crevice creates a micro-environment in which certain polar residues acquire special properties which are essential for catalysis.



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❖ **Activation of Enzymes**

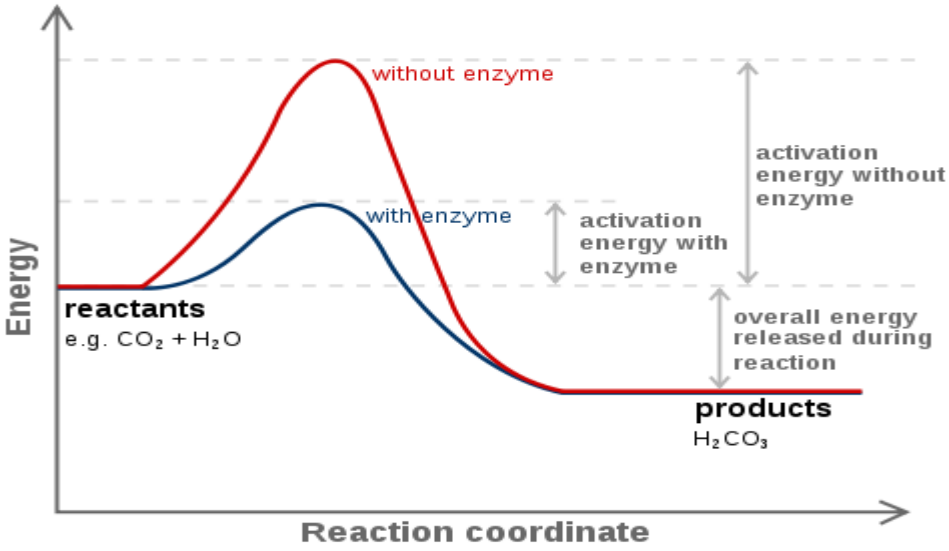
The activation energy required to achieve the transition state is a barrier to the formation of products; it is the minimum, necessary, amount of energy required for a reaction to proceed.

Enzymes lower the activation energy to a point where a small amount of available heat can push the reactants to a transition state.

Enzymes are large proteins that bind small molecules. When bound to an enzyme, the bonds in the reactants can be strained (that is stretched) thereby making it easier for them to achieve the transition state. This is one way for which enzymes lower the activation energy of a reaction. When a chemical reaction involves two or more reactants, the enzyme provides a site where the reactants are positioned very close to each other and in an orientation that facilitates the formation of new covalent bonds. This technique also lowers the needed activation energy for a chemical reaction. Straining the reactants and bringing them close together are two common ways the enzymes use to lower the activation energy. There are other methods that the enzymes use to facilitate

a chemical reaction. Changing the local environment of the reactants is one of these methods. In some cases, enzymes lower the activation energy by directly participating in the chemical reaction. For example, certain enzymes that hydrolyze ATP form a covalent bond between phosphate and amino acid in the enzyme that may have a charge that affects the chemistry of the reactants. This is very temporary condition. The covalent bond between phosphate and the amino acid is quickly broken, releasing phosphate and returning the amino acid back to its original condition.

A catalyst is something that lowers the activation energy; in biology it is an enzyme. The catalyst speeds up the rate of reaction without being consumed; it does not change the initial reactants or the end products.



The graph above shows how the activation energy is lowered in the presence of an enzyme (blue line) that is doing the catalysis, exempflified with the carbon anhydrase reaction. The transition state is usually the most unstable part of the reaction since it is the one with the highest free energy. The difference between the transition state and the reactants is the Gibbs free energy of activation, commonly known as activation energy.

Enzymes (blue line) change the formation of the transition state by lowering the energy and stabilizing the highly energetic unstable transition state. This allows the reaction rate to increase, but also the back reaction occurs more easily.

❖ Mechanism of enzyme action

The basic mechanism by which enzymes catalyze chemical reactions begins with the binding of the **substrate** (or substrates) to the active site on the enzyme. The **active site** is the specific region of the enzyme which combines with the substrate. The binding of the substrate to the enzyme causes changes in the distribution of electrons in the chemical bonds of the substrate and ultimately causes the reactions that lead to the formation of products. The products are released from the enzyme surface to regenerate the enzyme for another reaction cycle.

The **active site** has a unique geometric shape that is complementary to the geometric shape of a substrate molecule, similar to the fit of puzzle pieces. This means that enzymes specifically react with only one or a very few similar compounds.

The specific action of an enzyme substrate can be explained using

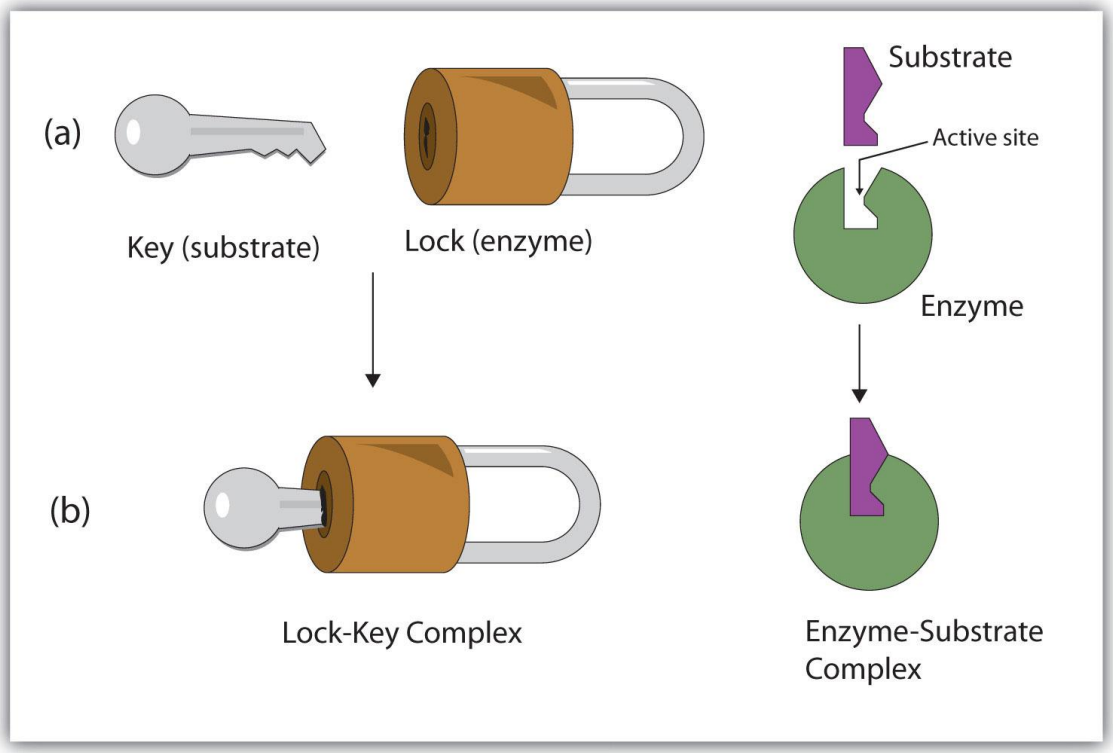
A) Lock and Key model by Fischer
B) Induced Fit Model by Koshland

A) Lock and Key model by Fischer

The specific action of an enzyme with a single substrate can be explained using a **Lock and Key** analogy first postulated in 1894 by Emil Fischer. In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized **key (substrate) fits** into the **key hole (active site)** of the **lock (enzyme)**.

Both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This early model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.

Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized substrate molecules) do not fit into the lock (enzyme). Only the correctly shaped key opens a particular lock.



In fact, the enzyme-substrate union depends on a *reciprocal fit* between the molecular structure of the enzyme and the substrate. And as the two molecules (that of the substrate and the enzyme) are involved, this hypothesis is also known as the **concept of intermolecuiar fit.** The enzyme-substrate complex is highly unstable and almost immediately this complex decomposes to produce the end products of the reaction and to regenerate the free enzyme. The enzyme-substrate union results in the release of energy. It is this energy which, in fact, raises the energy level of the substrate molecule, thus inducing the *activated state.* In this activated state, certain bonds of the substrate molecule become more susceptible to cleavage.

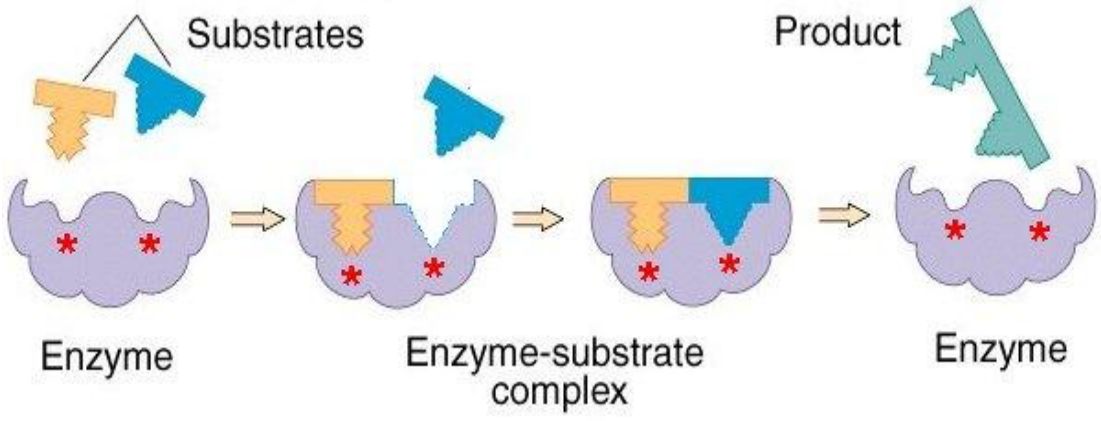
Not all experimental evidence can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theory

1. Active site is a single entity.
2. There is no separate catalytic group.
3. Active site is static.
4. Development of transition state is not considered.
5. It does not visualize the weakening of substrate bonds.
6. It does not explain the mechanism of non activity in case of competitive inhibitor.

B) Induced Fit Model by Koshland

An important but unfortunate feature of Fischer's model is the rigidity of the active site. The active site is presumed to be pre-shaped to fit the substrate. In order to explain the enzyme properties more efficiently, Koshland, in 1958, modified the Fischer's model. Koshland presumed that the enzyme molecule does not retain its original shape and structure. But the contact of the substrate *induces* some configurational or geometrical changes in the active site of the enzyme molecule. Consequently, the enzyme molecule is made *to fit* completely the configuration and active centres of the substrate. At the same time, other amino acid residues may become buried in the interior of the molecule.

Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge distribution is determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proofreading mechanism.



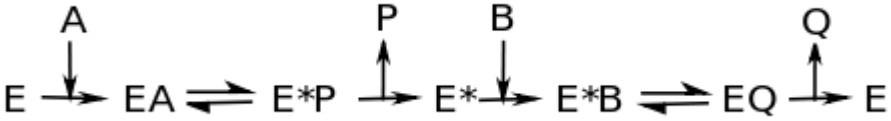
In this mechanism, the substrate wraps over the enzyme bringing about the change in the enzyme molecule. Here substrate determines the final shape of the enzyme. ES complex is formed after binding. ES complex breaks to give

rise to enzyme and products. Enzyme undergoes no change. Exactly to say the active site is modified during Enzyme-Substrate complex formation.

1. Active site is made up of two components.
2. A separate catalytic group is visualized.
3. Active site is not static.
4. It considers the development of transition state of before the reactants undergo change.
5. Catalytic group is believed to weaken the substrate bonds by nucleophilic and electrophilic attacks.
6. It explains the mechanism for non action over complete inhibitor.

❖ **Ping Pong mechanism**

Ping-pong mechanism, also called a double-displacement reaction, is characterized by the change of the enzyme into an intermediate form when the first substrate to product reaction occurs. It is important to note the term intermediate indicating that this form is only temporary. At the end of the reaction the enzyme MUST be found in its original form. An enzyme is defined by the fact that it is involved in the reaction and is not consumed. Another key characteristic of the ping-pong mechanism is that one product is formed and released before the second substrate binds. The figure below explains the Ping Pong mechanism through an enzymatic reaction.



This image shows that as substrate A binds to the enzyme, enzyme-substrate complex EA forms. At this point, the intermediate state, E* forms. P is released from E* , then B binds to E*. B is converted to Q, which is released as the second product. E* becomes E, and the process can be repeated. Often times, E* contains a fragment of the original substrate A. This fragment can alter the function of the enzyme, gets attached to substrate B, or both.

Enzymes with ping–pong mechanisms include some oxidoreductases such as thioredoxin peroxidase, transferases such as acylneuraminate

cytidylyltransferase and serine proteases such as trypsin and chymotrypsin. Serine proteases are a very common and diverse family of enzymes, including digestive enzymes (trypsin, chymotrypsin, and elastase), several enzymes of the blood clotting cascade and many others. In these serine proteases, the E* intermediate is an acyl-enzyme species formed by the attack of an active site serine residue on a peptide bond in a protein substrate.

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❖ Nomenclature and classification of enzymes

With the continuous increase in our knowledge of enzymology, various systems have evolved to name and classify the enzymes, using one or the other criterion as the basis. However, many of the enzymes were known before these systems of naming enzymes were adopted. The names of such enzymes were not changed under the new systems. In this category belong: bromelin, chymotrypsin, diastase, emulsin, papain, pepsin, ptyalin, rennin, trypsin etc.

The earlier names are given on the basis of

A. Substrate acted upon by the enzyme:

The substance upon which an enzyme acts is called the substrate. Duclaux (1883) named the enzymes by adding the suffix *-ase* in the name of the substrate catalyzed. For example, enzymes acting upon carbohydrates were named as *carbohydrases*, upon proteins as *proteinases*, upon lipids as *lipases*, upon nucleic acids as *nucleases* and so on. A few of the names were even more specific like *maltase* (acting upon maltose), *sucrase* (upon sucrose), *urease* (upon urea), *lecithinase* (upon lecithin), *tyrosinase* (upon tyrosine) etc.

B. Type of reaction catalyzed.

The enzymes are highly specific as to the reaction they catalyze. Hence, this has necessitated their naming by adding the suffix-ase in the name of the reaction; for example *hydrolases* (catalyzing hydrolysis), *isomerases* (isomerization), *oxidases* (oxidation), *dehydrogenases* (dehydrogenation), *transaminases* (transamination), *transaldolases* (transaldolation), *transketolases* (transketolation), *phosphorylases* (phosphorylation) etc.

C. Substrate acted upon and type of reaction catalyzed.

The names of some enzymes give clue of both the substrate utilized and the type of reaction catalyzed. For example, the enzyme *succinic dehydrogenase* catalyzes the dehydrogenation of the substrate succinic acid. Similarly, *L-glutamic dehydrogenase* indicates an enzyme catalyzing a dehydrogenation reaction involving *L-glutamic* acid.

D. Substance that is synthesized.

A few enzymes have been named by adding the suffix *-ase* to the name of the substance synthesized, viz., *rhodonase* that forms rhodonate irreversibly from hydrocyanic acid and sodium thiosulphate, and also fumarose that forms fumarate irreversibly from L-malate.

E. Chemical composition of the enzyme.

Based on their chemical composition, the enzymes been classified into following three categories:

1. Enzyme molecule consisting of protein only— *e.g.*, pepsin, trypsin, urease, papain, amylase etc.
2. Enzyme molecule containing a protein and a cation— *e.g.*, carbonic anhydrase (containing Zn^{2+} as cation), arginase (Mn^{+}), tyrosinase (Cu^{2+}) etc,
3. Enzyme molecule containing a protein and a nonprotein organic compound known as prosthetic group—Tauber (1950) has further subdivided them, on the basis of the nature of prosthetic group involved:
    a) Iron prophyrin enzymes— catalase, cytochrome *c* peroxidase I and II.
    b) Flavoprotein enzymes— glycine oxidase, pyruvate oxidase, histamine.
    c) Diphosphothiamin enzymes — p-carboxylase, pyruvate mutase.
    d) Enzymes requiring other coenzymes— phosphorylase, amino acid decarboxylase.

F. Substance hydrolyzed and the group involved.

1. Carbohydrate-hydrolyzing enzymes
    (a) Glycosidases—cellulase, amylase, sucrase, lactase, maltase
    (b) p-glucorinidase

2. Protein-hydrolyzing enzymes
    (a) Peptide bonds
        I. Endopeptidases
            Animals— pepsin, trypsin, rennin
            Plants—papain ficin, bromolin
        II. Exopeptidases—dipeptidase, tripeptidase
    (b) Nonpeptide C—N linkages (amidases)
        urease, arginase, glutaminase
3. Lipid-hyrolyzing enzymes Upases, esterases, lecithinases
4. Other ester-hydrolyzing enzymes
    (a) Phosphatases
    (b) Cholinesterases
    (c) Chlorophyllases
    (d) Sulfatases
    (e) Pectinesterases
    (f) Methylases
5. Oxidation-reduction enzymes
    hydrases, mutases, oxidases, dehydrogenases, peroxidases
6. Miscellaneous enzymes
    catalase, carboxylase, carbonic anhydrase, thiaminase, transpeptidase

G. Over-all chemical reaction taken into consideration.

The chemical reaction catalyzed is the specific property which distinguishes one enzyme from another. In 1961, *International Union of Biochemistry (I.U.B.)* used this criterion as a basis for the classification and naming of enzymes. Although complicated, the I.U.B. system is precise descriptive and informative.
The major features of this system of classification of enzymes are as follows:

(a) The reactions and the enzymes catalyzing them are divided into 6 major classes, each with 4 to 13 subclasses.

Each enzyme name has 2 parts—the first part is the name of the substrate(s) and the second part which ends in the suffix *-ase,* indicates the type of reaction catalyzed. Additional information regarding the nature of the reaction, if needed, is given in parenthesis For example, the enzyme *malate dehydrogenase* catalyzes the following reaction:

L-malate + NAD^+ ⟶ Pyruvate + CO_2 + NADH + H^+

This enzyme has now been designated as L-malate: NAD oxidoreductase (decarboxylating). Each enzyme has been allotted a systemic code number called Enzyme Commission (E.C.) number. The E.C. number for each enzyme consists of a series of numbers at *4* places: the first place numbers representing the major class to which the enzyme belongs, the two median numbers denoting the subclass and the sub-subclass of the enzyme within the major class. The last place number or the fourth digit represents the serial number of the enzyme within the sub-subclass. Thus E.C. 2.7.1.1 represents class 2 (a transferase), subclass 7 (transfer of phosphate), sub-subclass 1 (an alcohol group as phosphate acceptor). The final digit denotes the enzyme, *hexokinase* or *ATP: D-hexose-6-phosphotransferase.* This enzyme catalyzes the transfer of phosphate from ATP to the hydroxyl group on carbon 6 of glucose.

ATP + D-hexose Hexokinase ⟶ ADP + Hexose-6-phosphate

Where no specific category has been created for an enzyme, it is listed with a final figure of 99 in order to leave space for new subdivisions. For example, 4.2.99 refers to "other carbon-oxygen lyases."

The 6 major classes of enzymes with some important examples from some subclasses are described below:

The classes of enzymes

1. Oxidoreductases

2. Transferases

3. Hydrolases

4. Lyases

5. Isomerases

6. Ligases

1. Oxidoreductases.

Oxidoreductases catalyze oxidation-reduction reactions in which one compound is oxidized and another reduced. Oxidation involves removal of hydrogen atom or an electron from a donor and given to acceptor. The

systematic name is based on donor: acceptor oxidoreductase. Recommended name will be dehydrogenase or reductase. Oxidase is used only when O_2 is acceptor.

The second digit in the EC number shows the type of chemical group acting as electron donor: 1 denotes a -CHOH group, 2 an aldehyde or Keto group and so on.

The third digit in the EC number shows the type of acceptor involved: 1 denotes NAD (P), 2 a cytochrome, 3 molecular oxygen and so on.

An enzyme from this class is given to illustrate the classification scheme. Recommended name is lactate dehyrogenase. The systematic name is L-lactate: NAD* oxidoreductase it catalyzes following reaction:

L-Lactate + NAD* Pyruvate + NADH

Here, -CHOH-group of L-lactate acts as the electron donor and NAD^+ as the electron acceptor. The EC number of this enzyme is 1.1.1.27, where the first digit (1) shows class name (oxidoreductase), the second digit

(1) shows the sub-class (electron donor -CHOH group), the third digit (1) shows the sub-sub-class (electron acceptor NAD*) and the fourth digit (27) shows the serial number of the enzyme in its sub-sub-class.

This class comprises the enzymes which were earlier called dehydrogenases, oxidases, peroxidases, hydroxylases, oxygenases etc. The group, in fact, includes those enzymes which bring about oxidation-reduction reactions between two substrates, S and S'.

$$S_{reduced} + S'_{oxidized} \longrightarrow S_{oxidized} + S'_{reduced}$$

More precisely, they catalyze electron transfer reactions. In this class are included the enzymes catalyzing oxidoreductions of CH—OH, C=O, CH—CH, $CH—NH_2$ and CH=NH groups. Some important subclasses are:

1.1 Enzymes acting on CH—OH group of electron donor. For example:

1.1.1.1 Alcohol: NAD oxidoreductase

[Common or Recomonded name, Alcohol dehydrogenase]

This enzyme catalyzes the following reaction:

Alcohol + NAD $\longrightarrow$ Aldehyde or Ketone + NADH + H^+

1.3 Enzymes acting on CH—CH group of electron donor. For example:

1.3.2.2 Acyl-CoA : cytochrome c oxidoreductase

acyl-CoA dehydrogenase

Acyl-CoA + oxidized cytochrome c ⟶ 2, 3-dehydroacyl-CoA + reduced cytochrome c

1.9 Enzymes acting on the heme groups of electron donors. For example:
1.9.3.1 Cytochrome c: O_2 oxidoreductase

cytochrome oxidase

4 reduced cytochrome + O_2 + $4H^+$ ⟶ 4 oxidized cytochrome c + $2H_2O$

1.11 Enzymes acting on H_2O_2 as electron acceptor. For example:
1.11.1.6 H_2O_2: H_2O_2 oxidoreductase

catalase

$$H_2O_2 + H_2O_2 \longrightarrow 2H_2O + O_2$$

2. Transferases

These enzymes transfer groups such as methyl, carboxyl, formyl, glycosyl, acyl and phosphate from a donor to an acceptor. The systematic name is based on donor: acceptor group transferase. The recommended names are formed according to acceptor group transferase or donor group transferase.

The second digit in the EC number indicates the group transferred: 1 denotes a one carbon group, 2 an aldehydic or ketonic group, 3 a glycosyl group and so on, 7 a phosphate group.

The third digit in the EC number gives more information on the group transferred: e.g., sub class 2.1 is subdivided into methyl transferases (2.1.1), hydroxymethyl and formyltransferases (2.1.2) and so on. In the subclass 2.7 the third digit indicate acceptor group for example, 2.7.1 denotes alcohol group, 2.7.2 a carboxyl group as acceptor and so on.

Example : Glucokinase is a recommended name, the systematic name is ATP : D-glucose 6 phosphotransferase. It catalyzes following reaction:

ATP + D-glucose ----- ADP + D-glucose 6-phosphate

In the reaction, phosphate group is transferred from ATP to alcohol group of D-glucose at 6th position. The EC number of this enzyme is 2.7.1.2,

where the first digit (2) shows class name (Transferase), the second digit (7) shows the sub class (phosphate group transferred), the third digit (1) shows the sub-sub-class (alcohol group at sixth position of D-glucose as phosphate acceptor), and the fourth digit (2) shows the serial number of the enzyme in its sub-sub-class.

Enzymes which catalyze the transfer of a group (other than hydrogen) between a pair of substrates, S and S' are called transferases.

$$S—G + S' \longrightarrow S + S'—G$$

In these are included the enzymes catalyzing the transfer of one-carbon groups, aldehydic or ketonic residues and acyl, glycosyl, alkyl, phosphorus or sulfur-containing groups. Some important subclasses are:

2.3 Acyltransferases. For example:

2.3.1.6 Acetyl-CoA : choline O-acetyltransferase

choline acetyltransferase

$$\text{Acetyl-CoA + choline} \longrightarrow \text{CoA + O-acetylcholine}$$

2.4 Glycosyltransferases. For example:

2.4.1.1 α-1, 4-Glucan: orthophosphate glycosyl transferase

phosphorylase

$$(\alpha\text{-1,4-Glucosyl})_n + \text{Orthophosphate} \longrightarrow (\alpha\text{-1,4-Glucosyl})_{n-1} + \alpha\text{-D-glucose-1-phosphate}$$

2.7 Enzymes catalyzing the transfer of phosphorus-containing groups. For example:

2.7.1.1 ATP: D-hexose-6-phosphotransferase

hexokinase

$$\text{ATP + D-hexose} \longrightarrow \text{ADP + D-hexose-6-phosphate}$$

3. Hydrolases.

These enzymes split C-O, C-N, C-C and some other bonds by addition of water. The systematic name always includes hydrolase. In many cases the recommended name is formed by adding -ase to the substrate.

The second digit in the EC number indicates the nature of the bond hydrolysed : 1 denotes acting on ester bonds, 2 acting on glycosyl compounds, 3

acting on ether bonds, 4 acting on peptide bonds, *5* acting on carbon nitrogen bonds, 6 acting on acid anhydrides and so on.

The third digit in the EC number normally specifies the nature of substrate, e.g., in 3.1 the carboxylic ester (3.1.1) and so on.

Example: Adenosinetriphosphatase is a recommended name, the systematic name is ATPphosphohydrolase. It catalyzes following reaction:

In the reaction, the substrate ATP is hydrolysed so as to split the bond (acid anhydride) which allows release of orthophosphate. The EC of this enzyme is 3.6.1.3 where the first digit (3) indicates class name (hydrolase), the second digit (6) indicates the sub-class (acid anhydride), the third digit (1) indicates sub-sub-class (phosphoryl anhydride), and fourth digit (3) indicates the serial number of the enzyme in sub-sub-class,

These catalyze the hydrolysis of their substrates by adding constituents of water across the bond they split. The substrates include ester, glycosyl, ether, peptide, acid-anhydride, C—C, halide and P—N bonds. Representative subclasses are:

3.1 Enzymes acting on ester bonds. For example:

3.1.1.3 Glycerol ester hydrolase

$$\text{A triglyceride} + H_2O \xrightarrow{\textit{lipase}} \text{A diglyceride} + \text{a fatty acid}$$

3.2 Enzymes acting on glycosyl compounds. For example:

3.2.1.23 β-D-galactoside galactohydrolase

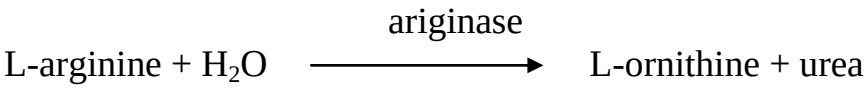
$$\beta\text{-D-galactoside} + H_2O \xrightarrow{\textit{β-galactosidase}} \text{Alcohol} + \text{D-galactose}$$

3.4. Enzymes acting on peptide bonds

Here the classical trivial names (pepsin, trypsin, thrombin, plasmin etc.) have been largely retained due to their consistent long usage and also due to dubious specificities which make systematic nomenclature almost impractical at this time.

3.5 Enzymes acting on C—N bonds, other than peptide bonds. For example:

3.5.3.1 L-arginine ureohydrolase



4. Lyases (Desmolases).

These enzymes break C-C, C-O, C-N and other bonds by elimination and introduce double bonds or add groups to double bonds. The systematic name is formed according to the pattern substrate-group lyase. In case of elimination of water, the recommended names include: decarboxylase, aldolase, and dehydratase. When the reverse reaction is much more important, then synthase is used.

The second digit in the EC number shows the bond broken: 1 denotes carbon-carbon-lyases, 2 carbon-oxygen-lyases and so, on.

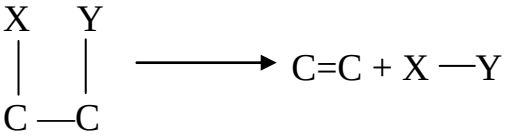
The third digit gives further information on the group eliminated, e.g., in 4.1 the carboxy lyases (4.1.1) the aldehlyde-lyases (4.1.2); in 4.2 the hydro-lyases (4.2.1) and so on.

Example: Fructose diphosphate aldolase is a recommended name. The systematic name is D-fructose 1,6-diphosphate D-glyceraldehyde 3-phosphate lyase. It catalyzes following reaction:

D-Fructose 1,6-diphosphate — D-glyceraldehyde 3-phosphate + dihydroxy acetone phosphate

The EC number of this enzyme is 4.1.2.13

These are those enzymes which catalyze the removal of groups of substrates by mechanisms other than hydrolysis, leaving double bonds.



In these are included the enzymes acting on C—C, C—O, C—N, C—S and C—halide bonds.

Important subclasses include:

4.1 Carbon-carbon lyases. For example:

4.1.2.7 Ketose-1-phosphate aldehyde-lyase

$$\text{ketose-1-phosphate} \xrightarrow{\textit{aldolase}} \text{Dihydroxyacetone phosphate} + \text{aldehyde}$$

4.2 Carbon-oxygen lyases. For example:

4.2.1.2 L-malate hydro-lyase

$$\text{L-malate} \xrightarrow{\textit{fumarase}} \text{Fumarate} + H_2O$$

4.3 Carbon-nitrogen lyases. For example:

4.3.1.3 L-histidine ammonia-lyase

$$\text{L-histidine} \xrightarrow{\textit{histidase}} \text{Urocanate} + NH_3$$

5. Isomerases.

These enzymes catalyze redistribution of chemical groups within a molecule. According to the type of isomerism, they may be called recemases, epimerases, cis-trans-isomerases, isomerases, tautomerases or mutases.

The second digit in the EC number fives the type of isomerism,

e.g., 1 denotes recemases and epimerases, *2* cis-trans isomerases, 3 intramolecular oxidoreductases and so on.

The third digit in the EC number gives the type of substrate e.g., in 5.3 the interconverting aldoses and kctoses (5.3.1), the interconvertingketo and enol group (5.3.2), etc.

Example: Triose phosphate isomerase is a recommended name. The systematic name is D-glyceraldehyde 3-phosphate ketol-isomerase. It catalyzes following reaction:

$$\text{D-glyceraldehydes3-phosphate} \rightleftharpoons \text{Dihydroxyacetonephosphate}$$

The EC number of this enzymes is 5.3.1.1.

These catalyze interconversions of optical, geometric or positional isomers by intramolecular rearrangement of atoms or groups. Important subclasses are:

5.1 Racemases and epimerases. For example:

5.1.1.1 Alanine racemase

L-alanine ⟶ D-alanine

5.2 *Cis-trans* isomerases. For example:

5.2.1.3 *All trans-retinene* 11-*cis-trans* isomerase

$$\text{All trans-retinene} \xrightarrow{\textit{retinene isomerase}} \text{11-cis-retinene}$$

5.3 Intramolecular oxidoreductases. For example:

5.3.1.9 D-glucose-6-phosphate keto-isomerase

$$\text{D-glucose-6-phosphate} \xrightarrow{\textit{glucosephosphate isomerase}} \text{D-fructose-6-phosphate}$$

6. Ligases *(ligare* = to bind) **or Synthetases.**

These enzymes catalyze the reaction in which two molecules are joined at the expense of energy source (usually ATP).The X and Y are substrates. The systematic names are formed on the system X-Y ligase (ADP-forming). The recommended name always contains the term synthetase with product.

The second digit in the EC number indicates the bond formed: 1 denotes C-O bond, 2 the C-S bond, 3 the C-N bond, 4 the C-C bond and so on.

Example: Glutamine synthetase is a recommended name. The systematic name is L-glutamate : ammonia ligase (ADP forming) it catalyzes following reaction:

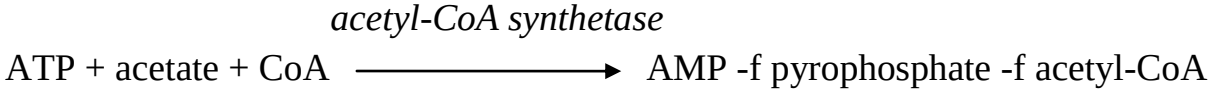
$$\text{ATP + L-glutamate + } NH_3 \rightleftharpoons \text{ADP+ Orthophosphate + L-glutamine}$$

The EC number of this enzyme is 6.3.1.2

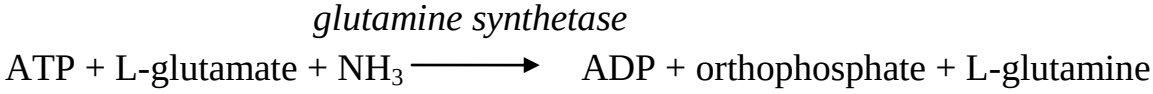
These are the enzymes catalyzing the linking together of two compounds utilizing the energy made available due to simultaneous breaking of a pyrophosphate bond in ATP or a similar compound. This category includes

enzymes catalyzing reactions forming C—O, C—S, C—N and C—C bonds. Important subsclasses are:

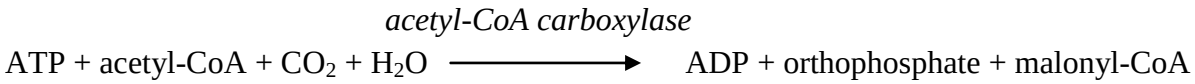
6.2 Enzymes catalyzing formation of C—S bonds. For example:
6.2.1.1 Acetate : CoA ligase (AMP)



6.3 Enzymes catalyzing formation of C—N bonds. For example:
6.3.1.2 L-glutamate : ammonia ligase (ADP)



6.4 Enzymes catalyzing formation of C—C bonds. For example:
6.4.1.2 Acetyl-CoA : CO_2 ligase (ADP)



To date, over 2,000 different enzymes are known, of which the oxidoreductases, transferases and drolases predominate. Because official names are often lengthy, the trivial names of enzymes are neraliy *used* after initial identification.

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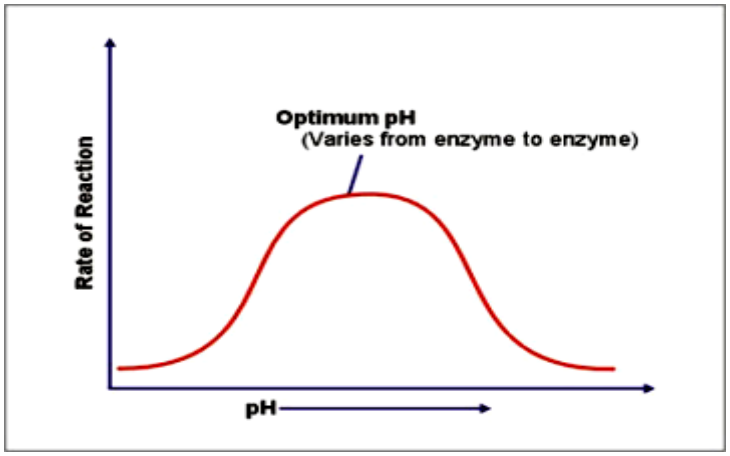
❖ **Factors affecting catalytic activity of enzymes**

Following factorsaffect the catalytic activity of enzymes

1. **pH**
2. **Temperature**
3. **Enyme concentration**
4. **Substrate concentration**
5. **Metal ions**
6. **Time**

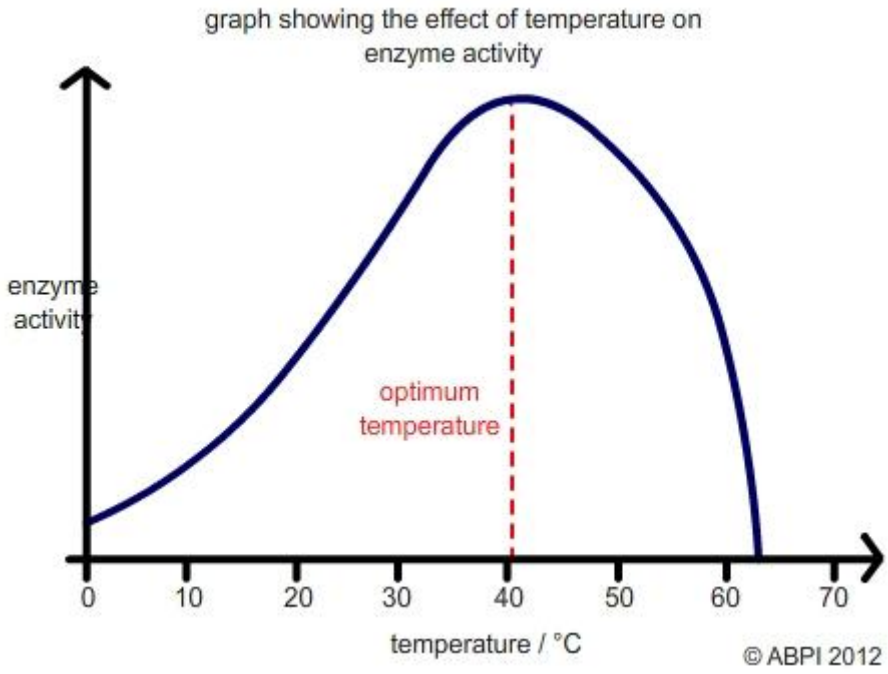
1. pH

- **pH** measures the **Acidity** and **Basicity** of a solution. It is a measure of the **Hydrogen Ion (H^+) concentration**, and therefore a good indicator of the **Hydroxide Ion (OH^-)** concentration. It ranges from **pH1** to **pH14**. **Lower pH** values mean **higher H^+** concentrations and **lower OH^-** concentrations.
- **Acid** solutions have pH values **below 7**, and **Basic** solutions (alkalis are bases) have pH values **above 7**. **Deionised water** is **pH7**, which is termed '**neutral**'.
- H^+ and OH^- Ions are **charged** and therefore **interfere** with **Hydrogen** and **Ionic** bonds that **hold together** an enzyme, since they will be **attracted** or **repelled** by the **charges** created by the bonds. This interference causes a **change** in **shape** of the **enzyme**, and importantly, its **Active Site**.
- **Different enzymes** have **different Optimum pH values**. This is the pH value at which the bonds within them are influenced by H^+ and OH^- Ions in such a way that the **shape** of their **Active Site** is the **most Complementary** to the **shape** of their **Substrate**. At the Optimum pH, the **rate** of reaction is at an optimum.
- Any **change** in **pH above** or **below** the **Optimum** will **quickly** cause a **decrease** in the **rate** of reaction, since **more** of the enzyme molecules will have **Active Sites** whose **shapes** are not (or at least are less) **Complementary** to the **shape** of their **Substrate**.
- **Small** changes in **pH** above or below the **Optimum** do **not** cause a **permanent change** to the enzyme, since the **bonds** can be **reformed**. However, **extreme changes** in **pH** can cause enzymes to **Denature** and **permanently** lose their function.
- Enzymes in different **locations** have **different Optimum pH** values since their **environmental conditions** may be different. *For example, the enzyme Pepsin functions best at around pH2 and is found in the stomach, which contains Hydrochloric Acid (pH2).*



2. Temperature

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. As shown in Figure 13, the reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.



- **Increasing temperature increases** the **Kinetic Energy** that **molecules** possess. In a **fluid**, this means that there are **more random collisions** between molecules per unit time.
- Since enzymes catalyse reactions by **randomly colliding** with **Substrate molecules, increasing temperature increases the rate of reaction,** forming more product.
- However, **increasing temperature** also **increases** the **Vibrational Energy** that molecules have, specifically in this case **enzyme molecules**, which puts **strain** on the **bonds** that **hold** them together.
- As temperature increases, **more bonds**, especially the **weaker Hydrogen** and **Ionic** bonds, will **break** as a result of this strain. Breaking bonds within the **enzyme** will cause the **Active Site** to **change shape**.
- This change in **shape** means that the **Active Site** is **less Complementary** to the **shape** of the **Substrate**, so that it is **less likely** to **catalyse** the reaction. Eventually, the enzyme will become **Denatured** and will **no longer function**.
- As **temperature increases, more enzymes' molecules' Active Sites' shapes** will be **less Complementary** to the **shape** of their **Substrate**, and **more** enzymes will be **Denatured**. This will **decrease** the **rate of reaction**.
- In summary, as **temperature increases, initially** the **rate** of reaction will **increase**, because of **increased Kinetic Energy**. However, the effect of **bond breaking** will become **greater and greater**, and the **rate** of reaction will begin to **decrease**.
- The temperature at which the **maximum rate** of reaction occurs is called the enzyme's **Optimum Temperature**. This is different for **different enzymes**. *Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.*

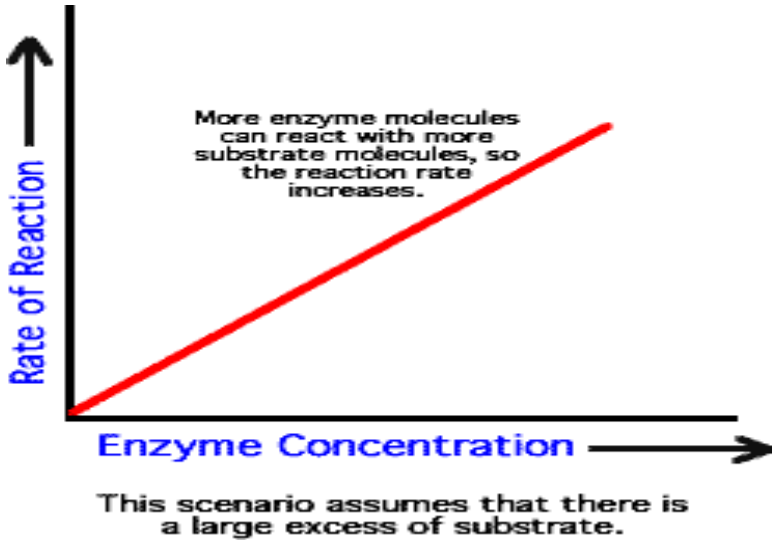
3. Enzyme concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present.

- Changing the **Enzyme** and **Substrate concentrations** affect the **rate** of reaction of an enzyme-catalysed reaction. **Controlling** these factors in a **cell** is one way that an organism **regulates** its **enzyme activity** and so its **Metabolism**.
- Changing the **concentration** of a **substance only** affects the rate of reaction if it is the **limiting factor**: that is, it the **factor** that is **stopping** a reaction from preceding at a **higher rate**.
- If it is **the limiting factor, increasing concentration** will **increase** the **rate** of reaction up to a **point**, after which any **increase** will **not affect** the rate of reaction. This is because it will **no longer** be the **limiting factor** and **another factor** will be **limiting** the **maximum rate** of reaction.
- As a **reaction proceeds**, the **rate of reaction** will **decrease**, since the **Substrate** will get **used up**. The **highest rate** of reaction, known as the **Initial Reaction Rate** is the **maximum reaction rate** for an **enzyme** in an **experimental situation**.

→ **Increasing Enzyme Concentration** will **increase** the **rate** of reaction, as **more enzymes** will be **colliding** with **substrate** molecules.

→ However, this too will only have an effect up to a **certain concentration**, where the Enzyme Concentration is **no longer** the **limiting factor**.



The graph shows that when a large excess of substrate is maintained, the reaction rate will increase as the concentration of enzymes is increased. This is a linear relationship.

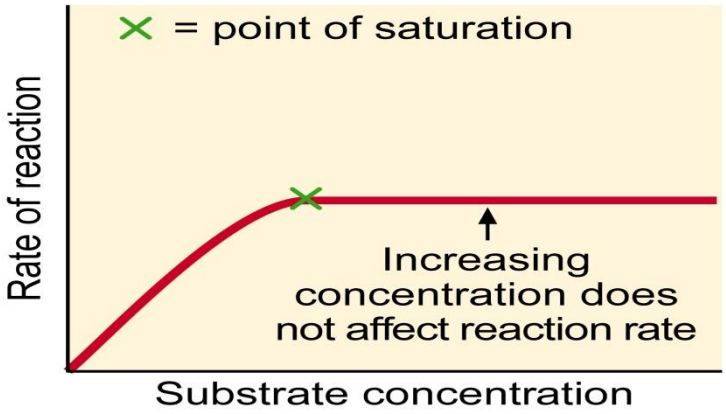
4. Substrate concentration

If you have a fixed number of enzymes, but slowly increase the amount of substrate, the rate of reaction will increase. This is because at low substrate concentration, some enzymes will have empty active sites, but as you increase the substrate concentration, these enzymes will start to be used.

When the substrate concentration reaches a certain point, adding more substrate makes no difference to the rate of reaction. This is because all the active sites are already being used – there are substrate molecules waiting for an active site to become free.

→ **Increasing Substrate Concentration increases** the **rate** of reaction. This is because **more substrate molecules** will be **colliding** with **enzyme molecules**, so **more product** will be formed.

→ However, after a **certain concentration**, any **increase** will have **no effect** on the **rate** of reaction, since Substrate Concentration will **no longer** be the **limiting factor**. The **enzymes** will effectively become **saturated**, and will be working at their **maximum possible rate**.



5. Metal ions

Most of the enzymes only become active in the presence of certain ions e.g. cations such as Mg, Ca, Zn, Na,or K. In some cases cations may get loosly bound to enzymes, while in other they get bound to substrate. Anions have also been found to increase the enzyme activity. E.g. Cl enhances the activity of salivary amylase.

The catalytic activity of certain enzymes is reversibly altered by certain inorganic and organic molecules called modifiers. Those molecules which increase the enzyme activity are called *positive modifiers* or *activators* and those which decrease the enzyme activity as *negative modifiers* or *inhibitors.*

Many metals act both as postitive and negative modifiers, whereas certain organic molecules retard enzyme activity, thus acting as negative modifiers.

Inorganic Modifiers (Enzyme Activators)

Certain enzymes, apart from a requirement of a coenzyme, also need a metal ion for full activity. Removal of the metal often results in partial or total loss of enzyme activity. The activity may, however, be restored by replacing the original or a similar metal ion. Some such metal ions (or cations) are K^{+}, Cu^{+}, Fe^{+}, Mg^{+}, Fe^{2+}, Vin^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} etc. Mg participates in phosphate-transfer reactions and Fe, Cu and Mo are required in oxido-reduction reactions. Certain mechanisms as to how the metal ions bring about activation are given beolw :

i. Direct participation in catalysis. Certain metals may directly participate in the oxidoreduction reactions by undergoing a valence change and thus function

in electron transport system. Fe, for exmaple, functions similarly in *cytochromes* or in *catalase.*

ii. Formation of a metallosubstrate. Sometimes a metal combines with the substrate to form a metallosubstrate (MS) which, in fact, is the true substrate for the enzyme and forms an enzyme-metal-substrate (EMS) complex. This complex, later on, decomposes to produce the reaction product (P) and regenerate the enzyme and the metal.

$$S + M \rightleftharpoons MS \xrightarrow{+E} EMS \longrightarrow E + M + P$$

iii. Formation of a metalloenzyme. A metal ion may first combine with an enzyme to form a metalloenzyme (ME) which then combines with the substrate forming an enzyme-metal-substrate (EMS) complex.

$$E + M \rightleftharpoons EM \xrightarrow{+S} EMS \longrightarrow E + M + P$$

iv.Alteration of equilibrium constant. Metals may also change the nature of the reactants so that the apparent equilibrium constant of the reaction is also altered.

v. Conformational change in the enzyme. Metal ions may also bring about conformational change in the enzyme molecule, converting it into an active form. In such a case, the metal may be linked at a point far remote from the substrate and may serve to maintain an active tertiary or quaternary structure.

6. Time

The longer an enzyme is incubated with its substrate, the greater the amount of product that will be formed. However, the rate of formation of product is not a simple linear function of the time of incubation.

All proteins suffer denaturation, and hence loss of catalytic activity, with time. Some enzymes, especially in partially purified preparations, may be noticeably unstable, losing a significant amount of activity over the period of incubation.

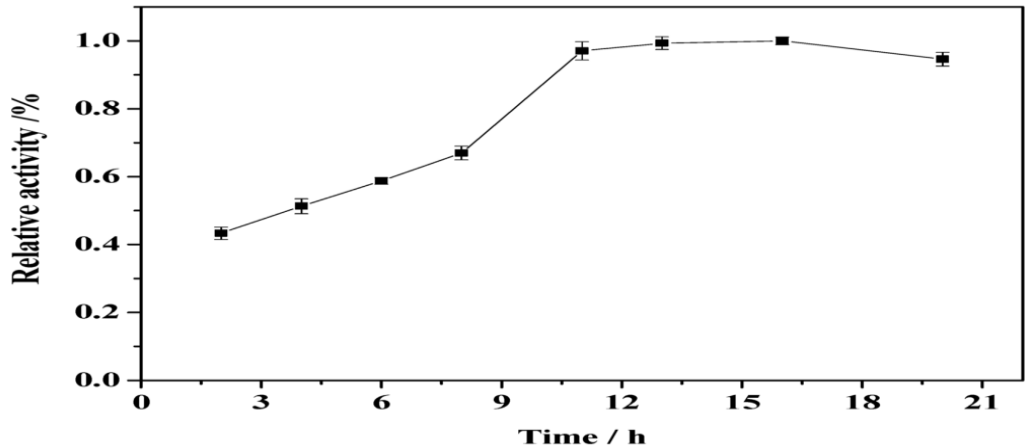
If the activity of the enzyme is such that much of the substrate is used up during the incubation, then, even if the concentration of substrate added was

great enough to ensure saturation of the enzyme at the beginning of the experiment, it will become inadequate as the incubation proceeds, and the formation of product will decrease.

Enzyme catalysed reactions are reversible. Initially, there is little or no product present, and therefore the reaction proceeds only in the forward direction. However, as the reaction continues, so there is a significant accumulation of product, and there is a significant rate of back reaction. As a result, the rate of formation of product slows down as the incubation proceeds, and if the incubation time is too long, then the measured activity of the enzyme is falsely low.

In some studies, especially when investigating the substrate dependence of the rate of reaction, it is usual to make measurements of the formation of product at relatively short time intervals (say every 10 seconds) for the first minute or so, then plot a graph of the amount of product formed against time, and determine the initial rate of reaction by drawing the tangent to the steepest part of the rate curve. However, in short incubations there can be a considerable error of timing; 1 second is a significant error in a short incubation, but negligible when the incubation time is several minutes. Similarly, in short incubations only a small amount of product has been formed, and analytical errors are magnified when the amount of product is extremely small.

Selecting an appropriate incubation time depends on a compromise between these various factors.As a general rule, the incubation should be long enough to permit a moderate amount of product to be formed, and long enough that the error in timing is insignificant, but not so long that there is detectable levelling off of the curve. You need to be sure that when you determine the rate of reaction (in mol of product formed / minute) the enzyme has been active at a more or less constant rate throughout your incubation.



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❖ Michaelis-Menten equation: Derivation and significance

The primary function of enzymes is to enhance rates of reactions so that they are compatible with the needs of the organism. To understand how enzymes function, we need a kinetic description of their activity. For many enzymes, the rate of catalysis V_0, which is defined as the number of moles of product formed per second, varies with the substrate concentration [S]. The rate of catalysis rises linearly as substrate concentration increases and then begins to level off and approach a maximum at higher substrate concentrations.

In 1913, a general theory of enzyme action and kinetics was developed by L. Michaelis and M.L. Menten, which was later extended by J.E. Briggs and J.B.S. Haldane. This theory is developed for the reaction in which there is only one substrate. The theory serves to explain how an enzyme can cause kinetic rate enhancement of a reaction and explains how reaction rates depend on the concentration of enzyme and substrate.

Michaelis-Menten equation

This theory assumes that the enzyme 'E' first combines with substarte 'S' to form the enzyme-substrate complex 'ES'. The ES then breaks down in a second step to form free enzyme and the product 'P'.

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} ES \quad \text{---------} \quad (1)$$

$$ES \underset{K_{-2}}{\overset{K_{+2}}{\rightleftharpoons}} E + P \quad \text{--------} \quad (2)$$

These reactions are assumed to be reversible. The rate constants for the forward and backward directions respectively have the positive and negative subscripts.

Michaelis-Menten equation expresses the mathematical relationship between the initial rate of enzyme-catalysed reactions and the concentration of the substrate and certain characteristics of the enzyme. This equation is the rate equation for reactions catalysed by enzymes having single substrate. In this derivation—

[E] ⟶ Concentration of free enzyme
[ES] ⟶ Concentration of enzyme bound to substrate
[E_T] ⟶ Total enzyme concentration (E + ES)
[S] ⟶ Substrate concentration, which is assumed to be far greater than [E].

The amount of S bound to E at any given time is negligible compared to with total concentration of S.

Now initial velocity V_0 is equal to the rate of breakdown of the enzyme substrate complex ES. According to equation (2) we can write the first order rate equation—

$$V_0 = K_{+2} [ES] \qquad \text{----------} \quad (3)$$

However, we cannot determine K_{+2} & [ES] directly. Therefore we have to find out alternate expression for V_0 in terms of other variable terms, which can be measured more easily.
For that, we first write a second order rate equation for the formation of ES from E & S.

$$\frac{d[ES]}{dt} = K_{+1} [E] [S]$$

$$\frac{d[ES]}{dt} = K_{+1} ([E_T] - [ES]) [S] \qquad \text{----------} \quad (4)$$

K_{+1} is the second order rate constant.
Although ES can also be formed from E and P, the rate of this reaction is neglected as we are considering the beginning of the reaction, when P is zero or close to zero.

Now, we may write rate equation for the breakdown of ES by the sum of two reactions; the reaction yielding the product (forward direction) and the reaction yielding E + S.

We then have ---

$$-\frac{d[ES]}{dt} = K_{-1} [ES] + K_{+2} [ES] \qquad \text{----------} \quad (5)$$

At steady state (concentration of ES remains constant) the rate formation of ES is equal to rate of breakdown.

$K_{+1}\ ([E_T] - [ES])\ [S] = K_{-1}\ [ES] + K_{+2}\ [ES]$ --------- (6)

Rearranging equation (6), we get

$$\frac{([E_T] - [ES])\ [S]}{[ES]} = \frac{K_{-1} + K_{+2}}{K_{+1}} = K_M \text{ ---------- (7)}$$

K_M is called Michaelis-Menten constant. From equation (7) the steady state concentration of the ES complex can be obtained by solving for [ES].

$$\frac{[E_T]\ [S]}{[ES]} - \frac{[ES]\ [S]}{[ES]} = K_M$$

$$\frac{[E_T]\ [S]}{[ES]} - [S] = K_M$$

$$\frac{[E_T]\ [S]}{[ES]} = K_M + [S]$$

$$[ES] = \frac{[E_T]\ [S]}{K_M + [S]} \text{ ---------- (8)}$$

We can substitute the value of ES in equation (3)

$$V_0 = K_{+2}\ \frac{[E_T]\ [S]}{K_M + [S]} \text{ ---------- (9)}$$

When the substrate concentration is so high that the entire enzyme is present as ES complex i.e. when enzyme is saturated, we reach the maximum initial velocity V_{max}.

$V_{max} = K_{+2}\ [E_T]$ ---------- (10)

Now substituting for $K_{+2}\ [E_T]$ its value from equation (10), we obtain

$$V_0 = \frac{V_{max}\,[S]}{K_M + [S]} \qquad \text{----------} \quad (11)$$

This is the Michaelis Menten equation, the rate equation for one substrate enzyme catalysed reaction.
When initial velocity $V_0 = ½\ V_{max}$

$$\frac{V_{max}}{K_M + [S]} = V_{max}\,[S]$$

By dividing the whole equation by V_{max} we get

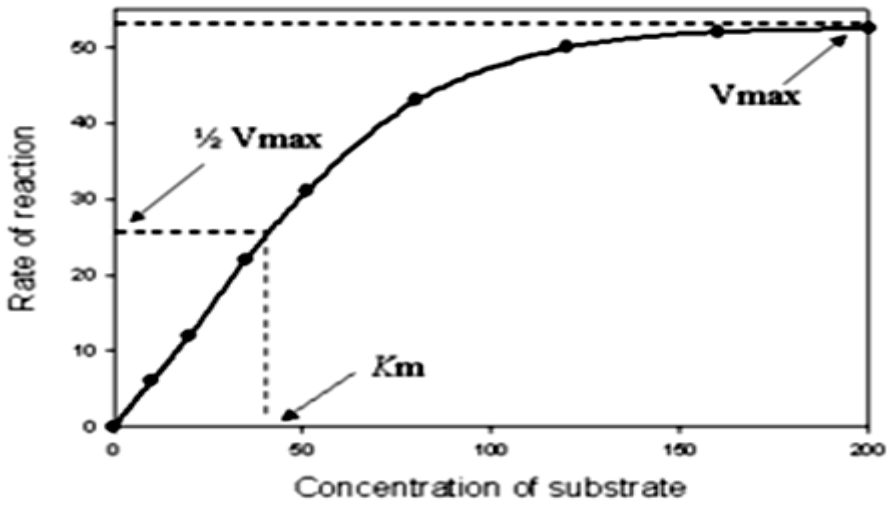
$$\frac{1}{K_M + [S]} = [S]$$

On rearranging it becomes

$$K_M + [S] = 2\,[S]$$

$$K_M = [S]$$

Thus Michaelis Menten constant K_M is equal to the substrate concentration at which the initial velocity is half maximum.



Significance of K_m

i) The K_m value is very useful in evaluating affinity of enzyme for substrate.
ii) The K_m value also gives an idea regarding the type of inhibition of an enzyme caused by inhibitor.
iii) It is used in medical research.
iv) This equation is used for experimental determination of rate of substrate concentration.
v) It is used to understand enzyme regulatory mechanism.
vi) It is useful in quantitating enzyme activity and purification.
vii) The Michaelis constant, K_M, has two meanings. First, K_M is the concentration of substrate at which half the active sites are filled. Thus, K_M provides a measure of the substrate concentration required for significant catalysis to occur.
viii) The maximal rate, V_{max}, reveals the *turnover number* of an enzyme, which is *the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.*
ix) The maximal rate, V_{max}, reveals the turnover number of an enzyme if the concentration of active sites $[E]_T$ is known.

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❖ **Types of enzymes**

On the basis of site of action, enzymes fall under following types:

(1) Exoenzymes: -

These are also called as extracellular enzymes. The principle function of exoenzymes is to perform necessary changes on the nutrients in the medium. Exoenzymes by hydrolysis decompose complex organic matter in the outer world, such as proteins, cellulose, and fats.

An **exoenzyme**, or **extracellular enzyme**, is an enzyme that is secreted by a cell and functions outside of that cell. Exoenzymes are produced by both prokaryotic and eukaryotic cells and have been shown to be a crucial component of many biological processes. Most often these enzymes are involved in the breakdown of larger macromolecules. The breakdown of these larger macromolecules is critical for allowing their constituents to pass through

the cell membrane and enter into the cell. For humans and other complex organisms, this process is best characterized by the digestive system which breaks down solid food via exoenzymes. The small molecules, generated by the exoenzyme activity, enter into cells and are utilized for various cellular functions. Bacteria and fungi also produce exoenzymes to digest nutrients in their environment, and these organisms can be used to conduct laboratory assays to identify the presence and function of such exoenzymes. Some pathogenic species also use exoenzymes as virulence factors to assist in the spread of these disease causing microorganisms. In addition to the integral roles in biological systems, different classes of microbial exoenzymes have been used by humans since pre-historic times for such diverse purposes as food production, biofuels, textile production and in the paper industry. Another important role that microbial exoenzymes serve is in the natural ecology and bioremediation of terrestrial and marine environment.

(2) Endoenzymes: -

These are also called as 'intracellular' enzymes. The endoenzymes are present inside the cell and are never given off during the life of cell. Endoenzymes are of two types:

i) Synthesizing of cell component enzymes; (ii) Bioenergetics (releases of energy from food stuffs)

An **endoenzyme**, or **intracellular enzyme**, is an enzyme that functions within the cell in which it was produced. Because the majority of enzymes fall within this category, the term is used primarily to differentiate a specific enzyme from an exoenzyme. It is possible for a single enzyme to have both endoenzymatic and exoenzymatic functions.

Example: Glycolytic enzymes, enzymes of Kreb's Cycle. Enzymes are a type of protein that speeds up chemical reactions in cells. Enzymes are specific to the job they do. Only molecules that are the correct shape can fit into the enzyme. This is called the lock and key model. enzymes work outside of the cell (extracellular enzymes) as well as inside the cell (intracellular enzymes) In most cases the term endoenzyme refers to an enzyme that binds to a bond 'within the body' of a large molecule - usually a polymer. For example an endoamylase would break down large amylose molecules into shorter dextrin chains. On the other hand, an exoenzyme removes subunits from the polymer one at a time from one end; in effect it can only act at the end points of a polymer. An

exoamylase would therefore remove one glucose molecule at a time from the end of an amylose molecule.

(3) Constitutive Enzymes: -

The enzymes which are produced in absence of the substrate are known as constitutive enzymes. **Constitutive enzymes** are produced constitutively by the cell under all physiological conditions. Therefore, they are not controlled by induction or repression.

Constitutive enzymes are produced in constant amounts without regard to the physiological demand or the concentration of the substrate. They are continuously synthesized because their role in maintaining cell processes or structure is indispensable.

(4) Induced Enzymes: -

These enzymes are produced in response to the presence of substrate in the environment only and hence are known as induced enzymes.

An **adaptive enzyme** or **inducible enzyme** is an enzyme that is expressed only under conditions in which it is clear of adaptive value, as opposed to a constitutive enzyme which is produced all the time. The Inducible enzyme is used for the breaking-down of things in the cell. It is also a part of the Operon Model, which illustrates a way for genes to turn ‘on’ and ‘off ’. The Inducer causes the gene to turn on (controlled by the amount of reactant which turns the gene on). Then there is the repressor protein that turns genes off. The inducer can remove this repressor, turning genes back on. The operator is a section of DNA where the repressor binds to shut off certain genes; the promoter is the section of DNA where the RNA polymerase binds. Lastly, the regulatory gene is the gene for the repressor protein.