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Biocatalysis and Membrane Reactors in the Pharmaceutical, Biotechnology and Waste Treatment Fields

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Over recent years, the use of biocatalysts has been progressively developed in many different fields, among which food processing, fine chemicals production, and organic synthesis, are the most representative [Drioli, 1999].

One of the technological problems to their wide use at large scale is their relatively low stability. To overcome this limit, studies are oriented to the use of thermostable enzymes, isolated from thermophiles, or to heterogenize the enzymes by loading them on supports that can improve their stability. Many scientific works in the literature evidenced the increase of stability of enzymes when immobilized on polymeric membranes as support. The advantage of immobilizing biocatalysts on membranes are also in the possibility to combine the biochemical reaction with the selective permeation through the membrane.

The integration of bioconversion and membrane separation, in so called biocatalytic membrane reactors, is a challenging opportunity for the production of high added value fine chemicals, such as pharmaceuticals, nutriaceuticals, food additives, etc. They also represent a feasible technology for the conversion of organic wastes into valuable products.

The use of biocatalysts is advantageous in terms of energy consumption, safety, pollution prevention, and materials prevention.

Besides expectations, the use of biocatalysts at industrial level is however not yet fully established. Nevertheless, some examples are known: the production of L-aspartic acid with *E. coli* cells entrapped in polyacrilamide [Chibata, 1974]; the lactase (β-galactosidase) entrapped into fibres of cellulose acetate, used for the hydrolysis of the milk and whey lactose [Pastore, 1976]; the synthesis of the dipeptide Aspartame™ realised using termolysin [Oyama, 1981]; the L-alanine produced by Tanabe Seiyaku using P*seudomonas dacunahe* immobilized with glutaraldehyde [Takamatsu, 1982]; the glucose-isomerase reticulate with glutaraldehide used for the production of fructose concentrated syrups [Carasik, 1983]; the L-amino acids production from racemic mixtures by means of an amino acylase immobilised on DEAE-Sephadex [Sato, 1993]; the production of L-malic acid with *Brevibacterium ammoniagenes* entrapped in polyacrilamide [Takata, 1993]; the production of (2R,3S)-*trans* isomer of methyl ester of 4-methoxyphenylglycidic acid (a chiral intermediate of diltiazem, an important calcium channel blocker used in the treatment of hypertention and angina) [Lopez and Matson, 1997].

Immobilised systems are not generically reliable, but their application needs individual investigations to overcome case by case the technological difficulties.

Various studies on the development of biocatalytic membrane reactors and their optimisation for the production of amino acids, antibiotics, anti-inflammatories, anticancer drugs, etc., in most cases as optically pure isomers, have been carried and are currently under development.

Many bioactive molecules are enantiomers, they are present in two different forms being one the non-superimposable mirror image of the other. The two enantiomers can have very different activity. Since recognition at molecular level is governed by chiral interactions, it is evident the importance of administering the proper enantiomer to living organisms and environment, in order to avoid undesired side effects.

The more and more clear necessity of pure enantiomers is orienting research efforts towards the development of new advanced technologies able to produce them at large scale. 25% of the drug market is, for example, represented by chiral molecules.

Although the most common method to produce enantiomers at industrial level is the chemical synthesis of a racemic mixtures followed by separation of the two enantiomers by diastereomeric christallization, new resolution processes are being considered. Among others, membrane processes are today investigated for this purpose. The coupling of enzyme enantiospecificity with membrane separation properties to obtain chiral membrane reactor systems, is one of the most promising strategies.

The catalytic action of enzymes is extremely efficient and selective with respect to ordinary chemical catalysts. Lipases have been widely used in multiphase reactions thanks to their ability to act at the organic/water interface with different substrates and to their resistance to organic solvents. Due to these peculiar characteristics, the lipases are feasible to be used immobilized at the interface of biphasic organic/aqueous membrane reactors. In these systems, the membrane provides the reaction site and fixes the interface, therefore it allows stable operation compared to heterogeneous emulsion systems. When the reaction products are soluble in water, this membrane reactor also acts as a separator and can simplify the downstream processing.

The major problems to overcome for their application at industrial level is the life time in terms of catalytic activity, enantiospecificity and stability. Immobilization has proved to increase stability, but it causes changes in the catalytic activity and enantiospecificity. Most probably, these effects are due to the interactions between the chemical groups of enzyme and membrane. The challenge is to obtain the proper interactions to guarantee a balanced efficiency between the molecular rigidity (that improves stability) and molecular flexibility (that improves the selectivity).
In this reference, a study on the effect of different membrane matrix, structure, morphology, pore size, additives, etc., on the catalytic properties of immobilized lipase has been carried out [Li 2002]. The kinetic resolution of racemic naproxen ester into (S)-naproxen acid has been used as a model reaction system. Membranes made of polyamide, polysulfone, and polypropylene, with symmetric or asymmetric structure, have been used. The pore size ranged from 0.2 microns to 10 kDa molecular weight cut-off. The performance of the enzyme-loaded membrane increased with the amount of enzyme immobilized. On the other hand, the pore dimension affected the loading capacity. Therefore, the immobilization on the thin layer of membranes with low molecular weight cut-off is not recommended for this kind of systems.
Furthermore, the influence of fluid dynamics conditions and physical-chemical properties of reaction mixture on the performance of the two-separate phase membrane reactor has been investigated. The efficiency has been evaluated in terms of productivity, enantioselectivity and stability. The influence of operating conditions (such as pH, temperature, substrate concentration, enzyme concentration etc.), immobilization procedure, and type of membrane on the catalytic activity and enantioselectivity of immobilized enzyme has been investigated. Membranes made of polyamide, polysulfone and polypropylene have been used [Giorno 1995, 1997; Sakaki 2001].
The performance of immobilized lipase has been also investigated in an o/w emulsioned membrane reactor, where enzyme is immobilized on polyamide membrane. The o/w emulsion was fed to the enzyme-loaded membrane in a cross-flow operation mode. Only water could permeate through the membrane whilst the organic phase (containing the racemic ester) was retained. The measure of (S)- and (R)-naproxen in the permeated aqueous phase as a function of time, allowed to calculate the enantiomeric excess of the produced naproxen acid. The performance of the two type of membrane reactors has been compared. Furthermore, a comparison between the enzyme-loaded membrane rectors and the free enzyme suspended in an o/w emulsion stirred tank reactor has been carried out.
As general results, the immobilized enzyme was more stable compared to the free one; the emulsioned membrane reactor showed higher enantioselectivity compared to the two-separate phase membrane reactor due to the fact that most immobilized enzyme is able to work at the o/w interface; Immobilizing the enzyme on hydrophobic membrane the efficiency of the two-separate membrane system in terms of productivity and enantioselectivity is improved, whilst the stability of the two separate organic/aqueous phases is decreased, due to swelling of the membrane with organic solvent.
The experimental study allowed to identify the parameters that mostly affect the kinetic and transport properties of multiphase enzyme-loaded membrane reactors.
The immobilization of lipase as an organic/enzyme/aqueous emulsion into the sponge layer of asymmetric membranes is likely to achieve the best performance in terms of stability and enantioselectivity. Furthermore, the transport of the organic reagent to the reaction site and the removal of product from the reaction site to the aqueous phase can be also improved.

In addition to the use of enzymes in organic-aqueous systems, their use in pure organic solvents is increasing. Enzymes in organic media are able to work in microenvironments that contain very little quantities of water (usually less than the solubility limit) and several studies have confirmed that it is possible to carry out biotransformations in organic media [Klibanov, 1985; 1990]. Immobilized enzymes operating in organic media show novel properties, such as enhanced stability and altered substrate specificity. A few studies carried out using lipases immobilised on DEAE-Sephadex [Yang, 1992], chitosan [Shaw, 1990], zirconia [Giorno, 1997] have demonstrated these effects. The use of a hydrophilic matrix can help to protect the biocatalyst because it helps to maintain the water molecules around the enzyme, and inorganic membranes are today available that are resistant to the majority of organic solvents. An investigation on transesterification reaction in anhydrous tetrahydrofuran carried out with free and immobilized lipase demonstrated that the enzyme immobilised on zirconia membranes is more active compared with when the enzyme is in suspension and that the selectivity towards a reaction intermediate changed, resulting in the production of a non-naturally available flavonoid [Natoli, 1996].
In many instances, when the product is obtained by fermentation, it is present as a component of a complex solution from which it needs to be separated and purified. In these cases, integrated membrane systems can be used for continuous production and downstream separation. For example, the production of L-lactic acid is obtained by continuous fermentation in a membrane fermentor. This consists of a traditional fermentor combined with an ultrafiltration unit. During operation of the bioreactor, its volume is kept constant by adding fresh medium at the same rate as it permeates through the ultrafiltration membrane. The solution recovered as permeate contains the product, L-lactic acid, together with other small molecules that are not retained by the membrane, whilst the cells and macromolecules are recycled back to the bioreactor. The product is then in a clarified solution, from which it needs to be further purified and concentrated. The purification process can take place by membrane-based solvent extraction carried out through two membrane modules [Giorno 1999]. This operation is based on the transport of the solute from an aqueous solution at acidic pH (feed) to another at basic pH (stripping) via an organic

phase (extracting). The phases are kept in contact at the pore entrance of the membrane situated between them. When specific carriers are used as the extractant phase, the separation can be highly selective.

In recent years, membrane technology has also attracted considerable attention for the treatment of water and wastewater [Brindle, 1996]. Many studies on the use of membranes and membrane bioreactors for treatment of activated sludge [Yamamoto, 1989] and the removal of nitrogen from wastewater [Chiemchaisri, 1993; Magara, 1992] have been carried out. Lu et al. combined highly concentrated activated sludge processes with a rotary disk ultrafiltration membrane for the treatment of high-strength fermentation wastewater [Lu, 1999]. The performance of the system for long time operation (approximately 130 days) demonstrated that the system is amenable for the treatment of fermentation waste-water. Zoh et al. at University of California, combined a membrane bioreactor to a ceramic cross-flow ultrafiltration module for treating a synthetic wastewater containing hydrolysis byproducts of high explosive RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine) compound [Zoh, 1999]. Among the highly explosive compounds that are manufactured, RDX is the most common, and is also classified as a possible human carcinogen. The hydrolysis by-products of RDX consist of acetate, formate, formaldehide and nitrite; nitrate can be removed by using a denitrifying (anoxic) biological process that converts the hydrolysates to harmless end-products, such as N_2 and CO_2. The ceramic membrane is used to recycle the cells back to the bioreactor while removing the treated water as clear effluent in the permeate. The permeation flux was between 0.15 and 2.0 $m^3 \cdot m^{-2} \cdot day^{-1}$ and was restored to the original flux following back-flushing. The results showed that the process was efficient in retaining the cells and producing a clear effluent [Zoh, 1999].
Mourato recently described an immersed hollow-fiber membrane system for water and wastewater treatment developed at the Zenon Environmental Systems (Canada) and known as ZeeWeed® (75). The immersed membrane is not housed in a pressurized vessel but operates in an open tank environment under a slight vacuum (-0.15 to -0.55 bar) to draw water through the membrane. In addition to other advantages this operating system allow to reduce membrane fouling, because contaminants are not forced into the membrane pores under high pressure. The membrane system uses reinforced membrane assembled in individual modules that can be combined to form cassettes (8-10 modules per cassette). The author reports that there are currently over 100 operating immersed membrane plants in operation and that typical application include municipal water treatment, municipal and industrial wastewater treatment, water reuse and reverse osmosis pre-treatment. The integration of a biological reactor with the immersed membrane allow to combine clarification and filtration of an activated sludge process into a single step process. The membranes form a barrier to solids and bacteria and retain them in the process tank. The system can operate at high levels of biomass (10,000 to 15,000 mg/l) and high sludge retention time (20-50 days).

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