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Membrane Bioreactors for Biotechnology and Medical Applications

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Biological membranes are well organized systems of lipids, proteins, and carbohydrates. They regulate the composition of the intracellular medium by controlling the flow of nutrients, waste products, ions, etc. into and out of the cell. The function of membrane proteins is mainly to allow the transport of ions and polar substances that would not otherwise permeate through the non polar membrane core.

The combination of artificial membranes with macromolecules of biological origin allow to obtain hybrid systems capable to imitate what nature has fine tune through aeons of evolution. In fact, these systems, based on their selective transport and catalytic properties, are able to carry out chemical reactions, selective separation, waste removal, etc.

The use of membranes and biological tools for improving traditional production systems is a possible approach for maintaining a sustainable growth. Typical examples include novel pharmaceutical products with well-defined enantiomeric composition; new foods with improved nutritive properties; medical devices for the care of crucial diseases; and the treatment of wastes.

Membrane reactors using biological catalysts can be used for production, processing and treatment operations. The recent trend towards environmentally friendly technologies makes membrane reactors particularly attractive because they do not require additives, are able to function at moderate temperature and pressure, and reduce the formation of by-products.

The catalytic action of enzymes is extremely efficient and selective compared with chemical catalysts; they demonstrate higher reaction rates, milder reaction conditions and greater stereospecificity. Many enzymes and micro-organisms have been used in membrane reactors to catalyze bioconversions for various application [1-18]. Biocatalytic membrane reactors can combine selective mass transport with chemical reactions and the selective removal of products from the reaction site increases the conversion of product-inhibited or thermodynamically unfavorable reactions.

In addition to the type of immobilization, biocatalytic membrane reactors are commonly distinguished on the basis of their operation mode. For example, ultrafiltration membrane reactors (UF-MR), two separate phase membrane reactors (TSP-MR) (or biphasic organic/aqueous membrane reactor) and so on. Ultrafiltration membrane reactors are used when the substrate has a high molecular weight compared with the product, and both are soluble in the same solvents. Hence, the product can be separated as it is formed. When the substrate and product have similar molecular size, they both pass through the membrane. Therefore, it is necessary to match the transport rate with the reaction rate to ensure that as the substrate reaches the enzyme it is converted and the product is transported to the other side. If the substrate has a different solubility compared to the product (as in the case of an ester and its hydrolysis products), then a biphasic membrane reactor can be used [19]. Typical reactions that require biocatalysts suspended in a solution include: (1) the hydrolysis of starch (by α- and β-amylase); (2) the fermentation of sugars (by yeast); (3) the hydrolysis of pectins (by pectinase); (4) the hydrolysis of K-casein (by endopeptidase); (5) the hydrolysis of collagen (by protease); and (6) the coenzyme-dependant reactions [20, 21]. In these cases, the biocatalyst is continuously flushed along the membrane which functions as a separative barrier removing the reaction products while keeping substrate and biocatalyst in the reaction bulk phase.

Immobilization has proved to increase stability of biocatalysts, although it may cause changes in the catalytic activity and enantioselectivity [22-24]. This is a common observation, nevertheless, it cannot be considered a general rule of the inverse relationship between stability and activity and enantioselectivity of immobilized enzymes. Most probably, these effects are related to the interactions between the chemical groups of enzyme and membrane: due to these interactions the molecule becomes more rigid, and therefore more stable, but the loss in flexibility might damages the specificity and activity.

The progresses made in the last years in membrane engineering and in the understanding of basic mechanisms for membrane transport phenomena, have a significant fall out not only in the industrial separation processes, in desalination, in gas treatments but also in biotechnology and in biomedical engineering.

The potential advantages of membrane technology over more conventional approaches, include higher efficiency and reduced costs owing to the integration of bioconversion and product purification, thus reducing equipment costs and processing steps as today requested by a process intensification strategy.

Enzymatic membranes is also contributing to the growth of new research area, such as nonaqueous enzymology, the use of antibodies as highly specific catalysts, use of biomimetic catalysts (such as cyclodextrines) and the development of novel biosensor for diagnostic purposes. The results reached in the development of biocatalytic membrane reactors for the industrial applications and for the realization of artificial organs are important examples. Further progresses will be realized by the integration of these systems, by the study of new more selective and resistant organic and inorganic membranes, by their further miniaturization [25].

References

[1] Takamatsu S et al. Production of L-alanine from ammonium fumarate using immobilized microorganisms - elimination of side reaction *Eur. J. Appl. Microbiol. Biotechnol.* 1982; 15: 147

[2] Boyaval P and Corre C. Continuous lactic acid fermentation with concentrated product recovery by ultrafiltration and electrodialysis *Biotechnol. Lett.* 1987; 9: 207-212

[3] Cantarelli C et al. Beverage stabilization through enzymatic removal of phenolics *Food Biochem.* 1989; 3: 203-213

[4] Illanes A et al. Immobilization of lactose for the continuous hydrolysis of whey permeate *Bioprocess Eng.* 1990; 5: 257-262

[5] Ikemi M et al. Sorbitol production in charged membrane bioreactor with coenzyme regeneration system:I. Selective retainment of NADP(H) in a countinuous reaction *Biotechnol. Bioeng.* 1990; 36: 149-154

[6] Battistel E et al. Enzymatic resolution of (S)-(+)-naproxen in a continuous reactor *Biotechnol. Bioeng.* 1991; 38: 659-664

[7] Livingston JA. A novel membrane bioreactor for detoxifying industrial wastewater: II. Biodegradation of 3-chloronitrobenzene in an industrially produced wastewater, *Biotech. Bioeng.* 1993; 41: 927-936

[8] Timmer JMK and Krompkamp J. Efficiency of lactic acid production by *Lactobacillus helveticus* in a membrane cell recycle reactor, *FEMS Microbiology Review* 1994; 14: 29-38

[9] Bouhallab S and Touze C. Continuous hydrolisys of caseino-macropeptide in membrane reactor: kinetic study and gram-scale production of antithrombotic peptides *Le Lait* 1995; 75: 251-258

[10] Moueddeb H et al. Membrane bioreactor for lactic acid production. *J. Membr. Sci.* 1996; 114: 59-71

[11] Alvarez V et al. Microfiltration of apple juice using inorganic membranes: process optimization and juice stability *Can. J. Chem. Eng*. 1996; 74: 156-159

[12] Giorno L et al. Study of fouling phenomena in apple juice clarification by enzyme membrane reactor *Sep. Sci. Technol*. 1998; 33: 739-756

[13] Giorno L et al. Study of enzyme membrane reactor for apple juice clarification *Fruit Processing* 1998; 8: 239-241

[14] Boyaval P, Bouhallab S, Lafforgue-Delorme, Goma G. Bioréacteurs à membrane, In *Les séparations par membrane dans les procédés de L'industrie alimentaire*, G. Daufin, F. René and P. Aimar (eds.), pp. 534-572, Lavoisier Tec & Doc, Publishers, London, New York, 1998

[15] Burton SG, Boshoff A, Edwards W, Rose PD. Biotransformation of phenols using immobilized polyphenol oxidase, *J. Molecular Catalysis B: Enzymatic* 1998; 5: 411-416

[16] Giorno L, Drioli E, Carvoli G, Cassano A, Donato L. Study of an enzyme membrane reactor with immobilized fumarase for production of L-malic acid, *Biotechnol. Bioeng.* 2001; 72(1): 77-84

[17] Liu W, Howell JA, Arnot TC, Scott JA. A novel extractive membrane bioreactor for treating bio-refractory organic pollutants in the presence of high concentrations of inorganics. Application to a synthetic acidic effluent containing high concentrations of chlorophenol and salt, *J. Membr. Sci* 2001; 181(1) 127-140

[18] Giorno L, Chojnacka K, Donato L, Drioli E. Study of a cell-recycle membrane fermentor for the production of lactic acid by *Lactobacillus bulgaricus*, *Ind. Eng. Chem. Res*. 2002; 41: 433-440

[19] Matson SL, Quinn JA. Membrane reactors, In *Membrane Handbook*, WSW Ho and KK Sirkar (eds.), Chapman and Hall Publisher, New York, 1992

[20] Drioli E, Giorno L. *Biocatalytic Membrane Reactors: Applications in Biotechnology and the pharmaceutical industry*, Taylor & Francis Ltd Publs London, 1999

[21] Sirkar KK, Shanbhag V, Kovvali S. Membrane in a reactor: a functional perspective, *Ind. Eng. Chem. Res.* 1999; 38: 3715-3737

[22] Chibata I, Tosa T, Sato T, Application of immobilized biocatalysts in pharmaceutical and chemical industries, in *Biotechnology*, H.-J.. Rehm and G. Reed, eds., VCH-Verlags, Germany, Vol. 7a, Chapt. 12b, pp. 654-708, 1987

[23] Giorno L, Molinari R, Drioli E, Bianchi D, Cesti P. Performance of a biphasic organic/aqueous hollow fiber reactor using immobilized lipase, *J. Chem. Tech. Biotech*. 1995; 64: 345-352

[24] Bower ST, Cuperus FP, Derksen JTP. The performance of enzyme-membrane reactors with immobilized lipase, *Enzyme and Microbial Technology* 1997; 21: 291-296

[25] L. De Bartolo, L. Giorno, E. Drioli. Membrane bioreactors for biotechnology and medical applications in "New Insights into Membrane Science and Technology: Polymeric, Inorganic and Biofunctional Membranes" Edited by: Dr. D. Allan Butterfield and Dr. D. Bhattacharyya. In press.