**Enzyme Production and Purification: Extraction & Separation Methods | Industrial Microbiology**

**In this article we will discuss about the production and purification of enzymes. Learn about the extraction and separation methods for isolation and purification of enzymes. The extraction methods are: 1. Extraction of Solid Substrate Cultures 2. Extraction of Cells and the separation methods are: 1. Solids Separation Techniques 2. Membrane Separation Techniques 3. Gel Filtration 4. Adsorption Techniques 5. Precipitation Techniques. And also glance over the below given article to get an idea about storability of enzymes and enzyme immobilization.**

In enzyme production there is a very unfavorable ratio between input of raw material and output of product. This requires the installation of concentration procedures. For economic reasons of enzyme application a concentration up to 10-fold is usually satisfactory for industrial enzyme preparations.

For example- enzyme products employed in detergents contain about 5-10% protease while amylase preparations for use in flour treatment contain only about 0.1% pure a-amylase. However, in applications where high purity enzymes are required, e.g., in enzymic analysis, 1000- fold purification is quite common.



In some applications, such as baking and dextrose manufacture, the presence of contaminating enzymes must be very low or rigidly controlled. Moreover, the raw enzyme solutions obtained from microbial cultures contain—independent of their source—different types of by-products. Separation of all these substances may be necessary because of the possibility of undesired effects.

Considering enzyme stability there is another reason for treatment of crude enzyme preparations. Since the trend in enzyme applications is toward use of liquid preparations, stabilization is an important procedure.

Techniques for the large-scale isolation and (partial) purification of enzymes from microbial sources make use mainly of traditional procedures. Most of the equipment can be found in food-processing plants. Large-scale equipment specific for enzyme isolation is not marketed.

Nearly all process operations are carried out at low temperatures (preferably 0°-10°C), with the exception of drying.



Separation processes are usually conducted in batches rather than continuously. However, the scale-up of batch operations inherently causes extended processing times which for many enzymes result in increased losses of activity due to denaturation of the enzyme protein.

For this reason the application of continuous operations seems to be useful, but the necessity for highly reliable machines and ingenious process control delays introduction of continuous methods. In addition the value of continuous processing is lost when a single process step is conducted batch wise, perhaps during precipitation.

**Extraction Methods:**

The first step in the isolation of enzymes is their extraction. Techniques that fall into this group are employed either to separate enzymes from solid substrate culture or to release enzymes from the interior of microbial cells.

**Extraction of Solid Substrate Cultures:**

Enzymes produced by solid substrate cultivation used to be of the extracellular type. It is therefore easily conceived that extraction of mold brans is rather a washing out process. Countercurrent techniques of percolation are the most frequently used unit operation.

In many cases the mold bran is dried prior to extraction. This is convenient when the utilization of the particular enzyme preparation is seasonal. The cultures can be produced in relatively small equipment all the year round, while the extraction is conducted in times of enzyme demand.

On the other hand, it is easily seen that extraction from dried bran will yield solutions with higher enzyme concentrations. And last, drying avoids interference caused by the activity of living cells of fresh cultures. This argument, however may not apply in continuously operated culture plants.

In all cases the extractant is water which, however, may contain acids (inorganic or organic), salts, buffer, or other substances to facilitate solubilization of the enzyme or to improve its stability in solution, or to exclude or minimize undesired effects caused by contaminating by-products or microorganisms.

**Extraction of Cells:**



The decision on whether to employ whole cells for a biochemical process or to use isolated enzymes depends on many factors. Technical difficulties and the related cost of large-scale isolation play an important role.

There are a number of methods for cell disruption, as reviewed by Hughes et al. (1971). Chemical and biochemical methods, such as autolysis, treatment with solvents, detergents, or lytic enzymes, have the disadvantage of being in principle batch operations. Their conduct is difficult to standardize and optimize. More recommendable are mechanical techniques.

At present, the APV-Manton-Gaulin homogenizer seems to be the most versatile type for cell disintegration. In this machine the cell suspension passes a homogenizing valve at the selected

operating pressure and impinges on an impact ring. The strong shearing forces combined with the sudden decompression lead to a disruption of the cell wall. Dunnill and Lilly (1972), who examined the disruption of yeast, found that release of protein can be described by a first order rate equation-

$$\log \frac{R_m}{R_m - R} = KNP^{2.9}$$

Where R is the amount of soluble protein released in g per kg cell mass, $R_m$ the maximum amount of soluble protein released, K a temperature-dependent rate constant, N the number of times the cell suspension has passed the homogenizer, and P the operating pressure. With industrial models, between 50 and 9000 liters of bacterial suspension per hr can be treated, depending on the size of the machine. Ball mills available on the market have a volume capacity of 0.6-250 liters.

**Separation Methods:**



It is possible here to give only the barest outline of methods that find wider application in the large-scale production of enzymes.

**Solids Separation Techniques:**

Such methods are involved in the clarification of culture liquors and extracts, in the separation of precipitates, and in the sterilization of liquid enzyme preparation by mechanical methods.



The solids to be separated may have a number of properties which make separation processes difficult. For instance, they may be greasy, sometimes colloidal, and often density differences between solid particles and liquid phase are very small. Therefore, pretreatment of the liquor is usually inevitable, as conducted by acidification, addition of water miscible solvents or liquid polyions, mild heating, etc.

The problems of large-scale solid-liquid separation are complex and diverse. There are two approaches- centrifugation and filtration. Industrial centrifuges are not ideal for removal of finely divided biological solids. Disc type centrifuges without solid discharge have proved most efficient for separation of easily settling suspensions of greasy particles. Decanters are used in cases where solids content is high but easily settling, e.g., in the production of dried acetone precipitates.

In cases of poorly settling protein precipitates, hollow bowl centrifuges are employed for separation from low solids suspensions as obtained during fractionated enzyme precipitation. In all cases flow rates must be determined empirically. Sometimes (e.g., with precipitates) the

throughput is reduced to less that 10% of the nominal capacity. This requires the integration of cooling devices.

Most frequently filters are more suitable for separation of biological particles. Generally large proportions of filter aids are required. In continuous processes vacuum drum filters are used, with diatomaceous earth, wood-meal, or starch as pre-coat materials. Batch operations are conducted with filter presses.



**Membrane Separation Techniques:**

Membrane processes allow separation of solutes from one another or from a solvent, with no phase change or interphase mass transfer. There are many different kinds of membrane processes, the classification of which is based on the driving forces that cause the transfer of solutes through the membrane. Such a force may be trans-membrane differences in concentrations, as in dialysis; electric potential, as in electro-dialysis; or hydrostatic pressure, as in microfiltration, ultrafiltration, and reverse osmosis.

At present, ultrafiltration is the only membrane process of importance in large-scale enzyme production. From normal filtration processes it differs just by the size range of the particles to be separated (molecular weight cutoffs between 500 and 300,000). Two types of ultrafiltration membranes are used, which differ in their transport mechanisms and their separation properties.

Isotropic porous membranes are the type most similar to conventional filters. They possess a spongy structure with extremely small random pores the average size of which is in the range of 0.05 to 0.5 microns. Molecules with a diameter smaller than that of the smallest pore will pass the membrane quantitatively, whereas particles larger than the largest pore will be retained at the filter surface. Molecules of intermediate size, however, will only pass to some extent.

Another proportion of these particles will be retained within the structure of the membrane. This leads, first, to a decrease in retention (or vice versa, in permeation) with a resulting fouling of the membrane, and secondly, to a reduced discrimination among solutes of different size. In order to minimize fouling it is useful to use membranes with a mean pore size well below that of the solute to be retained. Therefore, porous membranes are advisable for the concentration of high molecular weight solutes (molec. Weight $< 1 \times 10^6$).

Diffusive membranes are capable of more selective molecular discrimination. They are essentially homogeneous hydrogel layers, through which the solvent as well as the solute is transported by molecular diffusion under the driving force of a concentration or a chemical potential gradient. The transportation of a molecule through the membrane requires considerable kinetic energy.

This depends, of course, on the dimensions of the diffusing molecule and on the mobility of the single polymer chains within the membrane matrix. As a rule, the rate of diffusion is high when the polymer segments of the matrix are only loosely interlaced, i.e., when the gel matrix is highly

hydrated. For this reason all membranes made from hydrophilic polymers and capable of swelling in water to a certain degree are principally suited as pressure filtration membranes for aqueous solutions.

In addition to the retention potential of the membrane the flux is important for economic reasons. Since in both the porous and the diffusive filter types the flux depends largely on the thickness of the membrane, it is necessary to keep the membrane as thin as possible.

This requirement has been fulfilled by the construction of anisotropic membranes. They consist of a highly consolidated but very thin (0.1-5 μm) active layer on a comparatively thick (1 to 20 microns) highly porous support. The advantage of these anisotropic membranes is that there is no reduction in solvent permeability at constant hydrostatic pressure because there is no blockage within the membrane.

In any ultrafiltration system, accumulation of solutes at the membrane surface occurs, which leads to formation of a "slime" that increasingly impedes solvent flow through the membrane, until convective transport of solute toward the membrane is equal to the rate of back diffusive transport away from the membrane. This phenomenon is called "concentration polarization".

Proteins and colloidal particles build up solid or thixotropic gels when concentrated beyond a certain point. The solute concentration on the membrane surface reaches an upper limit which is typically between 20 and 70% solute by volume. In order to reduce the polarization effect, in industrial ultrafiltration equipment the feed solution passes the membrane surface at high flow rate.

When a macromolecular solution is ultra-filtered, flux of solvent is described by the relationship-

$$J = A\,(\Delta P = \Delta\pi)$$

Where A is the membrane constant (dependent on temperature, independent of pressure over the normal operating range), $\Delta P$ is the hydrostatic pressure driving force, and $\Delta\pi$ is the osmotic pressure difference across the membrane. For macromolecular solutions with concentrations over 1% w/v, osmotic pressures exceeding 10 to 50 psi are not uncommon.

There are several basic types of ultra-filters – thin channel, tubular, helical tubes, spiral wrapped, and hollow fiber systems. Suitability of a single type depends on the properties of the system to be treated.

**Gel Filtration:**

The technique of ultrafiltration has the advantage of combining both separation of impurities and concentration of the desired enzyme. However, due to its principle, it is a rather nonselective process. Better results, regarding separation of molecules from each other, can be obtained by gel filtration, but in many cases its application is not economically feasible.

In principle, gel filtration is the diffusional partitioning of solute molecules between the readily mobile solvent phase and that confined in spaces within the porous gel particles that make up the stationary phase. Diffusional exchange of solutes takes place between the stationary and mobile phases. The extent to which a molecule penetrates the stationary phase is represented by the partition coefficient $K_{av}$, according to the equation

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

Where $V_e$ is the volume of solvent required to elute solute from the gel column, $V_0$ is the void volume (i.e., the volume of liquid external to the gel particles), and $V_t$ is the total volume of column bed. $K_{av}$ is inversely proportional to the log of the molecular weight, as shown empirically.

Gel filtration works rapidly and preservingly, without mechanical stress as in ultrafiltration. However, precipitation of proteins within the gel column may occur as a consequence of desalting. In some cases it has been observed that the metal bridges of enzyme quaternary structures were uncoupled.

**Adsorption Techniques:**

Because of the possibility of highly selective separations, adsorption processes are increasingly used. Properties of enzyme molecules as different as, e.g., lipophily, electric charge, specificity, etc., are the basis of separation. This results in a great number of adsorbents, such as active carbon, hydroxyapatite, ion exchangers, carrier fixed substrate analogs, and so forth.

A common feature of all adsorption techniques is the principle of adsorption followed by desorption or elution. Separation is achieved by adsorption and elution of either enzyme or impurities. Two methods are available, batch wise adsorption or column chromatography. The latter process has greater separation efficiency and, in addition, offers the possibility of semi-continuous operation.

Among the different adsorption techniques, affinity chromatography is of very great interest, but far from being applicable on a large scale. Ion exchangers available for large-scale processes are of the resin, large-pore gels, or cellulose types. In particular, ion exchange resins exhibit useful properties for industrial production of enzymes.

It must, however, be taken into consideration that the proximity of a resin matrix with a high charge density can affect the structural integrity of enzymes. Large-pore ion exchangers and cellulose exchangers have a number of properties which make them very suitable for enzyme separation processes, but the former are very costly. Obviously, they are only suitable for batch processes because they are compressed in columns.

**Precipitation Techniques:**

Separation from solution by salting out is one of the oldest and yet most important procedures of concentration and purification of enzymes. The logarithm of the decrease in protein solubility in concentrated electrolyte solutions is a linear function of increasing salt concentration (ionic strength), as described by the equation-

$$\log s = B^1 - K^1 s \frac{\tau}{2}$$

where s is the solubility of the protein in g/liter solution; $\tau$ the ionic strength in moles per liter; $B^1$, an intercept constant, is dependent on pH, temperature, and the nature of the protein in solution; $K^1$ is the salting out constant which is independent of pH and temperature, but varies with the protein in solution and the salt used. From the preceding relationship it can be derived that precipitation of protein of known concentrations will occur when the ionic strength satisfies the equation

$$\frac{\tau}{2} = \frac{B^1 - \log s}{K^1 s}$$

This means that the electrolyte concentration required for protein precipitation varies with protein concentration.

The influence of the most important precipitation parameters can be outlined shortly as follows – Higher valency salts produce higher ionic strength than lower valency salts. At a constant ion strength, protein solubility increases with increasing distance (in both directions) from its isoelectric point. As a result, lower ionic strength is required for precipitation when carried out at the isoelectric point of the protein.

The commonly used salt for precipitation is ammonium sulfate. The reasons can be found in the high solubility of this salt and in its low price. In addition, ammonium sulfate is nontoxic for most enzymes and in many cases it acts as a stabilizing agent. In ammonium sulfate solutions precipitated enzymes are often storable for years without significant loss when kept at low temperatures.

In contrast to neutral salts, solvents are less customary for large-scale precipitation of enzymes. The reason is higher costs of raw materials and equipment. Explosion proof equipment and recycling of the solvents are inevitable requirements.

Solvent precipitation is based on the fact that the solubility of enzymes decreases with the decreasing dielectric constant ($\epsilon$) of the solvent. The concentration required is lower the less hydrophilic the solvent is. Thus, an increasing precipitating effect can be achieved in the series methanol ($\epsilon_{25} = 33$), ethanol ($\epsilon_{25} = 24$), isopropanol ($\epsilon_{25} = 18$). Besides aliphatic alcohols, acetone ($\epsilon_{25} = 20$) is often used as a precipitant.

Solvent precipitates are distinguished from salt precipitates by the ease with which they settle. However, at temperatures above 4°C denaturation of the enzyme protein can occur. Therefore, it

is quite normal to work at temperatures below zero. This, however, requires large cooling capacity in industrial manufacture.

All these difficulties can be avoided by using polyethyleneglycol with a molecular weight of 6000. This precipitant does not effect enzyme denaturation and is relatively independent of temperature and electrolyte concentration. However, there is a strong dependence on hydrogen ion concentration. The best results are obtained at the isoelectric point of the enzyme to be precipitated.

As the solubility of a protein molecule is lowest at its isoelectric point, successive precipitation of different enzymes from a solution can be achieved by changing the pH. These precipitates settle easily and can easily be separated from the solution by centrifugation.

**Conversion to Storage Form:**

Storability of an enzyme requires the preparation of a suitable storage form. Commercial enzyme products are available either in solution or in solid state. Generally, users prefer solutions because of their easier handling, but enzymes are usually very unstable in aqueous solution.

For this reason stabilization of dissolved enzymes is a very important step in the manufacture of liquid enzyme preparations. The storage stability is affected by the following two factors- microbial deterioration of the enzyme solution and denaturation of the enzyme protein. These two problems seem to be closely related to each other.

Many treatments have been tried in order to prevent growth of microorganisms. The methods include, for instance, incorporation of chemical preservatives, pasteurization, addition of salts and polyhydric alcohols, and irradiation. But some of those treatments are undesirable due to legal aspects. Therefore, the most suitable method to repress microbial growth is to dissolve the enzyme in a highly concentrated solution of salts and sugars.

With liquid preparations, storage at low temperatures and at suitable pH is essentially inevitable. It is well known that substrates almost invariably protect the corresponding enzyme against physical, chemical, or physicochemical agents. This can be attributed to either conformational stabilization or steric or competitive protection.

From a number of publications it can be seen that almost any effect on enzyme stability may a priori be an allosteric one due to attack at sites other than the active site of the enzyme. For example- in thermolysin, a bacterial protease, Ca ions stabilize the enzyme molecule, while Zn ions are required for activity. The enormous value of Ca ions in stabilizing bacterial α-amylase has long been known.

A number of techniques are available for stabilization.

**Some of them are presented in the following list, immobilization methods excluded:**

(1) Conformational or charge stabilization and/or protection from dilution-dissociation by using buffers, glycerol, substrates, or inhibitors.

(2) Protection of active site thiol via disulfide exchange by thiols, redox dyes, oxygen-binding agents, or chelating agents.

(3) Miscellaneous methods include, e.g., inhibition or removal of proteolytic enzymes; protection from light by photosensitive dyes; lowering activity of water by viscosity effectors, salts, or sugars; lowering surface energy by antifoams; cooling and crystallization protection by antifreeze; removal of harmful agents; and sterilization for protection against microbial attack.

Commercially available solid enzyme preparations are dried mold brans, dried precipitates, or dried solutions. Spray drying is the preferred method for removal of water from enzyme solutions due to economic reasons. However, it is only applicable to enzymes sufficiently resistant to the temperature conditions of this process. On the other hand, freeze drying is most preserving, but its use is limited by cost considerations as well as by the fact that unless the salt concentrations of the enzyme solution are sufficiently reduced, eutectic mixtures may be formed.

This may lead to incomplete drying or to severe foaming and protein denaturation. A specific method of drying sometimes used is granulation in a fluidized bed with milk sugar or maltodextrin as carrier. In this case, of course, sufficiently high specific activity of the enzyme is required in order to ensure satisfactory activity of the commercial preparation.

**Enzyme Immobilization:**

In commercial applications enzymes are used commonly in the soluble or "free" form. This practice, however, is very wasteful, because the enzyme is discharged at the end of the reaction, although its activity is scarcely lessened in reactions carried out under optimum conditions.

Immobilization prevents diffusion of the enzymes in the reaction mixture and permits their recovery from the product stream by simple solid-liquid separation methods. As a consequence, reaction products are free of enzyme and reuse of the enzyme is possible. Another advantage of immobilized enzymes is that they can be used in continuously operated reactors.

**Methods of Immobilization:**

In principle, immobilization of an enzyme can be achieved by fixing it on the surface of a water-insoluble material, by trapping it inside a matrix that is permeable to the enzyme's substrate and products, and by cross-linking it with suitable agents to give insoluble particles. Bound enzymes may be prepared by covalent coupling to active matrices or by heteropolar and/or van der Waals binding to adsorbents or ion exchangers.

Covalent coupling to activated carrier materials is achieved by methods known in peptide and protein chemistry. Some examples of enzymes immobilized in this pattern are given in Table 15.3. The formation of covalent bonds has the advantage of an attachment which is not reversed by pH, ionic strength, or substrate. However, covalent binding offers the possibility that the

active site of enzyme may be blocked through the chemical reaction used in the immobilization reaction and the enzyme rendered inactive.

TABLE 15.3. ENZYMES IMMOBILIZED BY COVALENT COUPLING

| Enzyme | Carrier Matrix | Binding Agent/Reaction |
|---|---|---|
| α-Amylase | DEAE-cellulose | Direct coupling |
| Amyloglucosidase | DEAE-cellulose | Cyanuric chloride |
| Cellulase | Polyurethane | Isocyanate |
| Glucose isomerase | Polyurethane | Isocyanate |
| Glucose oxidase | Porous glass | Isothiocyanate |
| Invertase | DEAE-cellulose<br>Polyaminostyrene<br>Porous glass | Direct coupling<br>Polydiazonium salt<br>Polydiazonium salt |
| Lactase | Cellulose<br>Polyurethane<br>Sephadex | Cyanuric chloride<br>Isocyanate<br>Cyanogen bromide activation |
| Pectinase | Polyurethane | Isocyanate |
| Pronase | CM-Sephadex | Carbodiimide activation |

There are a large number of methods of covalent attachment. The groups of enzymes that take part in the formation of the chemical bond are- amino, imino, amide, hydroxyl, carboxy, thiol, methylthiol, guanidyl, imidazole groups, and the phenol ring. Methods have been developed for covalently attaching enzymes to inorganic carriers such as alumina, glass, silica, stainless steel, etc.

Adsorption of enzymes at solid surfaces (Table 15.4) offers the advantage of extreme simplicity. It is carried out according to the principles of chromatography. The conditions of adsorption involve no reactive species and thus do not result in modification of the enzyme. The binding of enzymes, however, is reversible and for this reason adsorbed enzymes present the problem of desorption in the presence of substrate or increased ionic strength.

Commonly used adsorbents include many organic and inorganic materials such as alumina, carbon, cellulose, clays, glass (including controlled-pore glass), hydroxyapatite, metal oxides, and various siliceous materials. Ion exchange resins bind enzyme by electrostatic interactions. The first successful commercial application of immobilized aminoacylase (for resolution of DL-amino acids) involved fixing of the enzyme by adsorption to DEAE-Sephadex as carrier.

TABLE 15.4. ENZYMES IMMOBILIZED BY ADSORPTION

| Enzyme | Carrier Matrix |
|---|---|
| α-Amylase | Calcium phosphate |
| Amyloglucosidase | Agarose gel, DEAE-Sephadex |
| Catalase | Charcoal |
| Glucose oxidase | Cellophane (followed by cross-linking with glutaraldehyde), inorganic adsorbents |
| Invertase | Charcoal, DEAE-Sephadex |
| Subtilisin | Cellulose |

Inclusion of enzymes in polymer gels, microcapsules, or filamentous structures has the advantage of relatively mild reaction conditions. This method is free from the risk of blocking active site groups on the enzyme molecule by chemical bonds; the enzyme is retained in its native state.

The major drawbacks of this immobilization technique are two- retardation of the enzymic reaction due to diffusional control of the transport of substrate and products (particularly with high molecular weight substrates and/or products); and continuous loss of enzyme due to the distribution of pore sizes. Materials used for entrapment include silicone rubber, silica gel, starch, and, preferably, polyacrylamides. Examples of enzymes immobilized by entrapment are given in Table 15.5.

TABLE 15.5. ENZYMES IMMOBILIZED BY ENTRAPMENT

| Enzyme | Polymer Matrix |
|---|---|
| α-Amylase (fungal) | Polyacrylamide gel |
| Amyloglucosidase | Cellulose triacetate, polyacrylamide gel, polyvinyl alcohol |
| Catalase | Cellulose triacetate, polyacrylamide gel |
| Glucose isomerase | Cellulose triacetate |
| Glucose oxidase | Cellulose triacetate, polyacrylamide gel |
| Invertase | Cellulose triacetate, polyacrylamide gel, polyvinyl alcohol |
| Lactase | Cellulose triacetate |
| Acid protease | Polyacrylamide gel |
| Alkaline protease | Polyacrylamide gel |
| Neutral protease | Polyacrylamide gel |

A variation of the inclusion method is encapsulation within semipermeable membranes. Materials such as collodion polystyrene, cellulose derivatives, and, most commonly, nylon have

been used to form thin, spherical, semipermeable membranes shaped into microcapsules which include the enzyme to be immobilized. The size of the capsules can range from μm to many μm. As has been demonstrated by Kitajima and Kondo (1971) with yeast, it is possible to encapsulate multi-enzyme systems from cell extracts and to carry out fermentation in such artificial cells.

Enzymes can be polymerized by cross-linking with low molecular weight multifunctional agents (see Table 15.6). This method leads to the formation of a three-dimensional network of enzyme molecules when the reaction is carried out in the absence of a support. However, usually it results in a considerable loss of activity. Commonly, enzymes are cross-linked after adsorption onto a suitable carrier.

TABLE 15.6. ENZYMES IMMOBILIZED BY CROSS-LINKING

| Enzyme | Cross-linking Agent |
|---|---|
| Catalase | Glutaraldehyde |
| Glucose oxidase | Glutaraldehyde and cellophane membrane |
| Rennin | Glutaraldehyde and aminoethylcellulose |
| Subtilisin | Glutaraldehyde |

Cross-linking agents most commonly used include diazobenzidine and its derivatives and particularly glutaraldehyde. On the other hand, enzymes can become immobilized by copolymerization, i.e., covalent incorporation into polymers. The methods most often employed involve copolymerization with maleic anhydride and ethylene. As with entrapped and microencapsulated enzymes, these derivatives show little or no activity toward macromolecular substrates.

An alternative to the immobilization of isolated enzymes is immobilization of whole microbial cells. This method provides a means of avoiding expensive enzyme purification operations. Entrapment of enzymes within whole cells may also be useful when various enzymes are involved in a given process.

And, finally, immobilized intact cells have proved effective in processes involving enzymes that require cofactors for mediating their catalytic action. Some examples of whole cell immobilization are shown in Table 15.7. Cells can be immobilized by fixing them to carriers, such as fibers or granular materials, or by entrapment.

**Properties of Immobilized Enzymes:**

After immobilization of an enzyme, its properties can be changed significantly. Such alterations may be attributed to (1) the physical and chemical nature of the carrier used, (2) the chemical and/or conformational changes in the enzyme structure, and (3) the "heterogeneous nature" of catalysis caused by immobilization.

TABLE 15.7. SOME IMMOBILIZED WHOLE CELL SYSTEMS

| Organism | Enzyme | Carrier Matrix | Immobilization Technique |
|---|---|---|---|
| Mold | Amino acid acylase | Cellulose nitrate | Entrapment |
| Fungal spores | Invertase | Cellulose | Adsorption |
| Mold | Glucose isomerase | Collagen | Complexation |

Effects of altered reactivity include kinetic constants (resulting from a change in activation energy), optimum pH, Michaelis constant, and substrate specificity. A matrix charge can affect the hydrogen ion concentration in the locus of the attached enzyme and thus change its apparent pH optimum in one direction or the other, depending on the use of either cationic or anionic carriers, and the apparent Michaelis constant if the substrates are also charged; it is increased if the matrix and the substrate charges are alike and decreased if they are opposite. Changes in enzyme specificity can result from conformational changes in the enzyme molecule caused by the attachment itself.

One of the more important results of enzyme immobilization is the retention of activity for considerable periods of time under suitable conditions of storage. The stability of immobilized enzymes to storage, heat, and pH basically depends on the nature of the carrier surface to which the enzyme is bound.

Among 50 immobilized enzymes, as compared with their soluble counterparts, Melrose (1971) found 30 more stable and 8 less stable than the soluble forms; 12 showed no difference from the free systems. The reasons for the observed increase in stability are not clear. It may be attributed, for example, to prevention of conformational inactivation or to shielding of active groups on the enzyme from reactive groups in solution.