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IMMOBILIZATION OF ENZYMES AND POTENTIAL APPLICATIONS IN FOOD INDUSTRY

ENZİMLERİN TUTUKLANMASI VE GIDA SANAYİNDE MUHTEMEL KULLANIM ALANLARI

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SUMMARY: In this review, a brief summary of the current technology of enzyme immobilization, commercial and potential applications in food industry are presented according to the recent scientific publications on the subject.

ÖZET: Bu derlemede, bugünkü enzim tutuklama teknikleri, günümüzde gıda sanayinde kullanılmakta olan veya kullanım olasılığı olan tutuklanmış enzimler hakkında kısa bir özet yakın geçmişe ait literatür bilgisine dayanarak verilmiştir.

INDRODUCTION

Immobilized enzymes may be defined as enzymes that are fixed on water insoluble inert supports. Immobilization of enzymes are carried out mainly due to the possibility of running enzymatic reactions continuously without a purification step to remove the enzyme from the product stream and to provide a more precise control of the reaction. Immobilized enzymes may exhibit enhanced pH, temperature, storage and operational stabilities and altered kinetic properties. They may be less sensitive than the native enzymes to inhibition by substrate and/or product. However, there are, several problems with immobilized enzymes, such as losses in activity, enzyme-substrate orientation, diffusional restriction problems and the cost of the immobilization step.

There is a variety of possible enzyme immobilization methods. These include physical adsorption, entrapment in natural or synthetic polymers, microencapsulation in a semipermeable membrane, covalent attachment to an inert support, ion exchange, incorporation directly into a polymer, intermolecular crosslinking of enzyme molecules and use of cells.

The first reported case of an immobilized enzyme was almost 80 years ago. The development of both theoretical concept and the laboratory use of immobilized enzymes has made slow but steady progress.

IMMOBILIZATION TECHNIQUES AND SUPPORT MATERIALS

The selection of a suitable immobilization method depends primarily on how the method affects the catalytic activity of the enzyme. Covalent and crosslinking methods create strong chemical bonds between the enzyme and the support. These methods can be relatively laborious and expensive. They may cause significant inactivation of the enzyme. In constrast, while methods of immobilization by adsorption and gel entrapment are simple and efficient, they do not create strong bonds between the enzyme and the matrix. Consequently, enzymes often leak from the supports. This problem can mostly be overcome by crosslinking the adsorbed or entrapped enzymes with glutaraldehyde.

Materials used as supports include both organic and inorganic materials. Organic supports do not have the most desirable flow properties and are often excessively affected by environmental factors like pH or deterioration by microorganisms. Cellulose, dextran, agarose, nylon, acrylamide-, styrene-, and maleic anhydride-based polymers are typical example of organic supports. Inorganic supports studied include porous and solid glass, diatomaceous earth, silica aluminas, sand and titania. These supports generally have fewer reactive sites than the organic supports but are more stable. They often have better flow properties. In some cases, organic materials have been first attached or immobilized to the inorganic support. This often has the advantage of obtaining the most desirable properties of both organic and inorganic supports in a single material.

Among the synthetic supports, many acrylic and vinylic polymers are used such as polyacrylamide, poly(hydroxyalkyl metharacrylates)s and poly(vinyl alcohol). A polymer has been synthesized by copolymerization of a mixture of acrylonitrile, acrylamide and methyl acrylate in a appropriate ratio and the coupled catalase showed a higher stability in comparison with the soluble enzyme (FILIPPO et al., 1990). Acrylamide and methyl acrylate were chosen as comonomers, because methyl acrylate provides the methyl ester groups which easily and selectively react with many chemicals and acrylamide gives marked hydrophilic character to the resulting polymer, very helpful in immobilization. Subsequent reaction of this polymer with a diamine or an amino-alcohol may be employed to obtain an amino- or a hydroxy-derivative which can further react with cyanogen bromide yielding a support capable of coupling for enzyme immobilization. The acrylic copolymer described can be easily prepared from cheap monomers, very durable against chemical and microbiological agents.

SKOVBY and KOPS (1990) reported an investigation on the porous beads that suspension polymerization was the method for synthesis of porous beads and these beads were suitable for physical binding of an enzyme. For preparation of the solid particles, suspension polymerization was chosen since a relatively uniform particle size distribution may be obtained. Such uniformity secure the flow and minimize the pressure drop over a packed column of such beads containing the enzyme. Porous beads were prepared by suspension polymerization of acrylic monomers and divinyl benzene in the presence of a porogen (octane, petroleum ether or cyclohexane). After preparation of beads, they were dried to remove porogen. Porosity varies greatly with the polymer composition, the amount and type of the porogen and the conditions for preparation. The enzyme binding capacity and activity has been determined for a lipase and the best results were obtained for crosslinked poly(methyl methacrylate) beads.

Most supports for enzyme immobilization are lattice type matrices and these restrict access of the substrate to the bound enzyme molecules. KUROTA et al. (1990) proposed immobilization by crosslinking enzymes adsorbed onto ion-exhangers not with crosslinking between enzymes and functional groups of ion exchangers, by using an ion exchanger as support. Trypsin was used in this study. Trypsin has positive charges below pH 10.5, therefore it was adsorbed only on the cation exchangers. Since cation exchangers have negatively charged groups they did not react with glutaraldehyde and adsorbed enzymes were crosslinked with each other by the glutaraldehyde treatment forming an envelope around the gel beads of the ion exchangers and inside the lattice caves. The method is an alternative to the immobilization of enzymes that are effective on macromolecular substrates, using large pore size supports.

Most of the supports for enzyme immobilization are artificial materials, and chemical reactions are also required to immobilize the enzymes in most cases. These support materials and chemical for immobilization are not be allowed to contaminate the final products, especially foods. Inexpensive edible proteins may be used as supports and the glycosylation products formed on these proteins can be used as the binding site for immobilization (KAMATA el al., 1990). For various proteins it was reported that in the initial stage of Maillard reaction nonenzymatic glycosylation of proteins occurs. In the later stages of the reaction, glycosylated proteins undergo complex chemical changes causing crosslinking. These crosslinking abilities can be applied to enzyme immobilization. The immobilization process is very easy, needing only high temperature incubation of the support with glucose or more conveniently with starch hydrolysate. Inexpensive protein can be chosen as suppor materials which compriese the major running cost of immobilized enzyme reactor systems. In the related reference, casein granules and dried egg white were used as support proteins for trypsin and α-amylase.

Cellulose is one of the ideal supports for immobilizing enzymes. For the immobilization of enzymes, reactive sites can be added to cellulose. However, this approach of producing such carriers is expensive and provides a potential for conformational change in the immobilized enzyme, resulting in decreased catalytic efficiency. An alternative to this approach is enzyme immobilization using a cellulose binding domain. Using molecular genetic techniques, a fusion protein has been produced which contains the cellulose binding domain of exogluconase from *Cellulomonas fimi* fused to a ß-glocosidase from *Agrobacterium sp.* (ONG et al., 1991). The simple stable coupling of any enzyme-cellulose binding domain fusion to a readily available inexpensive cellulosic support can be used for enzyme immobilization. The

absence of or minimal change in the conformation of an enzyme fused to the cellulose binding domain results in the maintenance of catalytic efficiency of the enzyme.

POTENTIAL USES OF IMMOBILIZED ENZYMES IN FOOD INDUSTRY

The commercialization of immobilized enzymes is slow and there are only a few industrial processes that have been commercialized as presented in Table 1. In principle they can be attributed to a combination of the several factors: soluble enzymes used in many industrial processes are relatively cheap; introduction of new capital equipment to existing processes is high; the disappointing performance in practice of immobilized systems in relation to the overall operational economy and plant design scale.

Table 1. Commercial Immobilized Enzymes[a]

Enzyme	Product	Immobilizing	Reactor type	Operating mode
Aminoacylase	L-Amino acids L-Amino acids	Adsorbed Membrane	Packed bed Membrane recyle reactor	Continuous Continuous
Aspartase[b]	Aspartate	Entrapped	Packed bed	Continuous
Fumarase[b]	L-malate	Entrapped	Packed bed	Continuous
Glucose isomerase	High fructose corn syrup	Adsorbed	Packed bed	Continuous
		Covalent	Stirred tank	Batch
Lactase	Lactose-free milk	Entrapped	Stirred tank	Batch

[a] Mehaia and Chervan (1990)
[b] Immobilized cells

Other uses of immobilized enzyme technology have been reported but have not been widely adapted. One is the use of immobilized α-galactosidase in the sugar beet indusry to remove raffinose from sugar beet extracts (OLSON and KORUS, 1977). This allows the crystallization of sucrose without interference from raffinose. Immobilized pectic enzymes have been suggested as clarifying agents for various fruit juices. LOZANO et al. (1987) developed a reactor configuration in which a cross-flow microfiltration system and an immobilized pectolytic enzyme system on a derivatized nylon membrane have been combined. The system allows depectinization and extraction of the clarified juice continuously and simultaneously. Pectinesterase and endo-D- polygalacturonase were covalently coimmobilized into a glycophase-coated controlled pore glass (ROMERO et al., 1988). Coimmobilization provided a more effective system than separately immobilized enzymes. Naringin is one of the bitter components of some citrus juices. Immobilized naringinase has been suggested for the removal naringin and fiber entrapment method could be most suitable for treatment of fruit juices (TSEN and YU, 1991).

Papain is used at present as a clarifying agent. However, when this proteolytic enzyme remains in beer for a long time, excess proteolysis occurs and this is undesirable. Utilization of immobilized papain has been studied for controlled treatment to prevent chill-haze in beer. Continuous preprocessing of alcoholic fermentation liquors using immobilized enzymes has also been investigated. In the production of beer, attempts have been made to hydrolyze starch by using immobilized amylases instead of malt, or to hydrolyze proteins or polypeptides by using immobilized proteolytic enzymes (CHIBATA et al., 1991).

Invertase could be immobilized by different methods for the production of invert sugar from sucrose. The enzyme was ionicaly immobilized on the poly(ethylene co-vinyl alcohol) hollow fiber inside surface, which was aminoacetalized with 2- dimenthylaminoacetaldehyde di-methyl acetal (SHIOMI et al., 1987). The enzyme reaction was carried out by letting the solutions pass or circulate through the inside of the hollow fiber. MANSFELD and SCHELLENBERGER (1985) investigated covalent binding of invertase via glutaraldehyde and benzoquinone to a macroporous polystyrene anion exchanger. The system are suitable for the production of glucose-fructose mixture in a packed-bed reactor.

Rennin is the milk-clothing enzyme from calf rennet used for cheese production. Continuous production of milk curd using immobilized rennin has been attempted. Rennin immobilized on CNBr-activated Sepharose or by carrier cross-linking using glutaraldehyde and AE-cellulose was prepared, and

studies on the continuous clotting of milk were carried out. Some problems remain regarding the stability of immobilized rennin and the type of reactor suitable for the recovery of curd (CHIBATA et al, 1991).

The application of immobilized enzymes for waste treatment has been studied for a number of years. Production of food from cellulosic residues involves depolymerization of the polysaccharides to sugars and related products. In recent years, extensive research has been directed towards developing enzymatic methods for the saccharification of cellulose. WILKINS and RAMESH (1991) investigated the biological conversion of wheat straw residue into ethanol. Saccharification was studied using a commercial enzyme preparation, Aspergillus niger, immobilized onto a support matrix-glass beads and S. cerevisia was used in the ethanol production by using fermentable sugars. This work provides information on the nature of treatments given to the substrate, enzyme stability and optimum operating conditions. Tests were conducted to ascertain the multiple usage of the immobilized enzyme and a loss in enzyme activity was observed. This study can be a model study for further investigations.

RUCKA and TURKIEWICZ(1989) studied the hydrolysis of sunflower oil by means of a hydrophobic membrane with lipolytic activity. Sorption of lipase on Polytetrafluorethylene (PTFE) membranes were used for lipase immobilization. Adsorption forces were not strong enough to retain lipase for a long time therefore adsorption was followed by cross-linking with glutaraldehyde.

High specifity of enzyme catalyzed reactions makes them ideally suitable for the production of complex chemicals which are difficult to synthesize by strictly chemical means. Immobilized enzyme technology could conceivably be developed to produce some complex molecules which are of importance in food industry and used in relatively small quantities such as flavor and color materials.

Futher industrial applications of immobilized enzymes can be grouped as those already in use or near application and those that will require a major research commitment before they can realistically become technically and commercially available.

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