# 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.

## INTRODUCTION

The importance to use optically pure isomers as pharmaceuticals, food additives, feeds, flavours, fragrances, agrochemicals, etc. is becoming more and more evident. 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.

To testify the increased attention to chirals production, is the fact that in the past decade several regulatory bodies around the world have issued 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 constantly expand the demand of chiral isomers, not only for pharmaceuticals and food pourposes, but also for agrochemicals. In fact, the possibility to halve the discharge of pesticides, herbicides and fertilisers into environment is of great importance.

Classical resolution still accounts for a large part of chiral production. However, asymmetric synthesis, biocatalysis and the use of chiral separation systems are becoming increasingly popular. 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.

## 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 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. When the enantiocatalyst is represented by a microrganism, 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 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 the carboxylic acid. An ultrafiltration process, which clarifies the fermentation broth, by separating the cells and macromolecules from small molecules. A membrane based solvent extraction system (realized by two hollow fiber membrane contactors in series), which separates the carboxylic acid from the other small molecules.

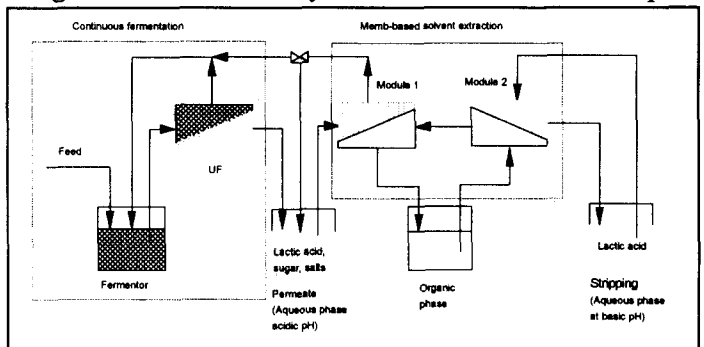


Fig. 1. Integrated membrane system for continuous downstream processing of fermentation broths.

Stereoselective hydrolases are widely used and specially attractive for large-scale preparations thanks to the fact that they are not very expensive and do not require cofactors.

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 contained in the *A. radiobacter* without further purification.

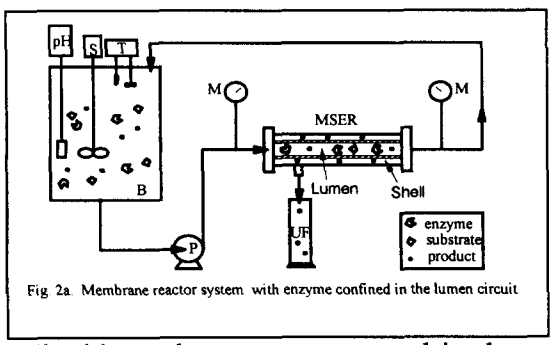


Fig. 2a. Membrane reactor system with enzyme confined in the lumen circuit

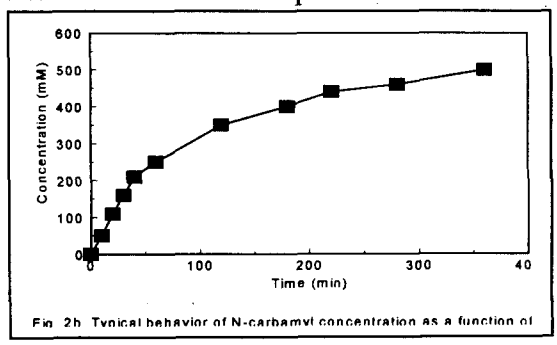
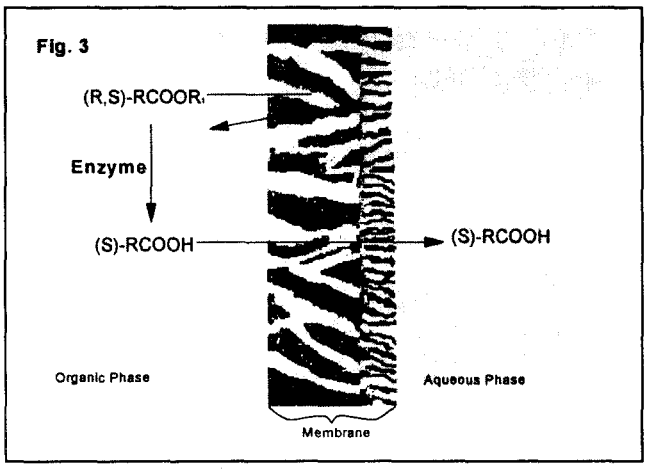


Fig. 2b. Typical behavior of N-carbamyl concentration as a function of

The biocatalyst was segregated in the reaction circuit by capillary membranes of 10 kDa cut-off. The scheme of the system and a typical behavoir of this biotransformation are reported in Fig. 2a and b, respectively.

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. 3). In these systems, enzyme-loaded membrane promote reactions between two separated phases, as a result of the properties of lipases to catalyse reactions at the organic/aqueous interface; the two phases are maintained separate but in contact at the membrane level. When the enzyme is also stereospecific, these systems are useful to produce optically pure isomers [3-5].




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.

The parameters which influence the selection of reactor components and the effects of operating conditions on the performance of biphasic membrane reactors will be discussed [6].

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 [7]. 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 realise liquid membranes or supported liquid membranes. Supported liquid membranes (SLMs) containing a chiral recognition carrier have been used to 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.

## CONCLUSIONS

The research efforts on enantiospecific membrane processes is costantly 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. On the basis of current literature data, kinetic resolution in

membrane reactors are 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 state final conclusions.

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