Biochemical Engineering

IMMOBILIZATION OF ENZYMES

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Enzyme immobilization

Definition:
Confining the enzyme molecules to a distinct phase from the one in which the substrates and the products are present

The process of attachment of an enzyme to a solid matrix so that it cannot escape but can still act on its substrate

Materials

The materials used for immobilization of enzymes referred as carrier matrices
usually inert polymers or inorganic materials.

The ideal carrier matrix has the following properties:

1. Low cost
2. Inertness
3. Physical strength
4. Stability
5. Regenerability after the useful lifetime of the immobilized enzyme,
6. Enhancement of enzyme specificity
7. Reduction in product inhibition
8. A shift in the pH optimum for enzyme action to a desired value for the process, and reduction in microbial contamination and nonspecific adsorption

Methods used for the Immobilization of Enzymes

1.Physical adsorption onto an inert carrier

- Adsorption of enzymes onto insoluble supports is a very simple method
- And of wide applicability and
- Capable of high enzyme loading (about one gram per gram of matrix).

➢Method of immobilization:

- Mixing the enzyme with a suitable adsorbent under appropriate conditions of pH and ionic strength
- Incubation for a sufficient period
- Washing off loosely bound and unbound enzyme will produce immobilized enzyme in a directly usable form
- The driving force causing this binding is usually due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule

- The particular choice of adsorbent depends principally upon minimizing leakage of enzyme during use
- Although the physical links between the enzyme molecules and the support are often very strong
- Link may be reduced by many factors including the introduction of the substrate

Physical Method

Adsorption: simplest way to immobilize enzymes

Enzymes can be adsorbed physically on a surface-active adsorbent by

- contacting an aqueous solution of enzyme with an adsorbent.
- Commonly employed adsorbents :

alumina, anion-exchange resins, calcium carbonate, carbon, cation-exchange resins, celluloses, clays, collagen, colloid-ion, conditioned metal, glass plates, diatomaceous earth, and hydroxyapatite.

- Care must be taken that

binding forces are not weakened during use by inappropriate changes in pH or ionic strength.

- Examples of suitable adsorbents are ion-exchange matrices, porous carbon, clays, hydrous metal oxides, glasses and polymeric aromatic resins.

Methods for producing immobilized enzymes with multifunctional reagents

- M1:Enzymes can be reacted with multifunctional reagent alone so that they are cross-linked by the reagent to form a water-insoluble derivative.
- M2:Adsorption of enzymes on a water-insoluble, surface-active support followed by intermolecular cross-linking with multifunctional reagents to strengthen the attachment.
- M3: Multifunctional reagents can be also used to introduce functional groups into water-insoluble polymers, which then react covalently with water-soluble enzymes.

Immobilization matrix materials
Examples:-

Commonly employed water-insoluble supports for the covalent attachment of enzymes include:

- *synthetic supports* such as acrylamide-based polymers,
- maleic anhydride-based polymers, methacrylic acid-based polymers,
- styrene-based polymers, and polypeptides, and
- *natural supports* such as agarose (Sepharose), cellulose, dextran (Sephadex), glass, and starch

2.Covalent binding to a reactive insoluble support

- Only small amounts of enzymes may be immobilized by covalent binding (about 0.02 g per g of matrix).
- The strength of binding is very strong, however, and very little leakage of enzyme from the support occurs.
- The relative usefulness of various groups, found in enzymes, for covalent link formation depends upon

- their availability and reactivity (nucleophilicity)
- in addition to the stability of the covalent link, once formed.

Chemical Method

Covalent Attachment:

- The covalent attachment of enzyme molecules via

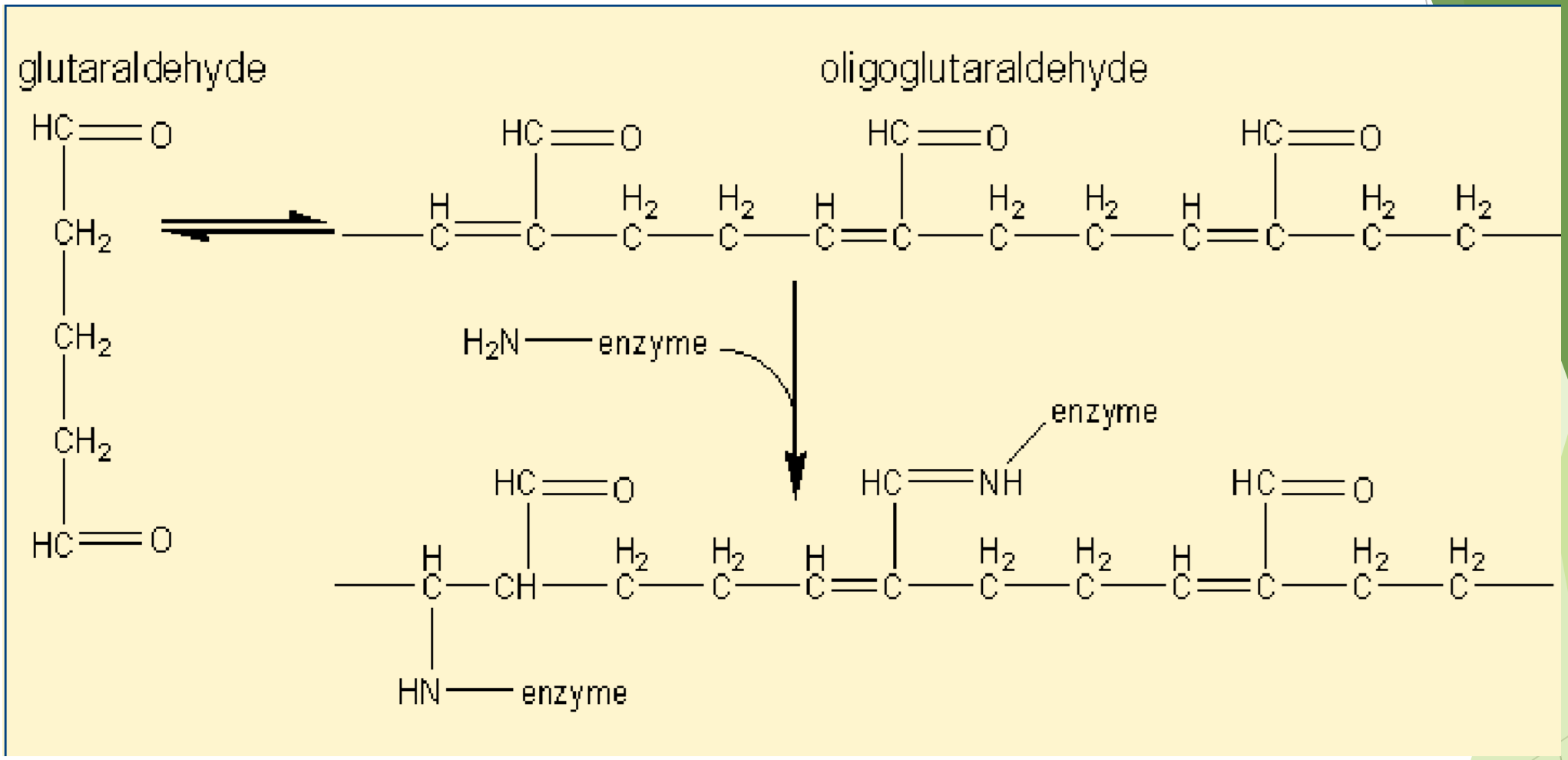
nonessential *amino acid residues* (that is, amino acids minus water) to water insoluble, functionalized supports are the most widely used method for immobilizing enzymes.

- *Functional groups* of the nonessential amino acid

residues that are suitable for the immobilization process are free α-, β-, or γ-carboxyl groups, α- or β-amino groups, and phenyl, hydroxyl, sulfhydryl, orimidazole groups.

- The reactivity of the protein side-chain nucleophiles is determined by their state of protonation (i.e. charged status) and roughly follows the relationship -S- > -SH > -O- > $-NH_2$ > -COO- > -OH > NH_3+
- where charges may be estimated from knowledge of the pKa values of the ionizing groups and the pH of the solution.

- Lysine residues are found to be the most generally useful groups for covalent bonding of enzymes to insoluble supports due to their widespread surface exposure and high reactivity especially in slightly alkaline solutions.
- They also appear to be only very rarely involved in active sites of enzymes.



Commonly used method for the covalent immobilization of enzymes

3.Inclusion in the lattices of a polymerized gel or entrapment or membrane confinement

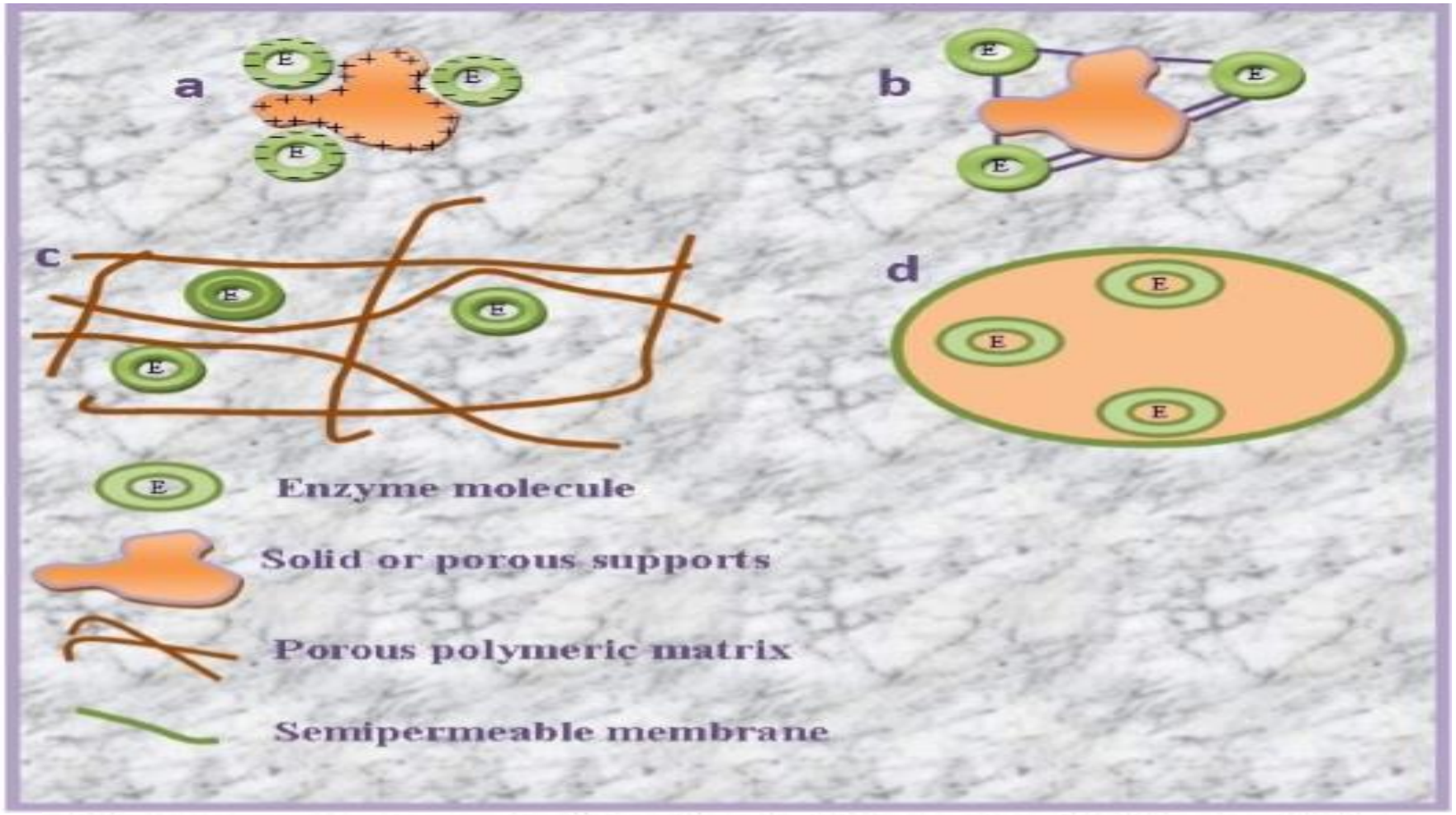
- Entrapment of enzymes within gels or fibers is a convenient method for use in processes involving low molecular weight substrates and products.
- Amounts in excess of 1 g of enzyme per g of gel or fibre may be entrapped.
- Large molecules have difficulty in approaching the catalytic sites of entrapped enzymes precludes use of entrapped enzymes with high molecular weight substrates.

- The entrapment process may be a purely physical caging or involve covalent binding.
- As an example of this later method the enzyme lysine residues may be derivatized by reaction with acryloyl chloride (CH_2=CH-CO-Cl) to give the acryloyl amides.
- This product may then be copolymerized and cross-linked with acrylamide (CH_2=CH-CO-NH_2) and bis acrylamide (H_2NCO-CH=CH-CH=CH-CO-NH_2) to form a gel.

- Enzymes may be entrapped in cellulose acetate fibres by, for example
- making up an emulsion of the enzyme plus cellulose acetate in methylene chloride
- followed by extrusion through a spinneret into a solution of an aqueous precipitant.

Entrapment is the method of choice for the immobilization of microbial, animal and plant cells where calcium alginate is widely used.

i. enzyme non-covalently adsorbed to an insoluble particle;
ii. enzyme covalently attached to an insoluble particle;
iii. enzyme entrapped within an insoluble particle by a cross-linked polymer;
iv. enzyme confined within a semipermeable membrane



(a) enzyme non-covalently adsorbed to an insoluble particle;
(b) enzyme covalently attached to an insoluble particle;
(c) enzyme entrapped within an insoluble particle by a cross-linked polymer;
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Immobilised enzyme systems

Utility of Enzyme Immobilization

- Use of immobilized enzyme eliminate enzyme separation step from the main process thus simplifying and increasing the overall process yield.
- Easy separation from reaction mixture providing the ability to control reaction times and minimize the enzymes lost in the product.
- Re-use of enzymes for many reaction cycles lowering the total production cost of enzyme mediated reactions.
- Ability of enzymes to replace multiple standard chemical steps and provide commercially pure products.

Properties of Immobilized Enzymes

the changes in physical and chemical properties which an enzyme would be expected to undergo upon immobilization for the best use of various immobilization techniques available are

- Changes have been observed in the stability of enzymes and in their kinetic properties because of microenvironment imposed upon them by the supporting matrix and by the products of their own action.

Stability

- The stability of enzymes might be expected to either increase or decrease on immobilization depending on whether the carrier provides a microenvironment capable of denaturing the enzymic protein or of stabilizing it.
- Inactivation due to autodigestion of proteolytic enzymes should be reduced by isolating the enzyme molecules from mutual attack by immobilizing them on a matrix.

- It has been found that enzymes coupled to inorganic carriers were generally more stable than those attached to organic polymers when stored at 4 or 23 °C.
- Stability to denaturing agents may also be changed upon immobilization.
- Kinetic properties changes in activity of enzymes due to actual process of immobilization have not been studied in detail.
- There is usually a decrease in specific activity of an enzyme upon immobilization and this can be attributed to denaturation of the enzymic protein caused by the coupling process.

- Once an enzyme has been insolubilized, however, it finds itself in a microenvironment that may be drastically different from that existing in free solution.
- The new microenvironment may be result of the physical and chemical character of the support matrix alone, or it may result from interactions of the matrix with substrates or products involved in the enzymatic reaction.

- Diffusion of large molecules will obviously be limited by steric interactions with the matrix, and this is reflected in the fact that relative activity of bound enzymes towards high molecular weight substrates has been generally found to be lower than towards low molecular weight substrates.

- an advantage in some cases since the immobilized enzymes may be protected from attack by large inhibitor molecules.

- The diffusion of substrate from the bulk solution to the micro-environment of an immobilized enzyme can limit the rate of enzyme reaction.

- The rate at which substrate passes over the insoluble particle affects thickness of the diffusion film which in turn determines the concentration of substrate in the vicinity of the enzyme and hence the rate of reaction.

- The effect of the molecular weight of the substrate can also be large.