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Immobilized Biocatalysts

The Research Program at Helsinki University of Technology

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Abstract

Recent work on immobilized biocatalysts at Helsinki University of Technology, Finland, is described, with starch processing, β-galactosidase, glucose isomerase, invertase, and the immobilization of live cells as special examples.

Index Entries: Immobilized biocatalysts; biocatalysts, immobilized; starch hydrolysis; immobilized cells; cells, immobilized; β-galactosidase; β-glucanase; glucose isomerase; invertase; catalysis, biological, by immobilized enzymes and cells.

Introduction

The beginning of a long tradition in the food industry applications-oriented research into enzyme and fermentation biochemistry and technology dates back to 1931, when the late professor Artturi I. Virtanen was invited to the first Chair of Biochemistry in Finland at the Helsinki University of Technology, Department of Chemistry, at a time when he was already developing the ideas that later brought him the Nobel Prize in biochemistry. Much of this early work already pointed to a promising future in what is today called as "enzyme engineering." Consequently, it is not surprising that enzymes, and immobilized biocatalysts in particular, are at the focal point of research interests in the Department's Laboratory of Biochemistry and Food Technology today. The research program includes improvements in

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and developments of immobilization techniques for various applications involving immobilized enzymes, multienzyme systems, cells, or live microorganisms for heterogeneous fermentation. Considerable importance is seen in process kinetics investigations for process optimization and control. Much of the work is applications oriented and shall be illustrated with a few examples.

Cellulose as Biocatalyst Carrier

Although a number of cellulose derivatives has been employed for the immobilization of various enzymes (*1*), it soon became evident that a suitable inexpensive carrier for whole cell entrapment under mild conditions would have definite advantages. Traditional methods for cellulose solubilization resulted in rapid enzyme inactivation. However, we succeeded in developing such solvent systems, based on *N*-ethylpyridinium chloride (NEPC) and dimethylformamide (DMF) or dimethylsulfoxide (DMSO), for α-cellulose that allowed the regeneration of the biocatalyst of high cell loading as beads from water (*2–7*). The resistance to all but cellulolytic enzymes, and their hydrophilic nature, made such biocatalysts suitable for a variety of applications. No bed compression nor particle deformation took place even during long periods of continuous column reactor operations (*4*). Similar techniques were also developed to prepare bead-shaped biocatalysts using cellulose di- or triacetates as carriers or in other applications (*7*). Activity yields of up to 85% were obtained, depending on the enzyme involved. The kinetic behavior of such biocatalyst systems for practical applications have been investigated in detail (*7, 8*).

Starch Processing

Our investigations on the effects of immobilization on enzyme kinetics and action pattern revealed that in certain cases the reaction mechanism changed on immobilization. Thus we observed that the action pattern of α-amylase was altered when the enzyme was covalently bound to cyanogen bromide-activated carrier (*9*). Although immobilized α-amylase may not be among the first materials to be applied in large-scale starch processing, the observation may lead to special applications, such as the manufacture of enzymatically modified starches. On the other hand, immobilized glucoamylase was found to offer an attractive alternative to soluble enzyme technology in continuous starch processing (*10, 11*).

β-Galactosidase

Our early work on heterogeneous biocatalysis included the immobilization of fungal *Aspergillus niger* β-galactosidase for the hydrolysis of whey lactose (*12*). The method developed has been commercialized, and a 600 L reactor system with phenol–formaldehyde–bound glutaraldehyde–crosslinked enzyme has been suc-

cessfully employed by dairy industry to process 40,000 L of whey daily. Considerable attention has been paid to the microbiological stability and process optimization of the system (*13*). On the other hand, soluble enzyme technology is still preferred in treatment of neutral dairy products such as milk (*14*), although we have recently been able to obtain promising results with immobilized *Kluyveromyces fragilis* whole cell yeast β-galactosidase (*15*).

Glucose Isomerase

Finland was among the first sites in Europe to apply (in 1975) continuous immobilized enzyme technology to High Fructose Syrup production in industrial scale. Consequently, immobilized glucose isomerase has been used as a model system in much of our kinetic research, and in the development of immobilization techniques (*3,4,16*). More recently we have investigated possibilities for increasing the fructose content of product above the normal 42–45%, and found that conversions of up to 90% could be obtained with 0.5*M* glucose containing 0.2*M* sodium tetraborate, and of up to 65% when a strong anion exchange resin in borate form was employed mixed with equal volume of biocatalyst beads in the reactor (*17,18*). The desired fructose content could also be obtained by processing with ion exchange resin in bisulfite form. The technique developed allowed continuous recycling of the glucose fraction because the higher glucose oligomers were removed in the fructose fraction. Futhermore, we have shown that immobilized glucose isomerase may be employed to increase the sweetness of β-galactosidase-hydrolyzed lactose syrup (*19–21*).

Invertase

Saccharomyces cerevisiae yeast cells of high invertase activity could be easily grown and entrapped in cellulose di- or triacetate or in alginate beads (*7,22,23*). The biocatalyst could be used for continuous treatment of up to 50% (w/w) sucrose solution or molasses, and the operational stability was excellent with extrapolated half-life of about 5 years. If crosslinking with glutaraldehyde was used, the ethanol fermentation mechanism was inhibited, allowing the utilization of whole cells for sucrose hydrolysis.

β-Glucanase

Recently, considerable efforts have been directed towards developing economic means for enzymatic hydrolysis of cellulose (*24*). However, few reports have been published on the immobilization of β-glucanases for the hydrolysis of soluble β-D-glucan polymers. We have shown that commercially available β-glucanases may be successfully immobilized by simple adsorption on phenol–formaldehyde resin. The immobilized biocatalyst is very stable after crosslinking with glutaraldehyde,

and can be used for continuous treatment of barley malt wort in a column reactor to improve viscosity and filtrability characteristics (*25–27*).

Immobilized Live Cells

All previous examples have involved relatively simple single-enzyme systems with no cofactor regeneration requirements. The immobilization of live cells would offer several additional advantages and possibilities, in particular the utilization of native multienzyme systems with cofactor regeneration *in situ*. Biocatalysts obtained by entrapment or adsorption of cells under conditions mild enough to preserve biological activity would provide a means for designing fermentation processes on a heterogeneous catalysis basis. We have employed entrapment in calcium alginate gel beads in most of our work with live cell immobilization. Such a biocatalyst with *S. cerevisiae* yeast has been shown to ferment glucose to ethanol with nearly 100% yield in a packed-bed column reactor (*28–30*). Pure glucose, molasses, sulfite spent liquor, cellulose and other polysaccharide hydrolyzates, and so on, may be used as substrates, and the technique can be also applied to alcoholic beverage manufacture. Other immobilized live cell applications investigated include the production of lactic acid with immobilized *Lactobacillus delbrueckii* with $CaCO_3$ for pH control, of citric acid with immobilized *A. niger* ATCC 9142 in a continuously mixed air-lift type of reactor, and of gluconic acid with immobilized *A. niger* NRRL 3 in a trickle-filter type reactor (*28, 29*).

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