Bioprocess Engineering 23 (2000) 503–512 © Springer-Verlag 2000

Technical aspects of separation and simultaneous enzymatic reaction in multiphase enzyme membrane reactors

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Abstract The technical aspects of the membrane extraction of a compound either from aqueous phase into apolar organic solvent phase or from the apolar phase to the aqueous one and the enzymatic conversion of the solute in a multiphase enzyme membrane reactor are considered. The application possibilities, the selection aspects of membrane material as well as the solvent phase, the water content and its control, the method of the enzyme immobilisation and the operation of the extraction/reaction system are discussed.

1 Introduction

Solvent extraction in biotechnology is usually performed in emulsions. The organic solvent is dispersed in the aqueous phase and the solute extracted from the continuous aqueous cultivation medium into the discontinuous solvent phase [1]. For simultaneous extraction and re-extraction, emulsion liquid membrane systems with aqueous droplets of some micrometer diameter are dispersed in globules of the organic phase of few mm diameter, which are contacted with the continuous aqueous organic phase [2] or solid supported liquid membrane systems with globules which are immobilised in the pores of membranes [3–5] can be used. Hydrolysis as well as synthesis reactions simultaneous with the extraction process are also performed by enzymes microencapsulated in reversed micelles [6, 7].

Continuous operation, product recovery and phase separation can be problems in such systems. Therefore, several research groups used membrane extraction. The aqueous and organic phases are separated by a membrane and the extraction is performed across the membrane [8–11]. In order to increase the driving force of the solute across the membrane, the solute is converted by an enzymatic reaction in the organic phase to the product [12, 13]. The latter idea is embodied as a concept of the so-called multiphase enzyme-membrane reactors: The emulsion system is replaced by a membrane bioreactor [14–16] in which the aqueous and organic phases are separated by the membrane and enzyme is immobilised in the solvent phase on the membrane by adsorption.

The application of enzymes in organic solvents has several advantages:

- the high solubility of hydrophobic substrates in the solvent,
- the ability to reverse the hydrolytic reaction into synthetic reaction,
- suppression of side reactions and
- easy product recovery.

However, enzymatic reactions in organic media are seriously limited by inactivation and denaturation of the enzymes by several solvents. Therefore, the selection of the solvent suitable both for the operation of biocatalyst and for the extraction is a key issue. With increasing hydrophobicity (decreasing polarity) of the solvent, the solubility of the organic phase in the aqueous phase and the aqueous phase in the organic phase decrease and the stability of the enzyme increases [17–19]. For ester formation, low water content in the organic phase is generally decisive, moreover it is important to remove the water formed during the reaction. These aspects affect the structure and the operational conditions of enzyme-membrane reactors, too.

The membrane extraction combined with enzymatic conversion seems to be promising for industrial application. Therefore, the present paper deals with the technical aspects of these systems.

2 Membrane extractor system

Membrane assisted solvent extraction is an emerging technique both in the chemical and the biochemical engineering. The laboratory scale applications are summarised in Table 1.

In the extraction modules, the two liquid phases are contacted at the interface immobilised by a membrane. In case of microporous membranes the pores are filled with the phase, preferentially wetting it, while the other immiscible phase is completely excluded, thus, no phase separation is required because the two liquid phases are never mixed. In all cases, hydrophobic membrane pores are assumed to be filled with the organic phase while hydrophilic membrane pores contain the aqueous phase.

For in situ extraction from a cultivation, cell containing medium is pumped from the bioreactor through the

Received: 2 November 1999

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Table 1. Application of membrane extractors

Application	References
Removal of volatile organic compounds	[20–22]
Extraction of toxic heavy metals, (e.g. Zn, Cu, Cr, Ni, Cd and Hg from aqueous solutions)	[23, 24]
Separation of inorganic and organic solutes and subsequent biodegradation	[25]
In situ removal of the product from the cultivation medium or from the mixture of enzyme catalysed reaction	[26–30]

module and back to the bioreactor [27]. In this case, hydrophobic membrane is recommended, to avoid the clogging of the pores by cells. For long range operation, the cell free medium should be used for extraction. In several cases, the cells grow in the organic phase and the water soluble substrate is transported through the hydrophylic membrane [31].

In case of hydrophobic microporous membranes, the water phase has an overpressure (0.3 bar) in order to avoid the break through of the solvent phase [32]. Concerning hydrophilic microfiltration membranes, the pores are filled with the aqueous phase and the solvent phase must have an overpressure. Producing biological surface active agents the control of transmembrane pressure drop can be difficult [31]. A possible way to neglect the control problem is the application of dense, non-porous membranes (e.g. silicone) that allow small hydrophobic molecules to permeate [34].

The extracted solute (the product of the bioprocess) is removed from the organic phase by evaporation [32] or enzymatic reaction (see the next chapter).

In most frequent configurations of the membrane extractors are

- the flat sheet membranes [29, 33] and
- the hollow fibres [26, 27],

but there are examples for membrane tubes wound spirally in the device [32].

Hollow fibre membranes are especially suitable for membrane extraction. The aqueous and the solvent phases are recirculated in counter-current mode through the hollow fibre module. High mass transfer rates per unit volume may be provided since the modules can contain very high surface area, e.g. Celgard and Cuprophan modules have surface areas from 5 to about 50 cm^2/cm^3 [20]. The interface in these modules can be accurately determined from the fibre dimensions and characteristics. Thus, the equipment can be easily characterised by pilot-plant tests for scale-up. This technique also overcomes other shortcomings of the conventional liquid extraction such as flooding limitations on independent variation of phase flow rates, requirements of density difference, stable emulsion formation or poor liquid–liquid contacting resulting in a low stage efficiency. The disadvantage of this extraction device could be the clogging by particles in the feed (prefiltration might be necessary).

To carry out in practice the membrane-assisted extraction process the mass transfer rate through the membrane is to be estimated which strongly depends on the flow rates in the tube and the shell side of the hollow fibre membrane and the characteristics of membrane material. The overall mass-transfer resistance can be considered the sum of three resistances in series [22]: tube side resistance, membrane resistance and shell-side resistance. The membrane mass transfer coefficient is most likely to be independent of aqueous or organic flow rates. However, the tube side mass transfer coefficient will vary with flow rate depending on whether aqueous or organic phase flows on the tube side. Similarly, the shell-side mass transfer coefficient will also depend on the flow rate. It has to be taken into consideration that the tube- and shell-side flow rates can be very different.

2.1 Correlation for mass transfer coefficients

The experimentally obtained membrane resistances may be described on the basis of the flat-membrane studies made by Prasad and Sirkar [20]. If mass transfer within the membrane is due to ordinary diffusion alone, the following expression may be used for calculating the mass transfer coefficient in the membrane:

$$k_{\mathrm{m}} = \frac{2D\varepsilon}{\tau[d_{\mathrm{o}} - d_{\mathrm{i}}]} \quad , \tag{1a}$$

where k_{m} is membrane mass transfer coefficient (m/s), D is diffusivity (m^2/s), ε is porosity (–), τ is tortuosity of membrane (–), d_{o}, d_{i} is fibre outside- and inside diameter (m), respectively.

The same mass transfer coefficient for asymmetric, cylindrical hollow fibre membrane considering that the effective diffusion coefficient $(D\varepsilon/\tau)$ of the two layers (subscript 1 and 2 refer to the skin- and sponge layer, respectively) are different can be expressed as follows [43]:

$$k_m = \frac{2D_1\varepsilon_1}{\tau_1 d_i} \frac{1}{(D_1\varepsilon_1\tau_2/D_2\varepsilon_2\tau_1)\ln(d_{\mathrm{o}}/d_{\mathrm{k}}) + \ln(d_{\mathrm{k}}/d_{\mathrm{i}})} \quad , \tag{1b}$$

where d_{k} is diameter of the skin layer of asymmetric membrane.

Several correlation exist for predicting the tube side mass transfer coefficient, k_{t}. For well defined laminar flow the Lévéque correlation is often used [42]:

$$\mathrm{Sh} = 1.62\mathrm{Re}^{1/3}\mathrm{Sc}^{1/3}(d_{\mathrm{i}}/L)^{1/3}, \quad (\mathrm{Re} < 2000) \tag{2}$$

where Sh is Sherwood-number (–), Re is Reynolds-number (–), Sc is Schmidt-number (–), L is length of hollow fibre (m).

For turbulent flow several correlations exist in the following form [36]:

$$\mathrm{Sh} = a\mathrm{Re}^b\mathrm{Sc}^c \quad . \tag{3}$$

Values for a, b and c are shown in Table 2.

The shell side mass transfer coefficient were correlated mostly by the following empirical equation:

$$\mathrm{Sh} = a\mathrm{Re}^b\mathrm{Sc}^{1/3}(d_{\mathrm{h}}/L)^c \quad , \tag{4}$$

where Reynolds- and Sherwood-numbers were calculated using average interstitial velocity and an hydraulic

Table 2. Values for the constants in Eq. (3)

a	*b*	*c*	Reynolds number	References
0.023	0.8	0.33	$Re > 10^5$	[37]
0.34	0.75	0.33	$10^4 < Re < 10^5$	[37]
0.0096	0.931	0.346	$10^4 < Re < 10^5$	[37]
0.0165	0.86	0.33	$Re > 10^4$	[38]

diameter d_h based on the shell cross-section. The *a*, *b* and *c* values are listed in Table 3.

Recently Qin and Cabral [44] have analysed theoretically the mass transfer coefficients in both sides of membrane solving numerically the mass balance equations and suggested approximated equations for these coefficients.

3 Enzyme membrane reactor system

3.1 Applications

As it has been shown in the chapters above the multiphase membrane systems for simultaneous extraction and enzyme catalysed reaction offer effective ways:

- For removing the product and/or by-product from the mixture of an enzyme catalysed reaction in order to shifting the equilibrium and/or reducing the product inhibition;
- For enhancing the driving force during the extraction by reacting the extractant in an enzymatic reaction.

Consequently, the application possibilities are connected to these two main cases as it is illustrated by the Table 4.

3.2 Membrane material

Both in multiphase enzyme reactors and membrane extractors, the membrane cut-off typically is in the ultrafiltration range because this pore size is the most suitable for the enzyme retention and it is small enough to avoid the mixing of the phases. The characteristic commercial membrane materials are asymmetric. Several treatments to modify the membrane structure often takes place. A summary for membrane materials is shown in Table 5.

From the publications referred in Tables 4 and 5, it can be learned that lipase is the most frequently applied catalyst in EMR-s and both hydrophilic and hydrophobic membranes have been found as suitable support for lipase,

Table 3. Values for constants in Eq. (4) for prediction of the shell side mass transfer coefficient

a	*b*	*c*	References
1.25	0.33	0.33	[33]
8.8	1	1	[40]
5.85 $(1 - \phi)$*	0.6	1	[20]
$(0.53–0.58\phi)$*	0.53	0	[41]

* ϕ-packing fraction of shell side of modules

Table 4. Enzymes and bioprocesses in multiphase membrane reactors

Biocatalyst	Reaction	References
Lipase	Hydrolysis of olive oil	[14, 15, 45, 46]
Lipase	Hydrolysis of triacylglycerols	[45, 46]
Lipase	Hydrolysis of butter oil	[47]
Lipase	Synthesis of ethyl caprylate	[32]
Lipase	Synthesis of n-butyl oleate	[48]
Lipase	Synthesis of ethyl oleate	[49, 50]
Lipase	Resolution of glycidyl butyrate	[51]
Lipase	Ibuprofen production	[52, 53]
α-Chymotripsin	Resolution of phenylalanine methyl esters	[29, 54]
Glycerol dehydrogenase	Resolution of 1-phenyl-1, 2-ethanediol	[26]
Thermolysin	Synthesis of aspartame precursor	[55, 56]

in spite of the face that is considered hydrophobic enzyme. Other enzymes are used in multiphase membrane reactors only in the last five years.

3.3 Solvent selection, extraction conditions, water activity control

The selection of organic solvent is determined both by the requirements of the extraction process and by the activity, stability and selectivity conditions of enzyme. The organic solvent used should not strongly distort the hydration shell of the enzyme molecule that is necessary for maintaining the native conformation [60]. The best solvent can be found empirically by testing the enzyme activities in various hydrophobic solvents or can be predicted on the basis of several quantitative parameters characterising the solvent hydrophobicity [17, 19]. In multiphase enzyme membrane reactors the organic phase is often the substrate itself (e.g. oleic acid [32, 50]).

The selectively extracted component is generally the product [26, 51, 55, 56] but often the extraction of by-product is more reasonable [29, 54] regarding the selectivity of the separation. In case of hydrolytic processes catalysed by lipase, the by-product alcohol is extracted by the aqueous phase through the membrane [45, 47, 53]. The esterification in EMR is accompanied usually by extraction of alcohol to the organic phase and water produced to the aqueous phase [32, 50]. The different cases of the realisation methods of the extraction process in enzyme membrane reactors are summarised in Table 6.

The activity and selectivity of enzymes in organic solvents depend on the water content in this phase [60–65]. In a biphasic emulsion system consisting of aqueous cultivation medium and a hydrophobic solvent, the water content of organic phase is given by the solubility patterns. In membrane devices, the distribution of water between the organic and aqueous phases depends on the membrane hydrophobicity, too.

The water activity control is an important task during enzymatically catalysed synthesis reactions. The removal (or addition) of water shifts the thermodynamic

Table 5. Materials applied in multiphase membrane extractors and reactors

Hydrophobicity	Material	Treatment method	References
Hydrophobic	Polyamide	–	[52, 53]
	Polypropylene	–	[26, 32, 57]
	Polyethylene	Glycidyl methacrylate grafting	[58]
	Polytetrafluoro-ethylene		[28]
Hydrophylic	PAN	–	[52, 55, 56, 59]
	Cellulose acetate	–	[45, 46, 49, 50]

Table 6. Extraction conditions in multiphase membrane reactors

Extracted components(s)	Extractant phase(s)	References
Product	Aqueous	[51]
Product	Organic	[26, 55, 56]
By-product	Organic	[29, 54]
Substrate/by-product	Organic/aqueous	[32, 49, 50]
Cosubstrate (water)/ by-product	Organic/aqueous	[45–47, 52, 53]

equilibrium in favour of the desired reaction. Some water is also required to maintain the catalytic activity of the enzyme. This requirement can be satisfied easily be presaturating the feed with water. However, this method does not ensure the optimum level of the water activity. Ujang et al. [66, 67] suggest a packed-bed hollow-fibre reactor, where the water activity control was achieved by recirculating air that had been bubbled through a column of saturated salt (NaCl, $Mg(NO_3)_2$, $MgCl_2$ or CH_3COOK) solution through the hollow fibres. A gas-phase hollow fibre reactor was also proposed [66] where the substrate mixtures pass through the fibre lumens and are subject of reaction with a constant humidity gas phase recirculated through the shell of reactor, acting as the medium used to control water activity. The simple device is suitable for use over a wide range of water activities.

3.4 Biocatalyst confinement in enzyme membrane reactors

In the multiphase membrane reactors, the appropriate location of biocatalyst is determined by the requirements that the membrane should be able both to separate and to contact the immiscible liquid phases and enzyme should be able to catalyse the reaction between the given components. The most frequent variations applied are listed in Table 7.

In the simplest case, enzyme is dissolved in the aqueous solvent [26, 68] or its aqueous solution is emulsified in the organic phase [69]. Since the cut-off value of the membrane is generally much lower than the size of the enzyme molecule, catalyst is maintained in the reactor working in continuous regime. Nevertheless, the immobilisation onto a solid support can be found to be typical in enzyme-membrane reactors. It is reasoned by the fact that the enzyme stability and activity can be improved by immobilisation because the conformation of the enzyme could be artificially fixed in such a way, to keep it in the native state.

Above all, enzyme is immobilised on the membrane itself to utilise this relatively large solid surface as well as the contacting area between the organic and aqueous phases. As it is shown in Table 6 the most common method of fixing is the cross flow filtration of enzyme solution through the membrane. The enzyme is solved in an appropriate (buffer) solution and fed into the membrane module operating as an ultrafiltration cell [70]. In case of lipases the enzyme-covered side of the membrane will be filled by the organic phase of the reactor.

The mechanical forces strengthening the immobilisation in case of the cross flow filtration method do not play a role in case of simple adsorption. Hydrophobic enzymes adsorbed on hydrophobic carriers result in fairly stable immobilizates with low leakage rate. Nevertheless, at the

Table 7. Enzyme confinement methods in membrane reactors

Phase for enzyme	Confinement type	Immobilisation method	References
Organic	Emulsion	Colloidal liquid aphrons	[69]
	Immobilised on the membrane	By cross flow filtration	[14, 15, 45, 46, 49, 50, 51–53, 59]
		By adsorption	[57–59]
		By covalent bond	[50, 59, 71, 72]
		Site-specifically, by genetic attachment	[71, 72]
		By cross-linking	[50, 59]
		By covering	[50]
	Immobilised on other support (packing, etc.)	By adsorption	[32, 59]
		By covering	[32]
Aqueous	Dissolved		[26]
	Immobilised	By surfactant coating	[29]

adsorption efficiency tests, the hydrodynamic conditions have to be taken into consideration besides the enzyme concentration [57]. Presumably, the new methods making more stable the adsorptive bond between the membrane and hydrophilic enzymes, developed recently by Kubota et al. for other proteins [58] will be useful in the future for enzyme immobilisation.

The formation of the covalent bonding is more complicated in membrane reactors than in the apparatuses with particulate solid support material. A simple but not selective chemical bond results by the spreading of a mixture of the concentrated enzyme solution and silicone on the membrane and by subsequent drying of the layer in nitrogen flow [50]. Special methods are which activate poly(ether)sulfone membrane blending it with polyacrolein as well as PVC sheets that embedded aldehyde-functionalised silica. The authors formed Schiff-base bonding to attach wild type subtilisin and used cross-linking agents in case of immobilisation the genetically modified one [71]. The site-directed immobilisation method suggested by Visvanath et al. [71, 72] resulted in a considerable enhancement of the activity in comparison with the traditional random immobilisation strategies. The technique based on recombinant DNA method can be hopefully applied in multiphase EMR systems, too.

Biocatalyst can be immobilised onto a separated packing built in the recirculation cycle of the multiphase membrane system [32]. *Mucor mihei* lipase (Palatase 1000L and 200L, NOVO Nordisk) has been absorbed on macroporous polypropylene carrier (Accurel EP100, Akzo Nobel) and coated with silicon layer. The enzyme immobilised on support particles is filled into a tubular reactor and operated as packed or fluidised bed. As alternative solution, the enzyme can be adsorbed in the solvent side on the surface and in the pores of the hydrophobic membrane module. A comparison of the activities of the lipase immobilised on 1.0–1.5 mm diameter Accurel EP 100 carrier and on the solvent side of the Cellgard X 10 (Celanese, Hoechst) hollow fibre membrane module, indicates that the latter exhibits higher activity. The pore size distributions of the polypropylene carrier and the polypropylene fibres are very similar, but because of the 7 m^2 large surface area of the fibres, the mass transfer controlled reaction rate is higher in the latter case.

4
Operation of membrane extractor/enzyme reactor systems

4.1
Hollow fibre unit as membrane extractor/enzyme reactor

The most common type of the apparatuses in question consists of a hollow fibre membrane module as a basic unit working as multiphase membrane extractor and an enzyme reactor at the same time. Considering as examples either esterification of a low molecular weight alcohol and a medium or long chain carboxylic acid or hydrolysis of triglycerides both one of substrates and products are present in the organic and the aqueous phase, respectively. Enzyme is immobilised on the membrane surface. The phases generally are recirculated through an external vessels [48, 49, 53, 55] – as it can be seen in Fig. 1. – or a single pass of the streams take place [15, 47]. The counter-current flow of the phases in the module proved to be of higher effectivity than the cocurrent flow [15].

4.2
Separated enzyme reactor/membrane extractor system

Extractive enzymatic synthesis of ethyl caprylate has been studied in a system containing a separated membrane extractor and a packed-bed immobilised enzyme reactor [32]. The scheme of the system is illustrated in Fig. 2. At first a Liquid Cell Flow Membrane Module (Cellgard X-10 microporous polypropylene hollow fibres of 240 μm internal diameter and 7 m^2 surface area) was filled with the aqueous solution of ethanol (9.6%, 1.56 mol dm^{-3}) and pumped through the module. Octane was chosen as solvent phase because of its high hydrophobicity (log P = 4.5) [17], low toxicity and low boiling point (126.6 °C). Ethanol was extracted into the octane phase in the membrane module. The ester (boiling point 208.5 °C) and caprylic acid(239.3 °C) can be easily separated from octane by fractionated distillation. The aqueous phase (4.7 mol ethanol in 3 dm^3 water) with a flow rate of 120 dm^3 h^{-1} was contacted in the membrane module with 1.28 mol acid in 2 dm^3 octane with a flow rate of 40 dm^3 h^{-1} in counter-current operation. The octane phase was recirculated through the packed bed enzyme reactor with a flow rate of 100 dm^3 h^{-1}. After about 10 h 40% equilibrium conversion is obtained. Unfortunately, the ester has a higher boiling point than the octane. Therefore the in situ recovery of the ester from the reaction mixture by flash distillation was not possible. This would be feasible with dodecane (boiling point of 216 °C) as solvent phase.

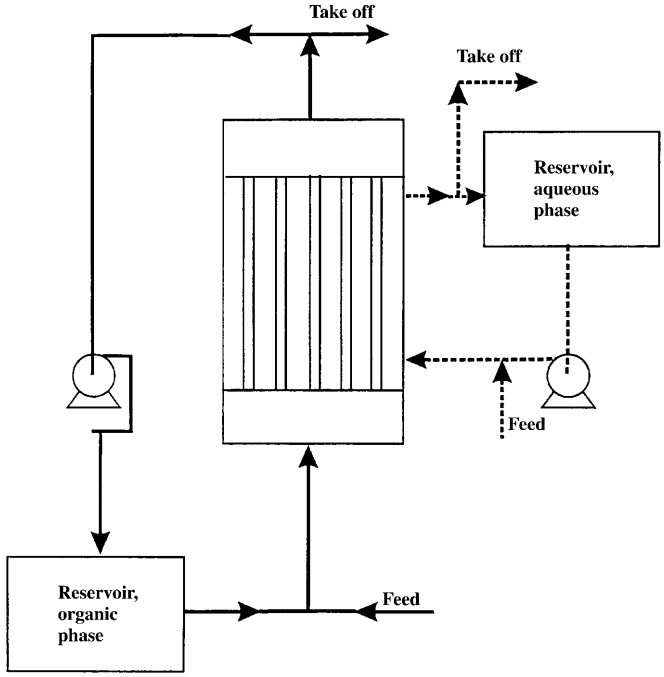

Fig. 1. Scheme of the multiphase enzyme membrane reactor

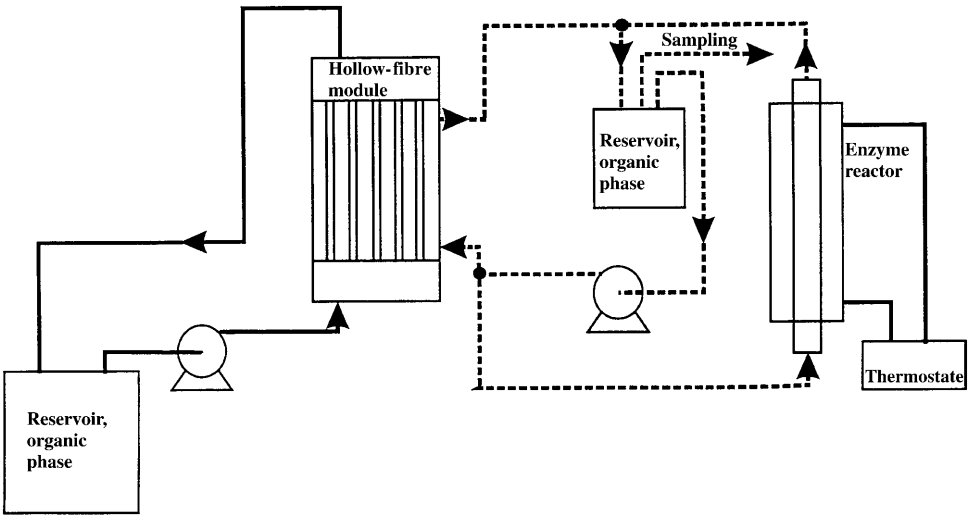

Fig. 2. Scheme of the membrane extractor and packed bed immobilized enzyme reactor system

4.3
Enzymatic membrane reactor/extractor integrated with immobilised cell fermenter

In the integrated system [50], a fluidised-bed reactor with Ca-alginate entrapped *Saccharomyces bayanus* IST 154 cells was selected as a fermenter of high operational stability and volumetric productivity. No distributor plate for the fluid flow was built into the reactor for the sake of simplicity. The effective volume of the reactor was changed in 0.3–2.5 dm^3 range, its diameter was 0.35–0.6 dm, the volume of the packing consisting of the immobilised cell particles was 0.05–0.5 dm^3. The enzyme-membrane reactor of hollow fibre type consisted of 40 fibres (diameter 2 mm, length 200 nm). The membrane was asymmetric cellulose acetate (cut off 40 000 Da), made in the Central Food-Industrial Research Institute (Budapest, Hungary). The module was jacketed and equipped by manometers to control the pressure difference between the primary and secondary sides. Lipase (*Mucor miehei*, Palatase 1000L, NOVO Nordisk) was immobilised on the membrane by different methods, e.g. by the ultrafiltration method mentioned above [70]. According to the so-called emulsion method, the undiluted enzyme solution was mixed with silicone solution and shaken in a test tube for 5 min. The emulsion was spread on the inner wall of the dry fibre applying alternated spreading and drying steps. In order to harden the silicone coating, the module was rinsed with inert gas for 24 h.

The immobilised cell fermenter and enzyme membrane reactor described above were combined as shown in Fig. 3. Comparing the different immobilisation methods especially the suspension method resulted in outstanding productivity. A relatively high ester yield (approximately 50% of the theoretical value) can be achieved even in case of low (30–40 mg/g) ethanol concentrations in the primary side of the enzyme membrane reactor.

4.4
Combination of membrane extraction, enzymatic conversion and electrodialysis for the integrated synthesis of ampicillin from penicillin G

The combination of membrane extractor/stripper with an electrodialysis cell is used for the separation of the co-product from the main product after an enzymatic reaction and change the pH value for a consecutive enzymatic reaction by Rindfleisch et al. [73].

The recovery of penicillin G (PG) from cell free cultivation medium was carried out by membrane extraction in a Hoechst-Celanese Liqui-Cel Extra-Flow hollow fibre membrane module. PG was removed from the aqueous phase across the membrane at pH = 5 into the Amberlite LA-2 isodecanol kerosene solution, and re-extracted in a second Hoechst-Celanese Liqui-Cel Extra-Flow hollow fibre module from the organic phase across a membrane into a phosphate buffer solution at pH = 7.5–8 (Fig. 4). In the stripping phase of the second module, PG was converted to phenyl acetic acid (PAA) and 6-amino penicillanic acid (6-APA) by the enzyme penicillin G amidase (PA) in phosphate buffer solution at pH = 8. The stripping phase cycle was integrated into the educt cycle of an electrodialysis module. PAA was separated from 6-APA by electrodialysis in the educt cycle of an electrodialysis cell system (Stan Tech GmbH, Hamburg) by means by bipolar anion and cation exchange composite membranes (Asahi Glass, Tokyo) and PAA was transferred into the product cycle. The pH of the phosphate buffer in the educt cycle of the electrodialyser was reduced to 6 by transferring phosphate into the product cycle. Ampicillin was formed by the reaction of 6-APA with phenylglycine ethyl ester (PGE) in the presence of PA at pH = 6 in the educt cycle of the electrodialyser. The stripping and conversion of penicillin G at pH = 8 and the ampicillin synthesis at pH = 6 are performed successively in a periodical continuous process (Fig. 5) [73].

5
Conclusions

Various unit operation techniques are often combined to increase the process performance. In biochemical engineering membrane separation is frequently linked with other separation processes, as pervaporation and membrane distillation for volatile components and membrane extraction for non volatile components. Membrane extraction is often connected with enzymatic reactions to enhance the mass transfer due to increased driving force across the membrane by converting the extracted

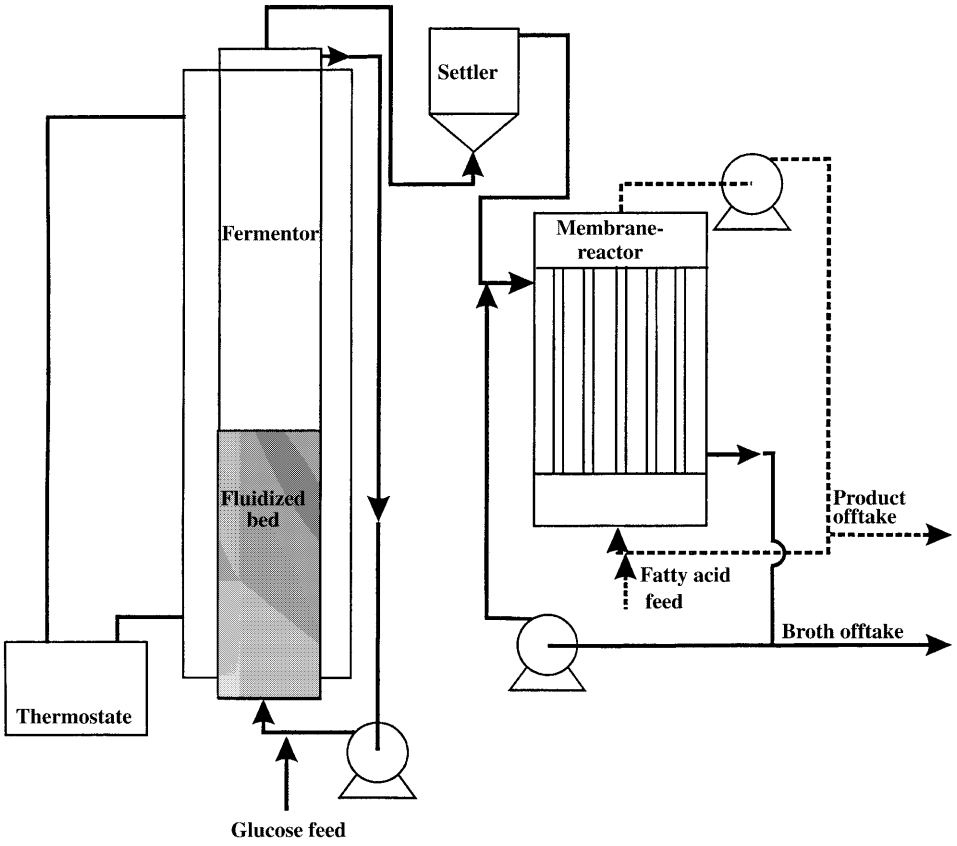

Fig. 3. Scheme of the integrated enzyme membrane reactor and immobilized cell fermenter

compound to a product and at the same time integrating successive stages of a production process. Several examples are presented in this paper. However, combined membrane processes are used for other purposes as well, e.g. to overcome the low solubility of substrates by dissolving the substrate in the organic phase and recharge it into the aqueous phase in an emulsion membrane reactor [74]. The aqueous phase is fed into an enzyme

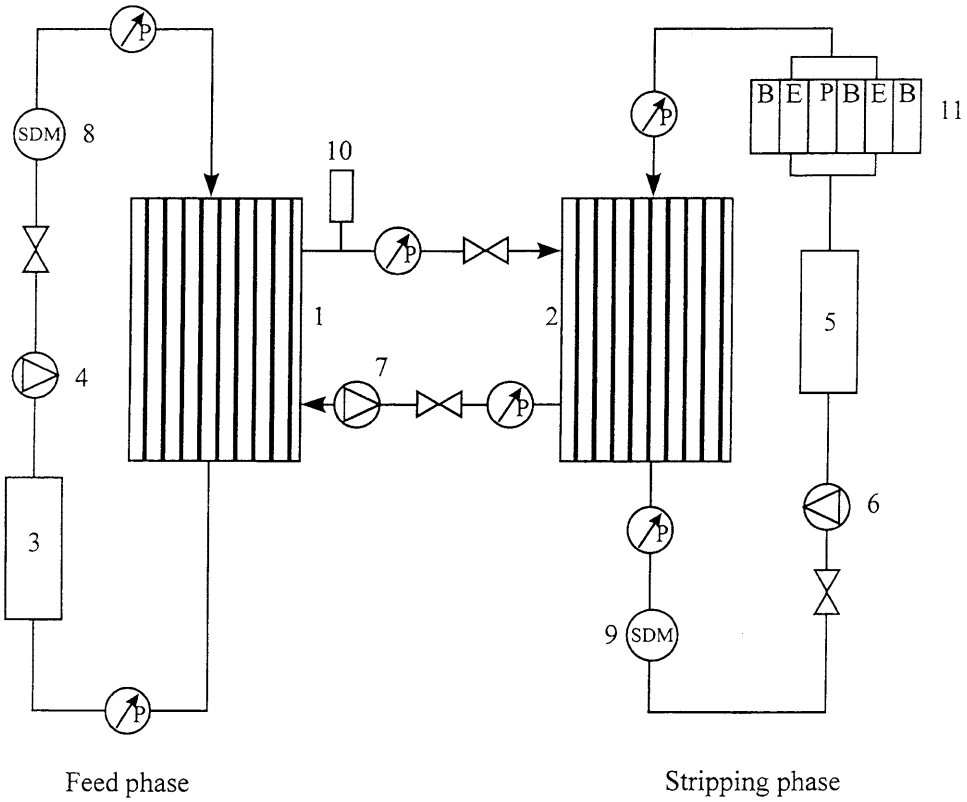

Fig. 4. Outline of the hollow fibre extraction system consisting of two membrane modules and the electrodialysis unit. 1, 2, two hollow fibre membrane modules; 3, 5, storage tanks for feed and extraction phases; 4, 6, 7, pumps; 8, 9, flow meters; 10, storage tank for the organic phase; 11, electrodialysis module with E (educt), P (product) B (base) and EL (electrode rinsing) cycles; SDM, flow meter [73]

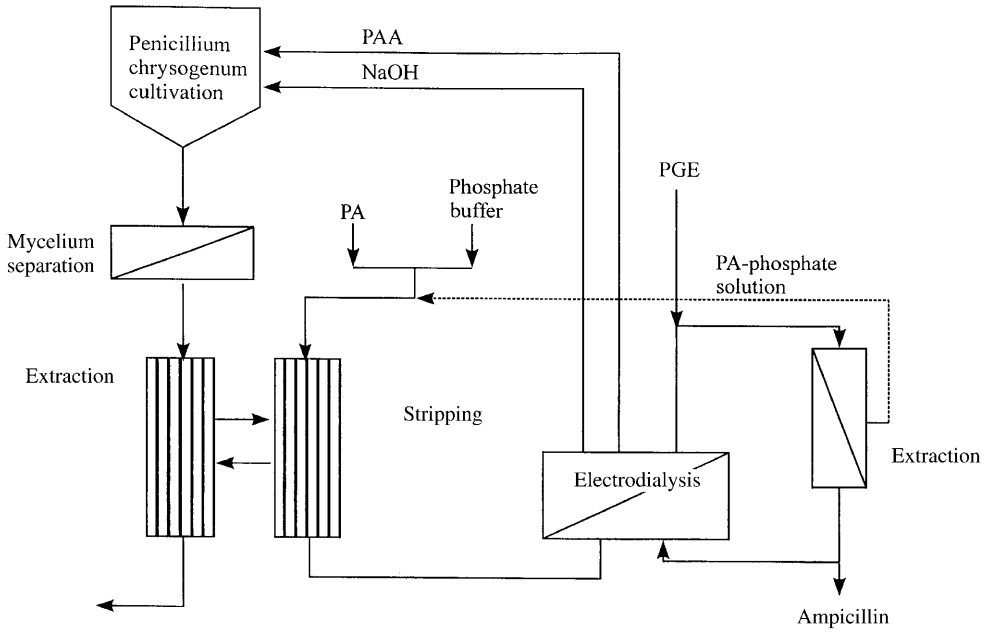

Fig. 5. Outline of the flow sheet for the integrated ampicillin production [73]

membrane reactor in which the substrate, 2-octanone is converted into the product, (S)-2-octanol by the enzyme carbonyl reductase from *Candida parapsilosis* and the NADH-cofactor is regenerated by the second enzyme, formate dehydrogenase. The product inhibiton is reduced by recycling the product into the emulsion membrane reactor and extracting it into the organic phase. The total turnover number could be increased by a factor of 9 by means of the additive use of the emulsion membrane reactor.

On the basis of the latter results and the other examples of the last few years, as also given in Table 4, it is expected that the combination of enzyme membrane reactors with various separation processes will have increasing importance in the future in biochemical engineering.

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