RADIOIMMOBILIZATION OF BIOCOMPONENTS

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INTRODUCTION

Owing to the shortage of natural resources and energy since the late 1970's, biotechnology has been received increasing attention. The term biotechnology comprises the techniques of fermentation technology, enzyme engineering and genetic engineering. Resulting from the immobilization of enzymes and microbial cells, a rapid and widespread rise in enzyme technology is coming about and wider applications of biocatalysts are expected.

The applications of irradiation techniques in biotechnology are spreading throughout the world and a number of different methodologies are available using radiation processing for the immobilization of enzymes and other biomolecules, such as haemoglobin. The products may be used for diagnostic, therapeutic and bioprocesses (fermentation, bioseparation, etc.) applications.

IMMOBILIZATION TECHNIQUE

The immobilization technique has the purpose of containing or including a functional component in a limitted space. The aims of the immobilization are the fixation or inclusion - for repeated uses or continuous processes, the stability against heat, solvent and other external stimulations, the molding and shaping for convenient uses and systems, the controlled slow release and the enhanced cell culture (1).

In the case of enzymes, many of the characteristics that make them difficult to use "in vitro" can be overcome by their immobilization on a solid support. Usually, immobilized

enzymes are more stable to changes in pH and temperature than are their soluble counterparts. The separation of products and reactants is also quite easy since these compounds are frequently soluble and can simply be rinsed from the insoluble - catalyst material.

Enzymes can be immobilized on nearly any material either organic or inorganic. The method of immobilization is dictated by the support and enzyme required for the particular application.

One of the oldest methods of immobilization consisted of adsorption of the enzyme onto a charged surface, such as an-ion exchange resin. The enzyme also can be intermolecularly cross-linked to form a sheet of enzyme material on the surface of the support. Alternativelly, enzyme molecules can be covalently bonded to the surface of chemically reactive support materials or enzyme molecules may be entrapped in the interstices - of polymer structures by copolymerization of enzyme species - with the suitable monomer (2). Fig.1 illustrates the general methods of immobilization.

RADIATION POLYMERIZATION

Two different basic methods are currently being - used: the physical immobilization by entrapment in a polymer matrix produced by radiation polymerization of a mixture of monomer and bioactive material, and chemical immobilization by combining radiation initiated grafting of polymer surface with chemically active groups and subsequent chemical bonding of materials being immobilized.

For the immobilization of biologically active materials, especially enzymes, drugs and cells, radiation polymerization is the most versatile and has many advantages over chemical immobilization (3):

1) The enzyme and its activity are less affectec by radiation than the chemical treatment:

2) Frozen solutions can be polymerized and there is no problem with heat of polymerization;

3) Various degrees of porousness textures can be produced

in the resultant gel without addition of special agents;

4) The polymerization is both easy to carry out and rapid.

RADIATION CURING

The electron beam (E.B.) curing process is the result of a transfer of energy grom the electrons as they penetrate matter. This energy transfer initiates free-radical polymerization of coatings or adhesives causing an almost instantaneous cure.

Recent development in radiation polymerization involves radiation cure processes where mixtures of oligomers or monomers are polymerized into a thin homopolymer film in a fraction of a second using ionizing radiation from electron beam sources. During E.B. curing of such films, concurrent grafting can occur with the substrate. Such grafting during radiation cure processing improves the properties of the resultin product.

The possibility of using radiation curing processes with electron beam source for immobilization has been explored and found to be possible. The curing system possesses great potential for the current immobilization methods since cimplete polymerization is achieved in a shor time. Thus, for the immobilization of biomaterials, E.B. irradiation also possesses many advantages. These includes speed of processing, simplicity and uniformity of attachment of insolubilized reagent on the polymer surface.

APPLICATION OF RADIOPOLYMERIZATION FOR IMMOBILIZATION OF ENZYMES

Hydrophilic glass-forming monomer, 2-hydroxyethylmethacrylate (HEMA) was used for the immobilization of cellulase and cellobiase, two enzymes involved in biomass conversion, following the method described early by others (4).

By using the irradiation technique at low temperatures with checks for enzymatic activity before and after irradiation (10^4 Gy from a ^{60}Co source) no damage to the enzyme occurred. Pellets of immobilized enzymes in the polymeric support

were qualitatively assayed for protein content by the nynhydrin reaction, showing the occclusion level of the proteic enzymes throughout the matrix. The subsequent biochemical determination of enzymatic activity of the polymers correlated well with the initial ninhydrin assay (5).

The dried immobilized particle changed from a rigid matrix to an expanded porous one, when immersed in the aqueous suspension of substrate. The changes in enzymatic activity with repeated use of cellulase reaction with carboxymethyl cellulose as a function of batch enzyme reactions are shown in Fig.2.

APPLICATION OF RADIOPOLYMERIZATION FOR IMMOBILIZATION OF YEAST CELLS

The preparation of hydrophylic polymer pieces was performed via gamma irradiation (10^4 Gy) polymerization at low temperature (-78° C) using 33% 2-hydroxyethyl acrylate (HEA) in aqueous medium following the method already described by - Fujimura and Kaetsu (6). The immobilization of yeast cells on the polymeric disc were done by means of a low dose gamma irradiation ($5x10^2$ Gy) of the swollen slices imbibed with yeast containing medium and monomer. This treatment resulted in super position of thin layers of polymer newly produced on porous - carriers, giving a soft surface which allows the diffusion of the substrate, glucose, into the inside of the carrier. The ethanol productivity was evaluated by the increase of optucal absorption in the supernatant as a result of NAD-alcohol reaction in the presence of alcohol dehydrogenase. Table I shows the results of the assays of one hour fermentative capacity of immobilized yeast cells measured at different intervals after immobilization (7).

APPLICATION OF RADIATION CURING FOR IMMOBILIZATION OF ENZYME

Urease was immobilized on cellulose fibril sheets by radiation curing of tetradecaoxyethylene glycol diacrylate (A-14G), an hydrophylic monomer. This enzyme is the catalytic agent for the hydrolysis of urea. It is used in clinical diag-

Table I - One hour fermentative capacity of immobilized yeast cells measured at different intervals after immobilization.

Interval (h)	Alcohol production (g/l/g polymer)	
	30% HEA	50% HEA
24	4.8	6.0
48	5.4	5.9
76	7.0	7.7
144	6.7	6.0
168	5.4	6.0

nosis for the detection of malfunctioning of the kidney. Immobilized urease has been used in dialysis equipment for the adsorption or decomposition of urea in the blood.

Since urease is a very labile enzyme for irradiation,a protective agent, soy bean powder, was added in the immobilization procedure as shown in Fig.3. Thin filter paper was used as support for the urease solution mixed with monomer that was coated onto the sheet using micro-automatic pipette. Urease paper discs were irradiated in a low energy self-shielded type electron accelerator, in which the electron beam acceleration - voltage was 0.3 MeV. The irradiation at 5 mA electron beam - current and speedof the belt conveyor at 12.5 m/min, gsve the irradiation dose of 10^4 Gy. The samples were irradiated at room temperature.

Various properties of urease immobilized onto the cellulose sheets were evaluated. such as pH stability, heat stability and sustenance of activity. The optimum pH for the enzyme reaction of the immobilized urease was identical to the native one, while the activity of the former urease decreased - less than that of the latter one at both acidic and alkaline - sides of the optimum pH, as shown in Fig.4.

Up to 30° C, due to denaturation of the enzymatic protein, a decline in the activity was observed, more deeply - for the free enzyme while the immobilized enzyme was stable -

from 50 to 70° C, as shown in Fig.5. It indicates that the folded active structure in the immobilized enzyme was much more stable.

The maintenance of the immobilized enzyme activity was examined by repeated batch enzyme reactions. The relationship between activity yield and number of batch enzyme reactions is shown in Fig.6.

CONCLUSION

1) The immobilized enzymes retained significant activity showed by the maintenance of the products yield in the repeated batch reactions.

2) The immobilization onto filter paper discs is a simple technique and applicable to different biocompounds. It does need large amount of bioactive substance and the preparation conditions are mild and fast by the use of E.B.irradiation.

3) Immobilized enzymes are useful not only for medical and pharmaceutical purposes but for various processes in the food industry. The potential advantages of water insoluble enzyme derivatives are expected to lead to automated continuous flow processes and to reduce the cost of both enzyme and finished products.

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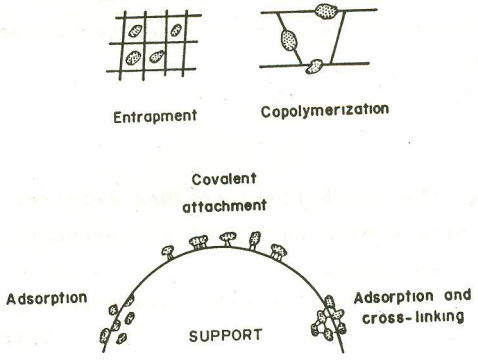

Fig.1 - General methods of immobilization

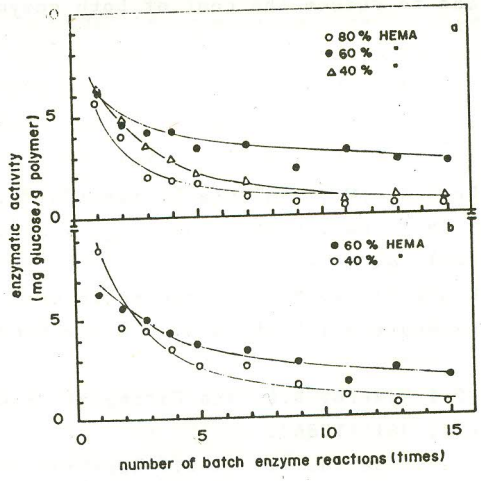

Fig.2 - Enzymatic activity as a function of repeated use of immobilized cellulase at different monomer concentrations. Substrate: 1% CMC. Enzyme: a) 10% b) 20%.

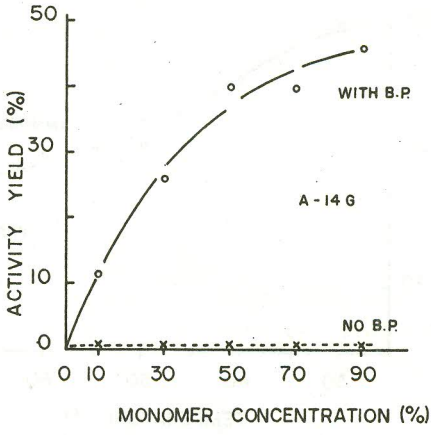

Fig.3 - Effect of addition of bean powder (B.P.) in the immobilization of urease. Monomer: A-14G. Bean powder:1% Enzyme: 0.025%. Coating volume: 2 ul. Irradiation : 10^4 Gy,r.t. Substrate: 0.2% urea.

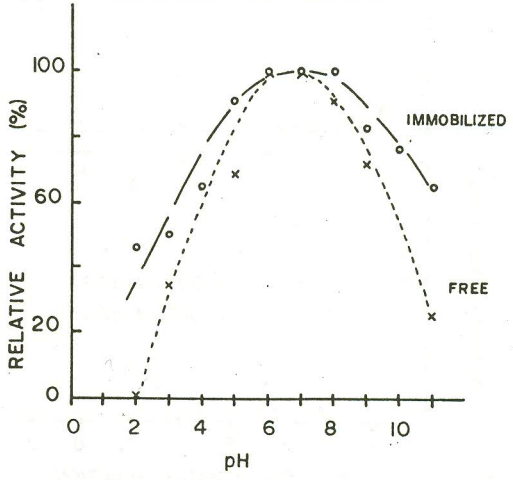

Fig.4 - pH stability of immobilized and free urease. Enzyme: 0.025%. Treatment time: 1 hour, 30°C. Substrate: 0.2% urea.

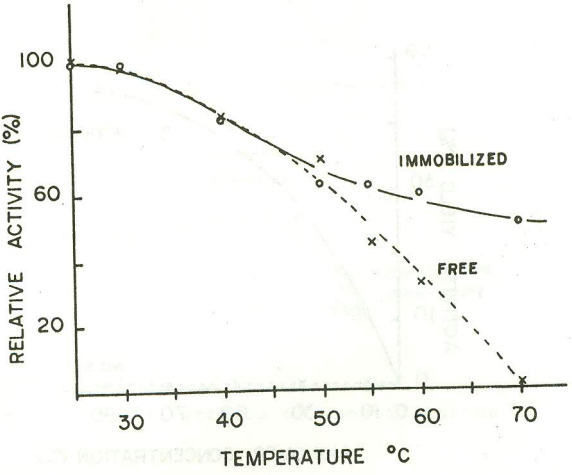

Fig.5 - Heat stability of immobilized and free urease.
Enzyme: 0.025%. Treatment time: 1 hour.
Substrate: 0.2% urea.

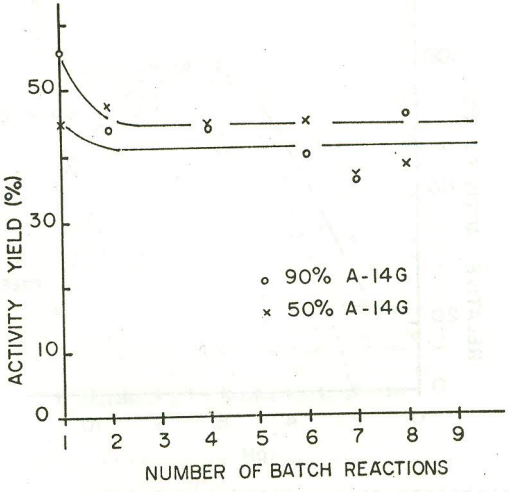

Fig.6 - Activity yield after repeated batch enzyme reactions.
Enzyme: 0.025%. Bean powder: 1%. Irradiation: 10^4 Gy,
Substrate: 0.2% urea.