**Biotechnology and Genetic engineering**

Dr. Abeer Fauzi Murad Lecture(3)

**Methods & Applications of Enzyme & Whole Cell Immobilization**

**What is enzyme immobilization?**

Immobilization is defined as the imprisonment of cell or enzyme in a distinct support or matrix. The support or matrix on which the enzymes are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecules. The practice of immobilization of cells is very old and the first immobilized enzyme was **amino acylase** of *Aspergillus oryzae* for the production of L-amino acids in Japan.

**Advantages of immobilized enzymes:**

**(1).**   *Increased functional efficiency of enzyme*

**(2).**   *Enhanced reproducibility of the process they are undertaking*

**(3).**  *Reuse of enzyme*

**(4).**   *Continuous use of enzyme*

**(5).**  *Less labour input in the processes*

**(6).**   *Saving in capital cost and investment of the process*

**(7).**   *Minimum reaction time*

**(8).**   *Less chance of contamination in products*

**(9).**   *More stability of products*

**(10)**. *Stable supply of products in the market*

**(11)**. *Improved process control*

**(12).** *High enzyme substrate ratio*

**Disadvantages of enzyme immobilization:**

**(1).**  Even though there are many advantages of immobilized enzymes, there are some disadvantages also.

**(2).**  High cost for the isolation, purification and recovery of active enzyme (most important disadvantage)

**(3).**  Industrial applications are limited and only very few industries are using immobilized enzymes or immobilized whole cells.

**(4).**  Catalytic properties of some enzymes are reduced or completely lost after their immobilization on support or carrier.

**(5).**  Some enzymes become unstable after immobilization.

**(6).**  Enzymes are inactivated by the heat generated in the system

**Applications of enzyme immobilization:**

**(1). Industrial production:** Industrial production of antibiotics, beverages, amino acids etc. uses immobilized enzymes or whole cells.

**(2). Biomedical applications:** Immobilized enzymes are widely used in the diagnosis and treatment of many diseases. Immobilized enzymes can be used to overcome inborn metabolic disorders by the supply of immobilized enzymes. Immobilization techniques are effectively used in drug delivery systems especially to oncogenic sites.

**(3). Food industry:** Enzymes like pectinases and cellulases immobilized on suitable carriers are successfully used in the production of jams, jellies and syrups from fruits and vegetables.

**(4). Research:** A Research activity extensively uses many enzymes. The use of immobilized enzyme allow researcher to increase the efficiency of different enzymes such as Horse Radish Peroxidase (HRP) in blotting experiments and different Proteases for cell or organelle lysis.

**(5). Production of bio-diesel** from vegetable oils.

**(6). Waste water management**: treatment of sewage and industrial effluents.

**(7). Textile industry:** scouring, bio-polishing and desizing of fabrics.

**(8). Detergent industry:** immobilization of lipase enzyme for effective dirt removal from cloths.

**Supports or Matrix used in immobilization technology:**

The matrix or support immobilizes the enzyme by holding it permanently or temporarily for a brief period of time. There are a wide variety of matrixes or carriers or supports available for immobilization. The matrix used should be cheap and easily available. Their reaction with the components of the medium or with the enzyme should be minimum as possible. The matrixes or supports for immobilization of enzymes or whole cells are grouped into three major categories

**(1).  Natural polymers**

**(2).  Synthetic polymers**

**(3).  Inorganic materials**

**(1). Natural polymers:**

**(a). Alginate:** A natural polymer derived from the cell wall of some algae. Calcium or magnesium alginate is the most commonly used matrix. They are inert and have good water holding capacity.

**(b). Chitosan and chitin:** They are structural polysaccharides occurring naturally in the cell wall of fungi and the exoskeleton of Arthropods. The various functional groups in enzymes can bind to the – OH group of chitin and can form covalent bonds.

**(c). Collagen:** It is the protenaceous support with good porosity and water holding capacity. The side chains of the amino acids in the collagen and that of enzyme can form covalent bonds to permanently hold the enzyme to the support.

**(d). Carrageenan:** It is a sulfated polysaccharide obtained from some red algae. Their good gelling properties together with its high protein holding capacity makes it good support for immobilizing enzymes.

**(e). Gelatin:** Gelatin is the partially hydrolyzed collagen with good water holding capacity.

**(f). Cellulose:** Most abundant polymer of nature and it is the cheapest support available as carrier of enzymes. The hydroxyl group of the monomer units (glucose) can form covalent bonds with that of the amino acids of enzyme.

**(g). Starch:** A natural polymer of amylose and amylo-pectin. It has good water holding capacity.

**(h). Pectin:** It is a structural polysaccharide of plants found in their primary cell wall and they also acts as the inter-cellular cementing material in plant tissues. Pectin is a gelling agent with good water holding capacity.

**(2). Synthetic polymers:**

They are ion exchange resins or polymers and are insoluble supports with porous surface. Their porous surface can trap and hold the enzymes or whole cells.  Example: Diethylaminoethyl cellulose (DEAE cellulose), Polyvinyl chloride (PVC), UV activated Polyethylene glycol (PEG)

**(3). Inorganic materials:**

**(a). Zeolites:** They are microporous, aluminosilicate minerals with good adsorbing properties and extensively used for immobilizing enzymes and whole cells.

**(b). Ceramics:** They are nonmetallic solids consisting of metal and nonmetal atoms held in ionic and covalent bonds. The composition and bonding pattern varies with different types.

**(c). Diatomaceous earth:** They are silicious sedimentary rocks formed by fossilized accumulations of the cell wall of diatoms. Celite is the trade name of diatomaceous earth. It is a good adsorbent and are resistant to high pH and temperature.

**(d). Silica:**

**(e). Glass:**

**(f).** Activated carbon

**(g).** Charcoal

**Methods of Immobilization:**

Based on support or matrix and the type of bonds involved, there are five different methods of immobilization of enzyme or whole cells.

**(1).  Adsorption**

**(2).  Covalent bonding**

**(3).  Entrapment**

**(4).  Copolymerization**

**(5).  Encapsulation**