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Enantiospecific Membrane Processes

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Abstract: Membrane technology can be applied in two ways to produce pure enantiomers. In one case, a membrane separation process can be combined with an enantiospecific reaction to obtain so-called 'enantiospecific membrane reactor'. These systems are useful to carry out asymmetric synthesis or kinetic resolution and simultaneously separate the produced enantiomer. As for general membrane reactors, the result is a more compact system with a higher conversion; in fact, removal of a product drives equilibrium-limited reactions towards completion. The other way to apply membrane technology to chiral production is the use of intrinsically enantioselective membranes that are able to distinguish between two isomers favouring preferential transport of only one isomer in absence of reaction. In this paper, the current development of chiral membrane processes will be discussed.

1. Introduction

The use of pharmaceuticals, food additives, feeds, flavours, fragrances, agrochemicals, etc. as optically pure isomers is today strongly recommended. This is due to the fact that bioactive compounds are in general enantiomers where often only one has beneficial effects while the other can be inactive and simply represents a waste or have serious undesired side-effects. Chiral compounds from natural origins usually exist as one predominant optical isomer. The presence of racemic pairs most often indicates adulteration or unnatural origin. Important chiral molecules are summarised in Table 1.

The increased attention towards production of chirals in the past decade, led several regulatory bodies to issue guidelines on the development of chiral drugs [1].

Although many compounds are still marketed as racemates, the improved knowledge on the impact of different enantiomers, the technological advances in stereosensitive analytical methods and the availability of technologies to produce on large scale optically pure compounds, will expand the demand of chiral isomers, not only for pharmaceuticals and food purposes, but also for agrochemicals. In fact, the possibility to halve the discharge of pesticides,

Table 1. Compounds Importan as Chiral Molecules

Compound	Function
Amoxicillin	Antibiotic
Ampicillin	Antibiotic
Cefratriaxone	Antibiotic
Cefalexin	Antibiotic
Captopril	*ACE-inhibitor
Enalapril	ACE-inhibitor
Diltiazem	Calcium antagonist
Ibuprofen	NSAID
Naproxen	NSAID
Atenolol	Beta-blocker
Albuterol	Bronchodilator
Lavastatin	Antihypercholesteremic

**ACE: angiotensin converting inhibitors*

Table 2. Technologies Available to Produce Enantiomers

Physical methods	Classical resolution (diastereomeric crystallization)
	Membrane separation
Biological methods (integrated in productive cycles)	Biotransformation-based processes (*e.g. biocatalytic membrane reactors*)
Asymmetric synthesis	Using chemical and biochemical catalysts

herbicides and fertilisers into environment is of great importance.

Concerning the technologies available to produce enantiomers, classical resolution still accounts for a large part of the chiral production. However, asymmetric synthesis, biocatalysis and the use of chiral separation systems are becoming increasingly popular. The different chiral technologies available at productive or laboratory scale are summarised in Table 2. In some cases, the combination of these technologies with membrane processes can provide alternatives to separations and to chemical conversions which are troublesome or impossible using classical methods.

In the following paragraph, some of the membrane systems used to produce and/or separate enantiomers will be discussed.

2. Discussion

Depending on the type of substance to treat, resolution of racemic mixtures can be carried out by enantioselective catalysis in membrane reactors or enantioselective separation through chiral membranes.

In biocatalytic membrane reactors the chiral system is represented by the biocatalyst that specifically recognises and converts only one of the two isomers. In these cases the membrane separates the preferentially obtained isomer, and in some cases also functions as a support for the enantiocatalyst. The biocatalyst can be represented by a micro-organism or an enzyme.

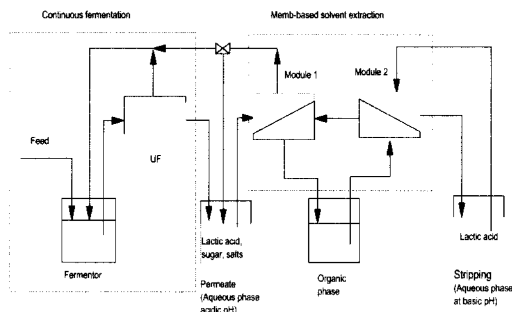

Fig. 1. Integrated membrane system for continuous downstream processing of fermentation broths.

When the enantiocatalyst is represented by a micro-organism, the reactor has a configuration of a continuous membrane fermentor and the product is separated by downstream processing. The kind of products obtained by fermentation of micro-organisms are, for example, D- or L-carboxylic acids, D-amino acids and chiral intermediates of antibiotics. The possibility of using integrated membrane systems for simultaneous production and downstream processing of fermented compounds is under investigation at laboratory level [2]. The overall process is depicted in Fig. 1. It is mainly constituted of three parts: a fermentation process, which produces a carboxylic acid (in our case D-lactic acid); an ultrafiltration process, which clarifies the fermentation broth, by separating the cells and macromolecules from small molecules; a membrane based solvent extraction system (realised by two hollow fibre membrane contactors in series), which separates the carboxylic acid from the other small molecules. In this system, the chiral agent of the process is represented by the micro-organism (*Lactobacillus bulgaricus*) that preferentially produces the D-lactic acid from glucose during its growth. The study is currently in progress, we first studied the continuous fermentation and the membrane extraction processes separately (afterwards, they will be combined and studied as a whole process). The behaviour of cell growth and amount of D-lactic acid produced during continuous fermentation with respect to batch fermentation are shown in Figs. 2 and 3, respectively. As it can be seen, the performance of

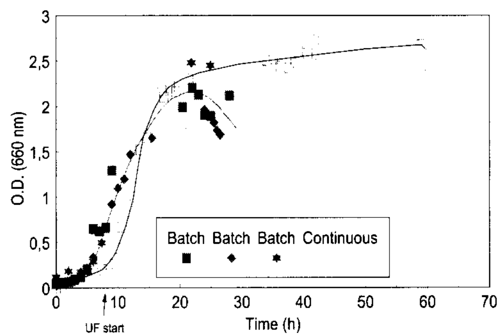

Fig. 2. Behaviour of cell growth in batch and continuous fermentor.

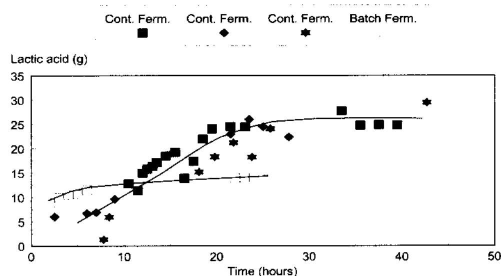

Fig. 3. Production of D-lactic acid in batch and continuous fermentor.

the continuous system is increased of about 40%. During continuous fermentation, an ultrafiltration membrane module made of polyamide capillary membranes with 50 kDa cut-off was used. At steady state, a permeate flux of about 30 l/h m^2 was obtained that allowed to work at a dilution rate of 0.04 h^{-1}. On the basis of substrate availability, amount of lactic acid produced, cell density and growth rate, the most convenient time to start the ultrafiltration resulted about 7 hours after the inoculum of the micro-organism into the fermentor.

For what concerns the membrane based solvent extraction, operating conditions (such as temperature, carrier concentration etc.) were studied and in part reported in Ref. 3. The best conditions resulted to be 37 (±2)℃, 10%w/v amberlite LA2 in *n*-heptane. The time course of lactic acid from ultrafiltered fermentation broth is reported in Fig. 4. An analysis of mass transport resistances through asymmetric ultrafiltration membranes is currently

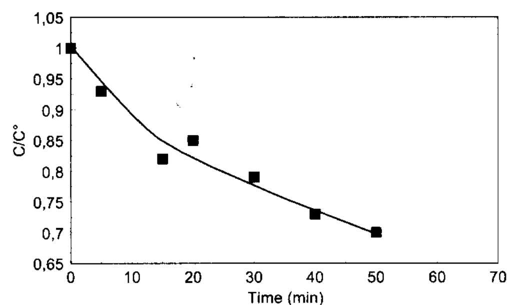

Fig. 4. Time course of lactic acid during membrane extraction.

under investigation.

When the biocatalyst is represented by an enzyme, it can be used as suspended or as immobilised. Two examples of membrane reactors using enzymes are illustrated in the followings. The first one refers to a system with the enzyme suspended in solution and compartmentalised by the membrane in a circuit of the membrane module; whilst the other refers to a system where the enzyme is immobilised within the membrane.

The production of D-para-hydroxy-phenyl-glycine (D-p-HPG) can be obtained by a subsequent enantioselective hydrolysis of 5-para-hydroxy-phenyl-hydantoin (5-p-HPH) into N-carbamil-hydrohy-phenyl-glycine (N-Carb-p-HPG) and to the corresponding amino acid using hydantoinase and carbamilase, respectively. The D-p-HPG is used for the production of semisynthetic penicillin and cefalosporin. The production of N-carb-p-HPG was carried out using the hydantoinase from *A. radiobacter*. The biocatalyst was segregated in the reaction circuit by capillary membranes of 10 kDa cut-off. The scheme of the system and a typical behaviour of this biotransformation are reported in Figs. 5a and b, respectively. The performance during subsequent reactions is reported in Table 3.

Among other hydrolases, lipases are the most used. In particular, they are useful for the bioconversion of low water soluble substrates using biphasic organic/aqueous membrane reactors (Fig. 6). In these systems, the enzyme-loaded membrane promotes reactions between two separate phases

thanks to the properties of lipases to catalyse reactions at the organic/aqueous interface. The organic phase (that contains the substrate) is maintained in contact with the aqueous phase (that extracts the reaction product) at the membrane level using a higher transmembrane pressure from the non-wetting phase. When the enzyme is also stereo-specific, these systems are useful to produce optically active isomers [4-6]. As an example, in the Fig. 7 is reported the time course of (S)- and (R)- naproxen acid extracted in the aqueous phase using a biphasic membrane reactor (with lipase immobilised on polypropylene).

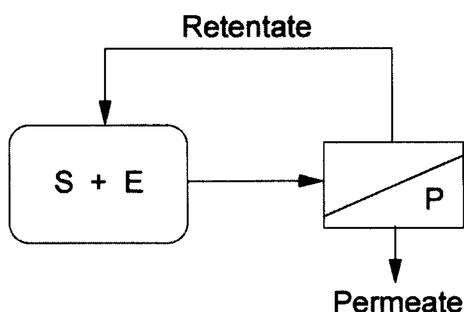

Fig. 5a. Scheme of enzyme segregated reactor.

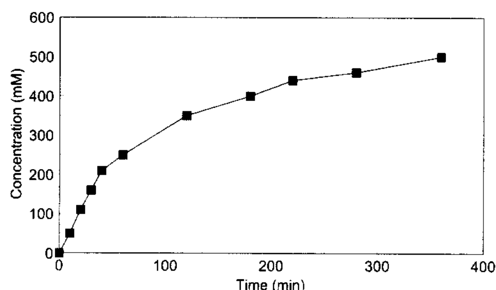

Fig. 5b. Production of N-carbamil-p-hydroxy-phenyl-glycine.

These systems represent one of the most promising areas for the development of phase transfer catalysis, a technique that allows reagents with markedly different polarities to come together without the need for a mutual solvent.

A discussion of the parameters that influence the

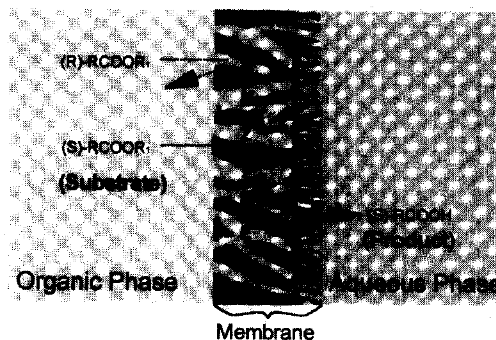

Fig. 6. Schematic representation of a biphasic organic/aqueous membrane reactor.

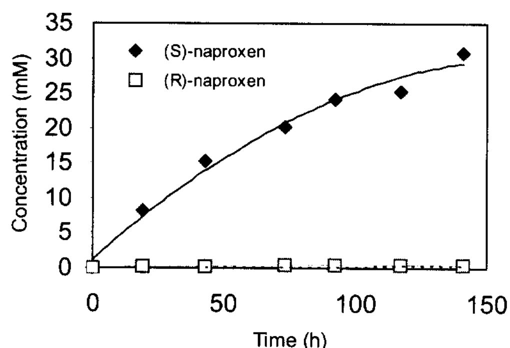

Fig. 7. Production of naproxen acid as a function of time.

Table 3. Performance of 5-p-hydroxy-phenyl-hydantoine of Hydrolysis During Subsequent Experiments

Experiment	Moles of 5-PHP	Moles of N-Carbamil (in the retentate and permeate)	Moles of N-carbamil (in the permeate)
1	0.400	0.365	0.300
2	0.400	0.367	0.320
3	0.416	0.347	0.302
4	0.509	0.360	0.358
5	0.520	0.500	0.491

selection of reactor components and the effects of operating conditions on the performance of biphasic membrane reactors are widely discussed in the Ref. [7].

In enantioselective separation without chemical transformation, the membrane represents the chiral system. Enantioselective membranes can be polymeric or liquid. The polymeric can be made of chiral polymers; chiral selectors can be loaded on the membrane; or stereoselective structure can be realised during membrane preparation, by adding imprinting molecules which are released after the membrane is formed [8]. Polymeric membranes with intrinsic enantioselective properties can be realised by chiral modification of achiral polymeric membranes. Cyclodextrins and crown ethers are useful chiral agents for this application. This is because the chiral rings of these molecules are able to host different chiral molecules. Due to the hydrophilic external surface of chiral ring of cyclodextrins, they are water soluble and because of their hydrophobic inner surface they may complex and carry over apolar molecules. On the other hands, the organic soluble crown ethers are able to transport polar molecules from organic to aqueous phase.

Chiral agents are also used to realize liquid membranes or supported liquid membranes. Supported liquid membranes (SLMs) containing a chiral recognition carrier have been used to separate amino acid enantiomers. In these membranes the chiral system is constituted by the carrier, which transports the selected enantiomer from a source phase to a receiving phase. The organic solvent in which the carrier is diluted does not have chiral properties, and both D- and L-isomers can diffuse through it. In order to obtain high resolution ratio, it is necessary to use high selective carrier diluted in a solvent through wich diffusion of non-complexed isomers is very low. Although SLMs have numerous advantages as a separation technique, they show low stability, and this represents a limit for application at industrial level.

3. Conclusions

The research efforts on enantiospecific membrane processes is constantly increasing. The technologies are still at an emerging step, but the cooperation between companies and research institutes focused on common objectives can favourite their development.

These techniques can offer several advantages in terms of productivity, purity of single isomer, ease to scale-up, environment safety. On the basis of current literature data, kinetic resolution in membrane reactors seem more likely to succeed with respect to enantioselective separation through intrinsically enantioselective membranes. This is due to the capacity of the former kind of process to operate in continuous at steady-state with good enantioselectivity with respect to the latter one. It should be noted, however, that studies on intrinsically enantioselective membranes are at a very early stage and not enough data are yet available to draw final conclusions.

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