Industrial Enzyme Encapsulation Into Polyvinylalcohol Matrix – Lentikats® Biocatalyst

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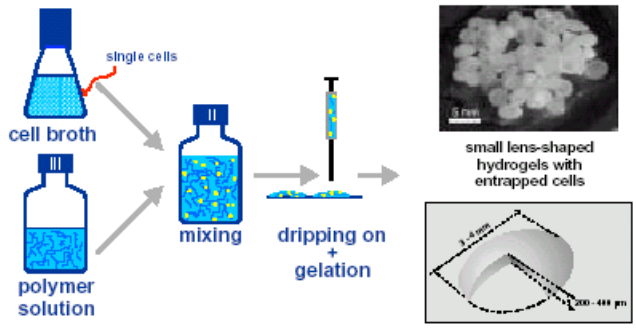
Introduction

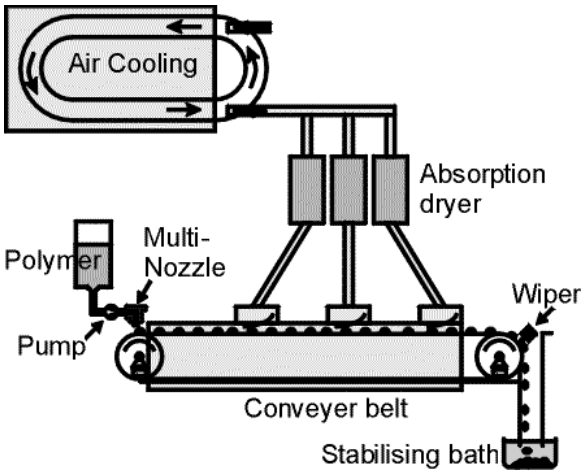
The method for industrial production of biocatalysts with biological material in the form of immobilized enzymes, or microorganisms immobilized into polyvinyl alcohol (PVA) gel, and their use is presented. The encapsulation of enzymes and bacteria using different kind of matrixes is known in the field. For industrial can be used the following matrixes: a) gels dissolved in water-encapsulation in hot water (agar, gelatine), b) gels (polysaccharides) isolated from seaweed (alginate, carrageenan), inorganic gels (silicic acid), built-in matrix of synthetic gel created by polymerization: polyacrylamide crosslinked by N,N/- methylene-bis(acylamide) and polyvinylalcohol (PVA) gelated by boric acid, cryotechnique and drying. The procedure for the production of a biocatalyst with biologically active material in the form of microorganisms, enzymes, spores and/or cells, which are placed in polyvinylalcohol gel is described in many publications. PVA-gel as a gel carrier for biologically active material is highly suitable for the production of chemical or biological robust biocatalysts. PVA-gel in the form of Lentikats® biocatalyst provides excellent physical and mechanical characteristics, enables long-term mechanical stability and PVA matrix is hardly biodegradable and non-toxic. In principle into robust Lentikats® biocatalyst is possible to encapsulate free cells (dead cells, viable non growing cells, viable and growing cells), free or immobilized (aggregated) enzymes. Size of Lentikats® biocatalyst porous is possible to change in accordance with additive type (size of macromolecule), additive concentration and PVA concentration.

Material and methods

Manufacturing procedure is shown in the following pictures and schemes and schemes:



Pic.1: Robust Lentikats biocatalyst principle production scheme



Pic.2: Robust Lentikats biocatalyst manufacturing scheme

Results and Discussion

The Lentikats® biocatalyst manufactured according to this presented industrial manufacturing proves robust biocatalysts with higher mechanical stability compared to other known biocatalysts, particularly in terms of abrasion resistance, tensile strength, biodegradability and mechanical stability. Owing to the above mentioned improved mechanical properties, the production of Lentikats biocatalyst in a reactive and kinetically suitable lens-shaped form is done. So the Lentikats® biocatalyst produced is firm and abrasion resistant for more than several months, even at high revolution stirring, compared to those formerly known. Due to the lenticular shape characterized by a large diameter and low height, physically, chemically or biologically active material is always situated closely beneath the surface, which provides its reactive and kinetically useful arrangement with high enzyme activity.

The usage is presented for the following biotransformation and processes:

Biotransformation of nitriles by *Rhodococcus equi* A4 immobilized in Lentikats® biocatalyst: whole cells of *Rhodococcus equi* A4, a producer of nitrile hydratase/amidase activities, were immobilized in lens-shaped hydrogel particles, Lentikats®. The immobilized biocatalyst was applied to the biotransformation of benzonitrile, 3-cyanopyridine, *(R,S)*-3-hydroxy-2-methylene-butanenitrile and *(R,S)*-3-hydroxy-3-phenyl-2-methylenepropanenitrile. The operational stability of the nitrile hydratase was dependent on the type of the substrate. The enzyme was the most stable during the transformation of *(R,S)*-3-hydroxy-2-methylene-butanenitrile. No significant loss of the amidase activity was observed within the biocatalyst operation.

Encapsulation of crosslinked penicillin G acylase aggregates in Lentikats® biocatalyst: the encapsulation of crosslinked enzyme aggregates (CLEA) of penicillin G acylase into a very rigid polymeric matrix based on PVA has been used successfully to improve the inadequate mechanical properties of CLEA. This encapsulation decreased CLEA activity by only around 40%. As compensation of decreased determined activity (about 78-100 U/g wet weight, 600-650 U/dry weight) a significant improvement in the long time stability of the CLEA in the repeated batch conversions (more then 600 repeated batch conversions) was detected. This permitted great improvement in the biocatalysts usage is used for hydrolysis of penicillin G to 6-APA production. Even more importantly, CLEA productivity is improved by using Lentikats® biocatalyst with PGA activity-encapsulated CLEA. Thus, this very simple technique not only provides an efficient

technique for solving the mechanical stability problem associated with CLEA, but also greatly improves the separation of CLEA from liquid media.

Yeast immobilization in Lentikats® biocatalyst for xylitol production from sugarcane bagasse: PVA-hydrogel matrix for yeast cell immobilization for xylitol bioproduction from sugarcane bagasse was tested. Five repeated-batch fermentation runs were carried out in medium based on sugarcane bagasse hemicellulosic hydrolysate with reuse of the entrapped biocatalyst. The system performance as well as the metabolic behaviour of cells entrapped into the matrix were evaluated. The biocatalyst remained stable and exhibited a similar fermentative profile in all the successive batches, demonstrating the viability of the system. At the end of the run, an average xylitol production was observed of 35.1 g l−1 and average xylitol yield and productivity of 0.58 g g^{-1} and 0.49 g l^{-1} h^{-1}, respectively.

A simple entrapment of glucoamylase into Lentikats® biocatalyst as an efficient catalyst for maltodextrin (particularly hydrolyzed starch solutions) hydrolysis: the glucoamylase from *Aspergillus niger* was immobilized into a poly(vinylalcohol) hydrogel lens-shaped capsules Lentikats® biocatalyst. Immobilization broadened the pH optimum of the enzyme. The glucoamylase activity increased after immobilization (approximately 1.5 times on maltose), which reflected the decrease in affinity of enzyme for its substrate. Immobilized enzyme retained excellent long-term operational stability for both, batch and continuous mode of hydrolysis. Specific enzymatic activity of the immobilized enzyme (0.67 $g.g^{(-1)}$ $h^{(-1)}$) was constant for 63 days of continuous operation at 45 degrees C.

Sucrose solution hydrolysis for glucose-fructose syrups production by the help of immobilized invertase: the invertase (SIGMA) was immobilized into PVA hydrogel lens-shaped capsules Lentikats® biocatalyst. Immobilization broadened the pH optimum of the enzyme. 12 % of volume of Lentikats® biocatalyst was added into 100 liters of saccharose solution with the concentration of 450 g/l (pH = 4.5) at the temperature of 30ºC. Hydrolysis occurred in the batch way, continuously stirred. The residual saccharose concentration is 10.1 g/l in that way after 360 minutes. Immobilized enzyme retained excellent long-term operational stability for both, batch and continuous mode of hydrolysis.

Lactose solution hydrolysis and the production of D-galactose, D-glucose and galactooligosaccharides from lactose solutions by the help of immobilized β-galactosidase: the invertase (SIGMA) was immobilized into PVA hydrogel lens-shaped capsules Lentikats® biocatalyst. Immobilization broadened the pH optimum of the enzyme. The lactose solution (pH = 6.5) and with addition of 2mM $MgCl_2$ was used in hydrolysis. 13% of weight of Lentikats® biocatalyst with β-galactosidase activity with the initial activity about 786 $U.cm^{-3}$ was added into 100 liters of thus prepared solution with lactose concentration of 100 g/l. Hydrolysis occurred continuously stirred (200 rev/min.) in the batch way at the temperature of 30ºC. The duration of conversion was constant. The Lentikats® biocatalyst activity decrease by 5% to 10% was observed after 25 batch hydrolyses, which generally lasted 160 hours. Immobilized enzyme retained excellent long-term operational stability for both, batch and continuous mode of hydrolysis.

Conclusions and References

PVA as a matrix for use in encapsulation of bacteria or enzymes has the following advantages: is low cost and nontoxic, gelation is done under optimal, cell-sparing conditions, allows production of hydrogels with excellent mechanical properties, with no side effects on biotransformation and fermentation, with high capacity for cell loading, is easily controllable, durability.

Finally the presented method for industrial production of biocatalysts with biological material in the form of immobilized enzymes, or microorganisms immobilized into polyvinyl alcohol (PVA) gel, and their use brings for end users economic benefits: improved return on investments (increased efficiency in final product production while dramatically reducing manufacturing investments), quality and durability (reusable catalysts, higher production stability), generated profit (decreased operational costs and increased revenues), environmentally friendly (reduced pollution and byproduct volume).