

Properties of immobilized enzymes

Based on the study of immobilization methods (previous chapter), it can be said that in order to achieve good performance in choosing an immobilization method, it is necessary to know the properties of the enzyme and those of the support.

1. Characteristic parameters of enzymes :

- *Biochemical properties :*
 - ~ Molecular weight ;
 - ~ Functional groups on the protein surface ;
 - ~ Purity (inactivation/protection against impurities).

- *Kinetic parameters :*
 - ~ Specific activity ;
 - ~ pH and temperature profile ;
 - ~ Kinetic parameters for activity and inhibition ;
 - ~ Enzyme stability with respect to pH, temperature, solvents, contaminants, impurities

2. Support properties :

- *Chemical characteristics :*
 - ~ Composition and chemical basis ;
 - ~ Functional groups and swelling behavior ;
 - ~ Pore size and accessible volume of the matrix ;
 - ~ Chemical stability of the support.

- *Mechanical properties :*
 - ~ Particle diameters ;
 - ~ Compression behavior of each particle ;
 - ~ Flow resistance ;
 - ~ Abrasion.

1. Properties of immobilized enzymes :

Thus, the immobilization of enzymes often leads to a change in their physical, chemical, and kinetic properties :

Physicochemical properties :

- Improved stability over time ;
- Resistance to denaturation.

Kinetic properties :

- The microenvironment determines all of the enzyme's catalytic activity ;
- Diffusion phenomena limit substrate access to the enzyme site, resulting in variations in maximum speed (V_m) and Michaelis constant (K_m) ;
- The pH of the enzyme can be modified when the reaction involves the release or consumption of protons.

2. Concept of stability :

There are four types of stability for an immobilized enzyme :

- **Storage stability :**

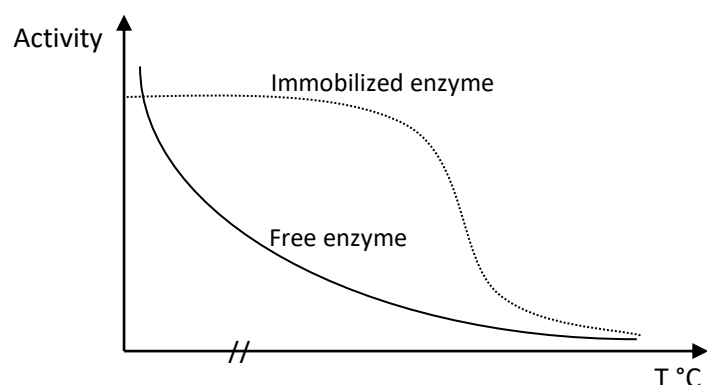
This is the change in activity recorded under normal storage conditions. This stability is attributed to an increase in the rigidity of the enzyme molecule.

- **Operational stability :**

This is the evolution of enzyme activity during repeated tests. Some immobilized enzymes are more stable during continuous operation, while others are more stable during discontinuous operation.

- **Thermal stability :**

It is the resistance to higher temperatures at which the enzyme is normally denatured.



- **Stability with respect to pH variations :**

Generally, the ionization state of the medium defines the stability range with respect to pH variations :

- ✓ Enzymes immobilized on cationic supports are stable in basic pH ranges.
- ✓ Enzymes immobilized on anionic supports are stable in acidic pH ranges.

In addition, certain techniques make enzymes resistant to the action of certain substances such as :

- **Effectors and denaturing compounds :**

The sensitivity of immobilized enzymes to inhibitors and activators may differ significantly from that of soluble forms. This difference is due to the interaction of the effector with the support and not to its direct action.

3. Concept of sharing and diffusion :

The immobilization of enzymes creates two environments : macroenvironment and microenvironment ; hence the appearance of two physical phenomena in the medium : sharing and diffusion. The first results from the interaction between the reactants and the microenvironment. The second is due to the mobility of the reactants in both environments.

Sharing :

This is the behavior of particles (solid bodies) in the presence of two immiscible liquids. This creates a discontinuous gradient in reactant concentrations.

Diffusion :

It is the movement (transfer) of a molecule or particle from a compartment where its concentration is high to another where its concentration is low. Diffusion will be faster when the concentration difference between the two compartments is greater. Therefore, a continuous gradient in reactant concentrations is created.

4. Kinetics of enzymatic reactions catalyzed by immobilized enzymes :

4.1. Effects of mass transfer on enzyme kinetics :

The restriction of solute mobility relates to mass transfer effects, which can be the first to reduce the reaction rate and, consequently, decrease the efficiency of the immobilized enzyme compared to the free enzyme.

There are generally two types of mass transfer (diffusional transport) :

- **External material transfers :**

This is the transport of molecules from the solution to the surface of a particle (non-porous supports).

- **Internal transfers of materials :**

This is the transport of molecules within a matrix (porous supports, gel, membrane...).

4.2. Fundamental coupling equation :

Near the surface of the support, where the enzymes are immobilized, the concentration of the substrate decreases and that of the product increases. A concentration gradient is established.

The diffusion rate of the substrate or product is generally of the same order of magnitude or lower than that of the enzymatic reaction.

In a steady state, the substrate flux (J_s) is equal to the reaction rate : $J_s = v$

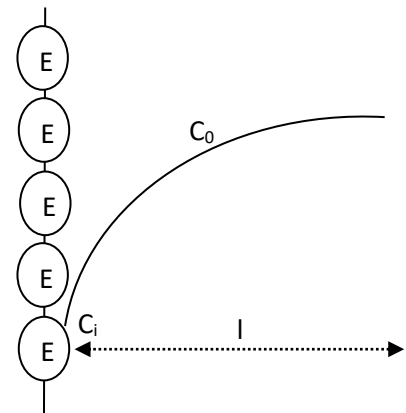
The figure opposite shows enzyme molecules attached to a rigid support :

The curve indicates the substrate concentration gradient.

Where :

C_0 : the concentration of the solute near the surface

C_i : the concentration of the solute at a distance l



According to FICK's two laws (of diffusion of solute particles in a solution), we arrive at the following expression for the flux :

$$J_s = h_d(c_0 - c_i) \quad \text{by posing: } h_d = D/l$$

Where :

D : diffusion coefficient

l : the distance traveled by the diffusing solute particle

Consider an enzyme fixed on an impermeable surface, membrane, or solid support. In the simplest case of a Michaelis enzyme, the rate equation is given by the relationship :

$$V = V_m \cdot S_i / (K_m + S_i) \quad (S_i \text{ is the local substrate concentration})$$

The net substrate flow reaching the immobilized enzyme is given by the relationship :

$$J_s = h_d(S_0 - S_i)$$

Where :

S_0 : the concentration of substrate in the solution

S_i : local substrate concentration

4.2. Simplification of calculations (kinetics of immobilized enzymes) :

By setting V_m and K_m as the kinetic parameters of a native enzyme in solution and V_m' and K_m' as its parameters modified by immobilization, general calculations can be made on the kinetic behavior of immobilized enzymes.

The average velocity observed per unit volume of medium is given by the relationship :

$$V = 1/\text{vol} \cdot \int (V_m' \cdot [S] / (K_m' + [S])) \cdot d\text{vol}$$

The Thiele module allows diffusion constraints to be characterized by comparing the reaction rate (V_m/K_m) and the diffusion rate (D/R^2) :

$$\phi = R^2 \cdot V_m' / D \cdot K_m'$$

Where :

R : support radius

D : diffusion coefficient of the substrate in the medium

In fact, if $\phi > 1$, diffusion constraints are significant.

The importance of the influence of mass transfer is usually expressed by the efficiency or yield factor (η) :

$$\eta = V_{imm} / V_{free}$$

Where V_{imm} and V_{free} are the reaction rates catalyzed by the same enzyme concentration (immobilized and free) and under identical conditions.