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Bioprocesses with Immobilized Biocatalyst-Engineering Aspects

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ABSTRACT

This article outlines some of the factors influencing the choice of a suitable reactor for using immobilized biocatalysts. We have concentrated on biochemical engineering parameters of immobilized biocatalysts, which are important with respect to their application in industrial processes.

Index Entries: Immobilized biocatalyst; bioreactor; deactivation.

INTRODUCTION

In the last two decades, the use of immobilized biocatalysts has become more of a reality and less of a curiosity of research laboratories. The recent developments in immobilization have led to commercially viable applications in industry and diverse other fields of social life. Many potential applications of biocatalysts are investigated by industrial concerns and therefore are not always published in the literature. Immobilized biocatalysts also have the potential for future industrial and commercial use in many areas of the food and fodder, pharmaceutical and chemical industries, and chemical specialities in processes that have no current equivalents. The article reflects the present state of the art in engineering aspects of bioreactors with immobilized cells. A biochemical reactor cannot be designated without knowledge of the engineering characteristics of the immobilized catalyst. These include methods of immobilization, carrier properties, mass transfer phenomena and reaction kinetics, choice of bioreactor, deactivation, and operation stability.

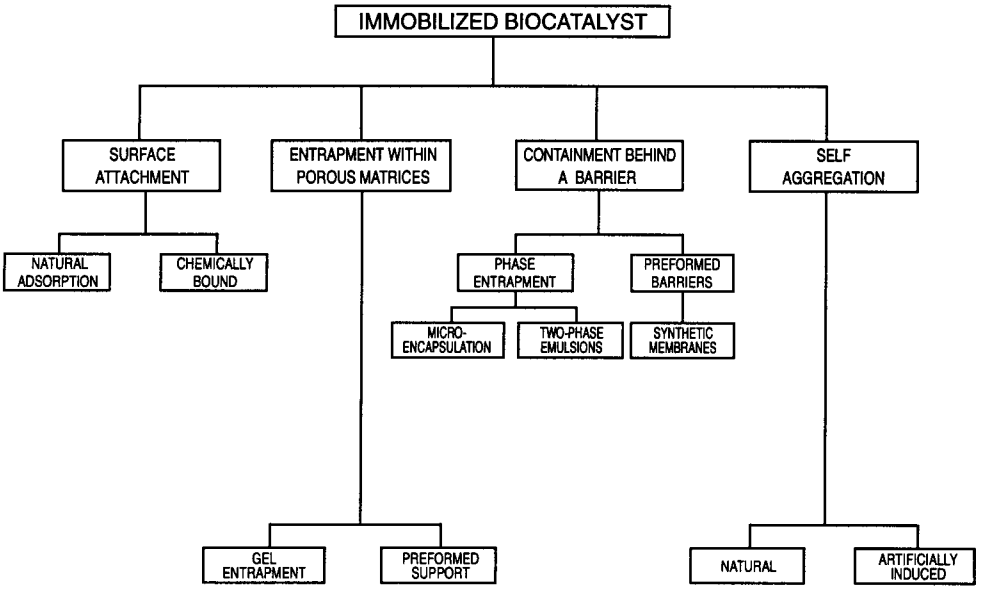

Fig. 1. Classification system for immobilization of enzymes and cells.

METHODS OF IMMOBILIZATION AND CARRIER PROPERTIES

There are several methods and techniques for immobilization of enzymes and cells (*1*). Their classification system is presented in Fig. 1. Immobilized enzymes and cells can be evaluated according to biochemical, physical, physicochemical, technological, and economic criteria. Specific activity can be considered as a fundamental biochemical criterion. Physical criteria include particle size or size distribution, shape, sedimentation velocity, fluidization features, mechanical stability, pore distribution, tortuosity, density, abrasion of particles, and compressibility. Optimal pH value, enzyme stability in relation to pH, and temperature are the most important physicochemical criteria for choice of carrier. Technological and economic criteria include the simplicity and number of operations in the preparation of biocatalysts, immobilization yield, stability during storage, operational half-time, raw material costs, equipment demands, and degradation of biocatalyst after using.

From a technological point of view for preparation of immobilized cells or enzymes is bottleneck the apparatus for biocatalyst particle production. If we need the volume of particles about cubic meter, it could be several problems for solution. Recommended apparatus are presented in ref. *2*.

Biocatalyst particles are usually compressible (alginate, carrageenan, pectate, and so on). Compressibility of the biocatalyst bed is why the height of the bed decreases by as much as 20% when the flow rates increase (3). So, appropriate attention should be paid to the pressure drop in the biocatalyst bed.

MASS TRANSFER PHENOMENA AND REACTION KINETICS

In heterogeneous biocatalytic reactors, biochemical reactions take on the external or internal surface of the catalyst. This depends on how the enzyme is immobilized, i.e., whether the biocatalyst is immobilized on the outer or the inner surface of the carrier. In order to participate in the reaction, the substrate has to overcome both the resistance against its passage through the porous structure of the biocatalyst (internal diffusion).

The concentration gradient between the outer surface of the carrier and the volume of the reaction medium is the driving force of external diffusion. When the reaction medium flows around the biocatalyst, a layer of liquid not disturbed by turbulence is formed near the surface of the carrier. Since the transport of substances in the laminar film is performed by molecular movements, it may be assumed that the resistance against the diffusion of the substrate is concentrated in the laminar film. It is evident that the thickness of the laminar film will depend on flow rates along the biocatalyst and will decrease with increasing film thickness. Hence, the film thickness depends on hydrodynamic conditions. Modeling of external mass transfer is presented in details in ref. *1*.

If the biocatalyst is entrapped in a porous carrier, then the substrate must diffuse through the pores to the surface of the biocatalyst. The products formed in the biochemical reaction must pass from the internal surface of the granule to its external surface. This transport of substances inside the carrier is called internal diffusion.

The driving forces of internal diffusion are the concentration gradients inside the granule in which the biocatalyst is immobilized. Concentration gradients inside the carrier may change the rate of biochemical reaction, substrate conversion, and so on. In this respect, three particular regions may be defined for the behavior of biocatalyst carriers.

In the kinetic region, the biochemical reaction proceeds at the same rate in the whole volume of the biocatalyst. Reaction rate is independent of carrier properties modulating the internal diffusion, such as pore size, granule density, pore shape, and the size of granules. The influence of diffusion is excluded.

In the region of internal diffusion, the reaction proceeds only in the layer below the surface of the carrier. A "nucleus of no use" is created inside the carrier without any biochemical reaction in this area.

The transient region lies between the kinetic and internal diffusion areas. The effectiveness factor, η_i, serves to quantify the effect of internal diffusion.

η_i = (actual apparent rate for the whole pellet) / (reaction rate evaluated without diffusion effects)

Consequently, η_i represents the ratio of the real reaction rate on the given carrier to the rate that could be reached if each molecule of immobilized enzyme was accessible to the substrate. In other words, it is the ratio of the reaction rate influenced by internal diffusion to the velocity with internal diffusion being excluded. In the kinetic region, the effectiveness factor is equal to one, whereas in the region of internal diffusion it is always below one.

CHOICE OF BIOREACTOR

The choice of an appropriate immobilized biocatalysts reactor is fundamental to the success of a process. The type of reactor chosen determines the type of immobilized biocatalyst preparation.

Batch reactor is particularly suitable for laboratory studies and/or for production of smaller amounts of products, e.g., for the pharmaceutical industry. Separation, usually filtration or centrifugation, causes losses in both the amount and the activity of expensive biocatalysts.

The advantage of *continuous stirred reactor* is its easy control. Filters preventing biocatalyst to pass out have to be placed in the output of the reactor. During continuous operation, the immobilized biocatalysts can be kept in a relatively constant environment, compared to a batch process. The major problem is related to the high rate of shear, which may severely damage the support particles, especially in the case of gel particles.

The most advantageous type of reactor is the *packed bed reactor*. Its advantages are easy attendance, high substrate conversion, and no friction of particles. This reactor is very useful for biochemical reaction with substrate inhibition. Among the disadvantages that should be mentioned: considerable pressure drop and problems with gas release by immobilized cells.

The last problem may be excluded by using a *fluidized bed reactor*. Biological fluidized bed treatment of water and wastewater has received considerable attention. Their attractive features include good mixing and transfer properties, thus gas–liquid contact and gas removal are facilitated.

For large-scale operations *air-lift bioreactors* are very attractive. They have shown low power consumptions and simple construction and operation. The use of air-lift bioreactors may be limited by particle density and friction difficulty.

The development of the *rotating disk and rotating cylinder reactors* is for immobilized aerobic cells. The support disks or cylinders are rotated in a trough partly filled with the growth medium.

Membrane reactors have been extensively used as enzyme reactors and immobilized cell reactors. Selectively permeable membranes have been used to divide reactors, either internally in one vessel or externally as part of a recycle loop, into separate compartments. It is obviously possible to construct an enormous variety of membrane reactors in the laboratory. How many of these could be scaled up to economic production volumes is a matter for considerable debate.

DEACTIVATION OF ENZYMES

One of the obstacles to the commercial use of immobilized enzymes in industrial biochemical processing is the loss of enzyme activity owing to deactivation. Therefore, it is important to determine the deactivation rate constant and study the performance of single immobilized enzyme catalyst under deactivation conditions. Deactivation of enzymes, especially in immobilized form, is a very important factor in the design of immobilized biocatalyst reactor. Enzyme deactivation using reactor operation may occur owing to one or more of several phenomena, such as thermal or pH stock, slow thermal denaturation, continuous exposure to inhibitory reaction product and/or substrate, formation of irreversible inactive complexes with substrate, microbial attack, and foreign materials, such as trace metals present in reaction solution.

Superimposed on these permanent losses of activity caused by interactions between the catalyst and the support are losses caused by the inefficient use of the biocatalyst activity, resulting from interactions between the support and solutes. Accessibility of substrate to the biocatalyst can be limited by slow diffusion from the bulk phase to the active site, or in extreme instances, the total exclusion of large substrate molecules by a microporous barrier. Some active sites may be occluded either by other large molecules, or because of the orientation of the enzyme on the support. The specific orientation of the substrate on the active site may also require a larger pore diameter than simple diffusion might imply.

CONCLUSION

Immobilized biocatalyst technology is now a mature technology in which application of the technique is of greater interest then the development of new methods. There is still a lack of fundamental engineering studies, which could be applied to analyze the overall performance of bioprocesses with immobilized biocatalyst.

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