Review

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Enzymatic membrane reactors: the determining factors in two separate phase operations

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Abstract

A two separate phase-enzymatic membrane reactor is an attractive process since it has a large interfacial area and exchange surfaces, simultaneous reaction and separation and other benefits. Many factors influence its successful operation, and these include characteristics of the enzyme, membrane, circulating fluids and reactor operations. Although the operating conditions are the main factor, other factors must be considered before, during or after its application. At the initial stage of reactor development, the solubility of substrates and products, type of operation, membrane material and size, enzyme preparation and loading procedure, and cleanliness of the recirculated fluids should be specified. The immobilization site, reactor arrangement, dissolved or no-solvent operation, classic or emulsion operation and immobilized or suspended enzyme(s) are determined later. Some factors still need further studies. Utilization of the technology is described for use from multigram- to plant-scale capacity to process racemic and achiral compounds. The racemates were resolved primarily by kinetic resolution, but dynamic kinetic resolution has been exploited. The technology focused on hydrolytic reactions, but esterification processes were also exploited.

Keywords: enzymatic membrane reactors; operational factors; immobilised and suspended enzymes; hydrolysis reactions; esterification; kinetic resolution

INTRODUCTION

Enantiomers of drugs often have different pharmacokinetic and pharmacodynamic properties. As in β-blockers, the distomers give no β-blocking effects, reduce the overall drug selectivity, produce side-effects as the eutomers or possibly cause adverse effects.[1–12] These facts emphasize they usually do not contribute to medications. Hence, enantiomers should be used individually.[13–15] The need for single enantiomeric synthetic drugs can be observed from the world market, where consumption of single enantiomers increased rapidly from US$ 74.4 billion in 1996[16] to US$ 225 billion in 2005.[17] Many methods have been applied to generate single enantiomeric synthetic drugs.[18–20]

Enzymatic resolution of racemic compounds in membrane reactors (enzymatic membrane reactors or EMR) give many benefits. Conversion and separation of the enantiomers can take place in a single operation,[21,22] thus selective removal of products from reaction sites increases conversion of product-inhibited or thermodynamically unfavourable reactions.[23,24] The EMR overcomes disadvantages of batch stirred tank reactors,[25–27] consumes low energy and is easy to scale-up.[28,29] Compared with other technologies, the EMR has high productivity and stability, better control of single enantiomers production, enriched and concentrated products and decreased reaction times.[30–32] They could provide an efficient and cleaner route to single enantiomers.[33] Studies show that one of the best approaches to improve economic and technical competitiveness is the EMR.[34–36] Therefore, it is an attractive alternative to the enzymatic processes.[37,38]

Work on two separate phase-enzymatic membrane reactors (TSP-EMR or biphasic EMR) were started many years after Rony[39] published a pioneering report on enzymes immobilization in hollow fibre membranes (HFM). Up to now, the TSP-EMR, which still uses the HFM as the enzymes dock, has processed racemic and achiral compounds such as drugs, acids, esters and oils. Production of the single enantiomers from an achiral substrate used this EMR type. The technology was encouraged in dynamic kinetic resolution of the racemic compounds,[20,40–42] and has been employed in plant-scale capacities.[43,44] This paper details various determining factors of the TSP-EMR, which should be examined in development of the technology. Different applications of the technology with its different characteristics are described.

TSP-EMR: BASIC NEEDS

Immobilization of enzymes at organic–aqueous interfaces is necessary for EMR implementation. Many enzymes are active at these interfaces.[45,46] When an enzyme is on the membrane,

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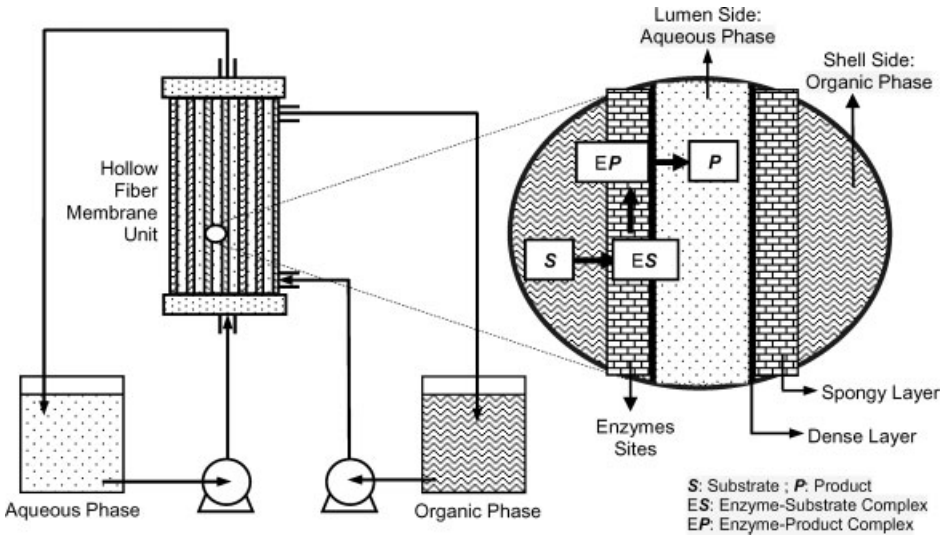

Figure 1. Materials transportation in an enzymatic membrane reactor.

in order for reaction to occur, substrate (***S***) has to be moved to enzyme sites, and product (***P***) has to be transported from the reaction sites to the other side of the membrane[29,47] as illustrated in Fig. 1. Catalytic activity of the enzyme on the interface makes it applicable to the two separate phase membrane-based system.

The TSP-EMR is formed by two immiscible fluids, a continuous organic phase and a continuous aqueous phase, separated by the enzyme-membranes interfaces.[48] This system is of interest when products are insoluble in the organic phase since the reaction and separation can be performed simultaneously, leading to a simplified process, reduction in production cost,[49,50] and reduction of down-stream processes.[51–53] Use of this biphasic EMR is highly attractive when HFM are employed.[54,55]

The HFM modules have four separate openings, an inlet and an outlet for each side of the membrane as shown in Fig. 1, which allow continuous flows across the membrane surfaces. Transport could be achieved using hydrophobic membranes and positive pressure on the aqueous side or hydrophilic membranes and positive pressure on the organic side.[50,56] Use of HFM to house the enzymes has advantages over conventional enzymes immobilization methods. Large exchange surface per unit volume is provided by these membranes.[32,57–59] The modules have high interfacial area, absence of emulsion, no flooding at high flow rates, no unloading at low flow rates and do not require density difference between phases.[50,57,60–62] They also have high volumetric productivity, simultaneous products separation, are easily reutilized, cheap enzyme fixation, little loss of the enzyme catalytic activity, high substrate concentration, and stable lipases activity.[62–66] However, they have several drawbacks, for instance solutions must be free of particulate materials, use of specific type of fibre,[63] diffusion resistance[64,67] and enzyme conformational changes.[68,69] Other membrane configurations having only one connection to downstream/permeate-side such as the spiral-wound are not suitable for the application.[35,70]

OPERATING CONSIDERATIONS: THE DETERMINING FACTORS

Development of the TSP-EMR involves operational strategy, enzyme loading procedure, membrane type and material, use of gas phase, etc. (Fig. 2). Operating conditions are the main issue, but other sensitive factors should also be considered before, during and after application.

Operating conditions

Operating conditions is one of the most important factors, involved in scale-up of a reaction process.[71] For the TSP-EMR, temperature, pH of the aqueous phase, concentration of the substrate(s) and immobilized enzyme, transmembrane pressure and flow rate of the organic and aqueous phase are conditioned during operation. Most of the factors could be managed externally to achieve the optimum performance; however, the immobilized enzyme activity during the processes is not controlled; this is determined by evaluating enzyme deactivation.[36,37,50]

Operating conditions of the TSP-EMR are described in Table 1. The technology is mostly applied in hydrolytic reactions, and run in batch recirculation mode. In general, operations are performed at mild temperatures, relatively long reaction times, low transmembrane pressures and medium flow rates in the shell- and lumen-side to give low to high conversions and medium to high selectivities. The organic and aqueous phases are fed through both sides of the HFM modules. All parameters are measured during experiments.

Fluids characteristics

Enzymes are stable and active in organic solvents,[89–92] but a certain amount of water bound on to enzyme surfaces is needed to keep them in catalytically active conformation.[93,94] Xin *et al*.[95] suggested an aqueous-organic biphasic system or a water saturated organic solvent reaction in hydrolysis of the racemic drugs. Lipases are found to be effective at the organic–water interfaces.[92,96] Although the reactor design for the biphasic system has several complications, the system overcomes the drug solubility problem and forms irreversible reactions.[97] It is undeniable that the solvent and water content may have effects on enantioselectivity.[98]

In the biphasic system, characteristics of the organic solvent are important since the enzymatic activity in the organic phase varies significantly.[99,100] Many lipases show high activity in hydrophobic solvents with low polarities.[57,101–103] Although higher

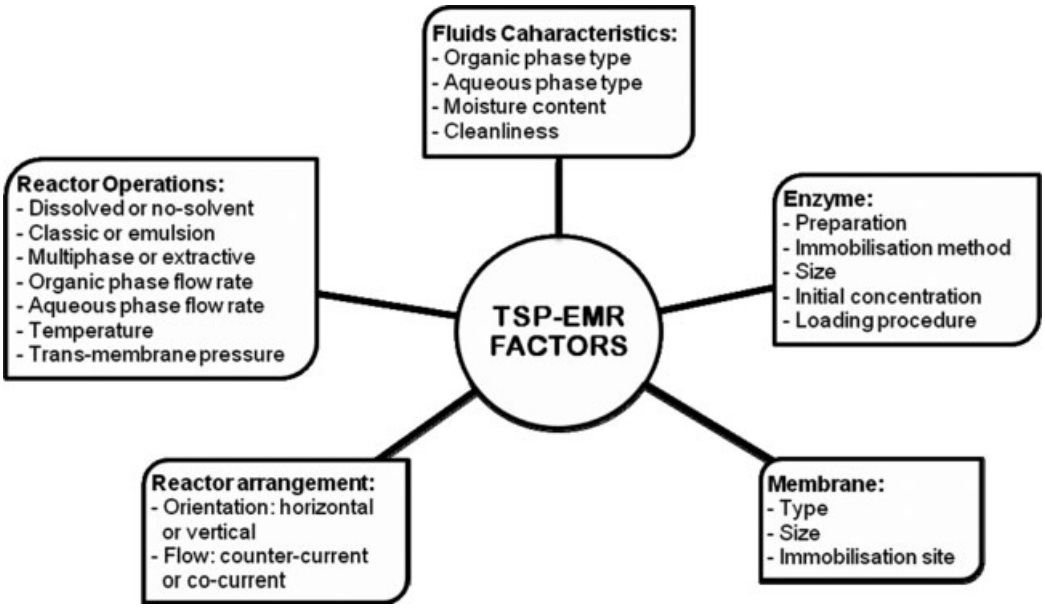

Figure 2. Factors in TSP-EMR operation.

enantioselectivity is found in hydrophilic solvents, hydrophobic solvents provide higher reaction rates.[103] Hydrophobic solvents are not dissolved easily in the aqueous phase, so good interfaces in the HFM modules can be formed. Thus, many TSP-EMRs are based-on strongly hydrophobic solvents such as those shown in Table 1. The moisture content plays a key role in enzyme-mediated esterification in organic media since high water activity (a_w) shifts the chemical equilibrium towards the reverse reaction.[104] Its requirement may vary as the solvent is changed.[105] Hydrophobic solvents have lower capability to remove water from the enzymes, so that in these solvents the enzymes have higher activity.[106]

As described in Table 1, a common aqueous phase is phosphate buffer solution at pH values of 5.5–8.5. Kamaruddin *et al*.[73] concluded that the pH of the aqueous phase during hydrolytic processes greatly influenced the lipase enantioselectivity, and a phosphate buffer at pH 8 resulted in the highest optical purity and enantioselectivity. During esterification of racemic ketoprofen, the conversion increased as pH was changed from 6.0 to 7.0, but it dropped at pH 8.[75] These results show that different reactions require a different aqueous phase. Lopez *et al*.[43] used bisulphite solution pH 8.5, which produced a significant increase in enantioselectivity, although pH 8.0 showed higher productivity. Dodds *et al*.[44] found similar results in which the aqueous phase at pH 8 gave high enantiomeric excess, although the buffer at pH 7 produced greater hydrolysis. Because of the importance of the aqueous pH, sodium hydroxide solution was frequently added to the aqueous phase during TSP-EMR runs to maintain buffer condition.[56,85,86] Albeit phosphate buffers were a good aqueous phase, they occasionally could not be used to provide a proper aqueous environment. As in hydrolysis of the racemic methyl-methoxy phenylglycidate (MMPG), the phosphate buffer led to base-catalysed hydrolysis of the substrate to form aldehyde by-product, which inhibited enzyme activity; hence in MMPG hydrolysis, researchers used a bisulphite solution to prevent aldehyde contamination.[44] This solution reduced the inhibitory product, shortened reaction time, increased the rate of reaction and improved the enantioselectivity.

Cleanliness of the liquid/air phase is also an important factor. The solution must be free of particulate materials.[63] This reduces membranes fouling caused by unwanted materials thus increasing the membrane service life. Installation of filters before the reactor units and use of non-corrosive materials prevents particulate matter. Breslau[107] suggested using an extremely pure liquid to avoid blockage or contamination of the membranes.

Dissolved- or no-solvent operation

Operating the TSP-EMR for drugs resolution uses the organic solvents to dissolve the racemic drugs and to form the organic phase (Table 1). Dissolved-solvent operations are applied when the substrates are solid compounds or viscous liquids. Some operations have been developed with no solvent added to the organic phase. For example, ethyl butyrate was hydrolysed by porcine liver esterase in several hours at the reaction rate of 9600 μ min^{-1}.[56] Hydrolysis of oils also proceeded in a no-solvent system.[76,77] The increase of olive oil concentrations led to the increase of catalytic efficiency.[48] Only a small conversion difference was found when this biphasic EMR was operated in the presence of an organic solvent (60%) or with the pure oil (50%).[108]

Multiphase or extractive TSP-EMR

In dissolved operation, substrates can be supplied either in the organic or aqueous phase. Matson[40] called the TSP-EMR with key reactant(s) fed through a water-immiscible organic phase a 'multiphase EMR'; while the aqueous phase dissolving key reactant(s) is referred to as the 'extractive' type. As described in Table 1, most operations are based on the multiphase type. Since TSP-EMR operations are based on simultaneous reaction and separation, the reactant(s) must not mix with the product(s). This means that in the multiphase TSP-EMR, the substrate(s) should have high solubility in the organic solvent and not dissolve in the aqueous phase. In contrast, the product(s) must dissolve easily in the aqueous phase, so that they can be transported without problems. Hence, the solubility behaviour of the substrate(s) and product(s) must be considered, since many commercial organic

Table 1. Operating conditions of chiral drugs and non-racemic substrates resolution in the TSP-EMR

Substrate			Enzyme		Membrane			Medium								
Type	Concentration	Locus	Type	Site	Material	MW CO (kDa)	Area (m^2)	Shell	Flow Rate (mL min^{-1})	Lumen	Flow Rate (mL min^{-1})	TMP (kPa)	T (°C)	Reactor Orientation	Operational Mode	Ref.
(±)-naproxen ester	ND	MP	CRL	Sponge	PA	50	0.076	Isooctane, Emulsion	ND	PO_4 buffer pH 7.0	ND	50	30	Horizontal, Counter Flow	Batch-Recirculation	21
(±)-naproxen ester	0.75 M	MP	CCL	Sponge	PAN	ND	0.85	Methyl Isobutyl Ketone	400–450	PO_4 buffer pH 8.50	300–400	ND	ND	ND	Batch-Recirculation	40,41
(±)-naproxen ester	ND	MP	CRL	Sponge, Lumen	PA	50	ND	Isooctane	300	PO_4 buffer pH 7.0	300	17-41	30	ND	Batch-Recirculation	52
(±)-naproxen ester	ND	MP	CRL	Sponge	PA	50	0.015	Isooctane, Emulsion	ND	PO_4 buffer pH 7.0	ND	0	30	Vertical, Counter Flow	Batch-Recirculation	60
(±)-naproxen ester		MP	CRL	Sponge, Lumen	PA/PS	10/30	0.005/0.004	Isooctane	330	PO_4 buffer pH 7.0	330	25	30	Horizontal, Counter Flow	Batch-Recirculation	65
(±)-naproxen ester	ND	MP	CRL	Sponge	PA	50	ND	Isooctane	ND	PO_4 buffer pH 7.0	ND	ND	ND	Horizontal, Counter Flow	Batch-Recirculation	72
(±)-Ibuprofen ester	25–100 mM	MP	CRL	Sponge, Lumen	PAN	50	0.093	Isooctane	50-250	PO_4 buffer pH 8	50–450	40	40	Horizontal, Counter Flow	Batch-Recirculation	32,33,73
(±)-ibuprofen ester	ND, No-Solvent	NSO	CCL	Sponge	PAN	ND	0.85	(±)-ibuprofen ester	400-450	PO_4 buffer pH 7.8	400–450	ND	ND	ND	Batch-Recirculation	40,41
(±)-ibuprofen ester	64.60 g L^{-1}	EX	CCL + Prozyme 6	Sponge	PAN	50	1.0	Cyclohexane	ND	PO_4 buffer pH 7.0	ND	ND	20	ND	Batch-Recirculation	41
(±)-ibuprofen ester	161 g L^{-1}	EX	Prozyme 6	Lumen	PAN	ND	0.85	Cyclohexane -Toluene	ND	PO_4 buffer pH 7.0	ND	ND	30	ND	Batch-Recirculation	41
(±)-ibuprofen ester	32.51 g L^{-1}	EX	Prozyme 6	Lumen	Cellulose	18	1.90	PO_4 buffer pH 7.0	ND	Hexane	ND	ND	RT	ND	Batch-Recirculation	41
(±)-ibuprofen ester	500 mM	EX	Seaprose	Sponge	PAN	50	ND	Cyclohexane	400	PO_4 buffer pH 7.0	400	ND	ND	ND	Batch-Recirculation	74
(±)-ibuprofen	2 M	MP	CCL	Sponge	PAN	ND	0.85	Pentanol	300	PO_4 buffer pH 5.5	300	ND	ND	ND	Batch-Recirculation	40
(±)-ketoprofen	20-35 mM	MP	CALB	Sponge	PS	10	0.015	Dichloropropane - hexane	40	PO_4 buffer pH 7	ND	ND	40	ND	Batch-Recirculation	75
(±)-glycidyl butyrate	ND, No-Solvent	NSO	PPL	Sponge	PAN	ND	0.85	Glycidyl butyrate	250-350	PO_4 buffer pH 7.8	250-350	ND	ND	ND	Batch-Recirculation	40,56
Ethyl butyrate	No-Solvent, 0.5 L pure substrate	NSO	PLE	Sponge	ND	ND	1.0	Ethyl butyrate	500	PO_4 buffer pH 8	500	45	ND	Horizontal, Counter Flow	Batch-Recirculation	56
Ethyl butyrate	ND, No-Solvent	NSO	PLE	Sponge	PAN	50	0.80-1.0	Ethyl butyrate	500	PO_4 buffer pH 8.0	500	ND	ND	ND	Batch-Recirculation	40,74

Table 1. (*Continued*)

Substrate			Enzyme		Membrane			Medium								
Type	Concentration	Locus	Type	Site	Material	MW CO (kDa)	Area (m^2)	Shell	Flow Rate (mL min^{-1})	Lumen	Flow Rate (mL min^{-1})	TMP (kPa)	T (°C)	Reactor Orientation	Operational Mode	Ref.
Palm oil	59-326 mM	MP	CRL	Lumen	CP	5	1.0	PO_4 buffer pH 7	2.55-20.5	Isooctane	11.7–50	ND	30	Vertical, Co-current Flow	Batch-Recirculation	35,38
Palm oil	No-Solvent, 0.5 L pure substrate	NSO	MML	Sponge	PS, RC	66	1.1, 1.0	PO_4 buffer pH 7	3	Palm oil	2.5	15	40	Vertical, Co-current Flow	Batch-Recirculation	76
Olive oil	ND, No-Solvent	NSO	CCL	Sponge	PAN	ND	1.0	Olive oil	250-340	Buffer pH 6.4	80	ND	RT-37	ND	Batch-Recirculation	40
Olive oil	ND, No-Solvent	NSO	CCL	Lumen	RC	ND	0.80	Olive oil	40	Buffer pH 6.4	20	ND	ND	ND	Batch-Recirculation	40
Olive oil	ND	MP	CRL	Sponge	MPP	ND	ND	Olive oil + Heptane	85	PO_4 buffer	335	30	40	Horizontal, Co-current Flow	Batch-Recirculation	48
Olive oil	No-Solvent, 0.5 L pure substrate	NSO	MML	Sponge	PS, RC	66	1.1, 1.0	PO_4 buffer pH 7	3	Olive oil	2.5	15	40	Vertical, Co-current Flow	Batch-Recirculation	76
Olive oil	No-Solvent, 0.2 L pure substrate	NSO	CRL	Sponge	PA	50	ND	Olive oil	80	PO_4 buffer pH 8	400	34	ND	ND	Batch-Recirculation	77
(±)-2-hydroxy octanoic acid ester	20, 100 mM	MP	CRL, PCL	Sponge	PAN, PES	10, 30	0.0030-0.0036	Isooctane	ND	PO_4 buffer pH 7	ND	ND	30	ND	Batch-Recirculation	53
(±)-2-hydroxy octanoic acid ester	100 mM	MP	PCL	Sponge	PAN, PES, PE	30,30, ND	0.0009-0.0007	Isooctane or Hexane	ND	PO_4 buffer pH 7	ND	20, 20, 5	30	Horizontal, Co-current Flow	Batch-Recirculation	78
(±)-1-phenyl ethyl acetate, (±)-1-phenyl-1 -propylacetate	50 mM	MP	P.Sp	Sponge	PA	50	0.0017	Heptane	5-20	PO_4 Buffer pH 8	5-25	ND	30	Horizontal, Counter Flow	Batch-Recirculation	79
(±)-1-phenyl ethyl propionate	100 mM	MP	P.Sp	Lumen	PA	50	0.0030	PO_4 buffer pH 7.2	ND	Heptane	ND	ND	ND	Horizontal, Counter Flow	Batch-Recirculation	80
(±)-octyl 2-chloro propionate	0.96 moles, No-Solvent	NSO	*Candida* lipase	Sponge	PAN	50	0.85	(±)-chloropro pionate octyl ester	400	0.05 M K_2PO_4	400	ND	ND	ND	Batch-Recirculation	40,41
(±)-butyl 2-chloro propionate	3.03 moles, No-Solvent	NSO	Candida	Sponge	PAN	ND	0.85	Butyl 2-chloro propionate	300	PO_4 buffer pH 7	300	ND	ND	ND	Batch-Recirculation	40
Phenoxy acetate methyl ester	ND, No-Solvent	NSO	*Candida* L-1754	Sponge	PAN	50	0.80-1.0	Phenoxyacetate methyl ester	150	0.1 M $NaHCO_3$	300	ND	ND	ND	Batch-Recirculation	40,74
Amyl acetate	ND, No-Solvent	NSO	Candida lipase	Sponge	PAN	50	0.80-1.0	Amyl acetate	50-150	0.05-0.1 M $NaHCO_3$	300	ND	ND	ND	Batch-Recirculation	40,74
(±)-methyl-me thoxy phenyl glycidate	12.6%-23 (w/w)	MP	OF 360	Sponge	PAN	30	0.75-60	Toluene	ND	Bisulfite pH 8.5	ND	31-51	15-19	Horizontal, Counter Flow	Batch-Recirculation	43

Table 1. (*Continued*)

Substrate			Enzyme		Membrane			Medium								
Type	Concentration	Locus	Type	Site	Material	MW CO (kDa)	Area (m^2)	Shell	Flow Rate (mL min^{-1})	Lumen	Flow Rate (mL min^{-1})	TMP (kPa)	T (°C)	Reactor Orientation	Operational Mode	Ref.
(±)-methyl-me thoxy phenyl glycidate	20.8 -2,340 g	MP	MAP, OF, Palatase M	Sponge	PAN	ND	0.75-12	Toluene	400-450	PO_4 buffer pH 7.0	400-500	ND	ND	ND	Batch-Recirculation	44
(±)-methyl me thoxy phenyl glycidate	ND	MP	Serratia marcescen	Sponge	PAN	50	0.75	Toluene	150	$NaHSO_3$ pH 8.5	ND	ND	ND	Vertical, Co-current Flow	Batch-Recirculation	81
Decanoic acid	100 mM	MP	CRL	Sponge	RC	ND	0.7, 1.0	Hexane	4.5	Air	ND	ND	25	Horizontal, Co-current Flow	Batch-Recirculation, Continuous	82
Decanoic acid	100-1000 mM	MP	CRL	Sponge	RC	10	1.0	Hexane	3.0	Air	0.90	ND	20	Horizontal, Co-current Flow	Batch-Recirculation	83
Decanoic acid	ND	MP	CRL	Lumen	CP	ND	0.77	Saturated sorbitol solution	20	Hexa-decane	30	ND	30	ND	Batch-Recirculation	84
cis-cycloxex -4-ene -1,2-di carboxylate	ND	MP	PLE	Sponge	PS	30	0.2	Hexane	ND	PO_4 buffer pH 7	ND	ND	25	Vertical, Co-current Flow	Batch-Recirculation	85
cis-cycloxex -4-ene -1,2 di carboxylate	230 mM	MP	PLE	Sponge	PS	30	0.49	Hexane	270	PO_4 buffer pH 7	102	30	25	Vertical, Co-current Flow	Batch-Recirculation	86
N-Benzoyl tyrosine	17 mmol	EX	Chimo trypsin	ND	PAN	ND	1.0	Octanol	400	PO_4 buffer pH 6	400	ND	ND	ND	Batch-Recirculation	40
(±)-BTEE	10-40 mM	MP	Chimo trypsin	Sponge	PAN	ND	1.0	Amyl acetate/Octanol	10-500	PO_4 buffer pH 7.0	250-500	ND	ND	ND	Batch-Recirculation	40,74
(±)-BTEE	0.2 mM	MP	Chymo trypsin	Sponge	PAN	ND	1.0	Silicone oil	ND	PO_4 buffer pH 7.8	1000	ND	ND	ND	Batch-Recirculation	40,74
(±)-ATEE	2.86 mM	MP	Chimo trypsin	ND	PAN	ND	1.0	Octanol	400	0.1 M K_2PO_4	400	ND	ND	ND	Batch-Recirculation	40
N-(benzyloxy carbonyl)-L-aspartic acid, L-phenyl alanine ester	ND	EX	Thermo lysin	Lumen	ND	50	0.70	Acetic buffer + butyl acetate (pH 5)	200	Butyl acetate + Acetic buffer	200	19.60	40	Vertical, Co-current Flow	Batch-Recirculation	87
Triacetin	ND	MP	CCL	Shell	PAN	50	0.75	Toluene	ND	PO_4 buffer pH 7.0	ND	50	30	Vertical, Counter-current Flow	Batch-Recirculation	88

CRL: *Candida rugosa* lipase, CALB: *Candida antarctica* lipase fraction B, OF-360: OF 360 lipase, PCL: *Pseudomonas cepacia* lipase, PPL: *Porcine pancreatic* lipase, PLE: *Pig liver* esterase, MML: *Mucor miehei* lipase, P.Sp: *Pseudomonas* sp.,
PAN: Polyacrylonitrile, PA: Polyamide, PS: Polysulfone, PES: Polyethersulfone, PE: Polyethylene, RC: Regenerated Cellulose, MPP: Modified Polypropylene, CP: Cuprophane, MWCO: Molecular Weight Cut-Off.
BTEE: N-Benzoyl-L-Tyrosine Ethyl Ester, ATEE: N-Acetyl-Tyrosine Ethyl Ester,
g: gram, w: weight, L: Litre, M: mole/Litre.
MP: Multiphase; EX: Extractive, NSO: No-Solvent Operation.

compounds have low solubility in the aqueous phase.[49,82,109] This factor must be considered in the first stage of development.

Reactor arrangement

Even though different specifications of HFM have been used, the TSP-EMR was primarily developed as a horizontal counter-flow or vertical co-current-flow arrangement (Table 1). In fact, the horizontal type is preferred, as it was found to resolve racemates, while the opponent modules were useful in oil hydrolysis. Counter-current flow reactors showed higher productivity/effectivity than the co-current TSP-EMR.[31,110] It is considered that HFM modules with vertical fibre orientation are better at controlling membrane fouling,[111] but this orientation requires a higher pressure input.[112]

Classic or emulsion TSP-EMR

Recently, the TSP-EMR integrated the use of emulsion in order to improve performance of the immobilized enzyme. In this case, an emulsion of oil-in-water (O/W) prepared by the emulsification technique is mixed with an enzyme solution. The mixture is then transferred to the HFM modules to form enzymatic reactors with an emulsion environment on the membrane surfaces. The TSP-EMR with emulsion optimized distribution of the immobilized enzyme improved the mass transfer.[21] Although the specific activity of the enzyme with and without the emulsion was same, the enantiomeric excess of the product increased from 74% (TSP-EMR) to 97% (TSP-E-EMR) for (*R*, *S*)-naproxen methyl ester, and from 96% (TSP-EMR) to 100% (TSP-E-EMR) for racemic naproxen butyl ester, and the overall mass transfer coefficient of the TSP-E-EMR was larger by 58%.[21] However, formation of the emulsion environment on the membrane surfaces may complicate workup,[113] whereas the classic biphasic EMR offers simple operation.

Immobilisation site

Either the shell-side (spongy layers) or lumen-side of the HFM can be used as asurface to dock the enzymes. The spongy layers are usually employed as described in Table 1. Immobilization of the enzymes on the shell-side gave higher protein attachment and enantiomeric excess than the lumen-side.[33,65] Besides, shell-side immobilization allowed higher conversion,[65] higher catalytic activity and stability,[114] and higher loading capacity.[115] Although immobilization of the enzymes on the lumen-side showed lower specific activity, it led to higher enantioselectivity.[52]

Enzyme preparation

The TSP-EMR enzymes were generally prepared by a simple method. First, the biocatalysts were dissolved in the phosphate buffer pH 7–8 with gentle steering for 45–120 min. Then the solution was centrifuged to remove the insoluble matter (3000–5000 rotation per minute, 5–15 min). After separation, the enzymes were immobilized.

The initial source of the enzymes could influence TSP-EMR performance. Most enzymes were used directly (crude or native enzymes). Sakaki *et al*.[52] compared a TSP-EMR using purified lipase with a TSP-EMR based on crude lipase, where both lipases were prepared by the above method. They found the reactions catalyzed by the crude lipase had lower enantioselectivity, and concluded that the crude lipase contains some hydrolases with low or no enantioselectivity, which act to reduce product purity. Another observation was that surfactant-coated lipase gave higher conversions (1.4 to 2 times) than the native type.[109]

Enzyme immobilization and loading procedure

The quantity of enzymes attached to the membrane surfaces is important.[33,115] For high enzyme attachment, a suitable immobilization strategy is required. Thousands of procedures have been reported.[116] Membranes have high surface area for enzyme loads and provide strong covalent attachment.[117] In the TSP-EMR, the preferred method to immobilize the enzymes is adsorption through cross-flow filtration. This method recirculates an enzyme solution from one side to the other side of the HFM (shell- to lumen-side or the other way) causing the enzyme to adsorb to the membrane layers. A diffusive mode of the enzyme immobilisation method was also developed.[74] In this method, the enzyme flows diffusively to the HFM surfaces without cross-flowing the enzyme solution. Immobilization of the enzymes onto chemically modified membrane surfaces has also been conducted, in which covalent bonds of the enzyme–chemical–membranes are formed. Results of enzyme immobilization inside the TSP-EMR are shown in Table 2. Relatively low enzyme concentrations attached to the HFM surfaces occur after the immobilization processes compared with the initial solution concentration.

The strongest immobilization method is obtained by the covalent bond.[118] However, for immobilization of *Candida rugosa* lipase on different membrane materials, Hollownia[66] found that the adsorption method provided higher process efficiencies than the chemical binding method, but the latter produced higher enzyme catalytic activity. These results were the same as the earlier observations.[40,74] The physical entrapment of the enzyme onto the HFM surfaces led to higher retention of the enzyme activity, while the covalent bond required a longer process, which led to a lower retention of the enzyme activity.[49]

Several enzyme loading procedures as shown in Fig. 3 were compared to evaluate protein distribution on the membrane surfaces, i.e. the axial and radial distribution of the enzyme since the enzyme activity depended on its distribution along the fibres.[85] The procedures (3a) and (3b) gave higher axial and radial enzyme distribution. However, enzyme immobilization could be done by recirculating the enzyme solution through both sides of the HFM.[119,120]

Membrane type

Since enzymes are more effective in an aqueous environment than in organic solvents, hydrophilic membranes are preferred.[40] Major differences between the hydrophobic and hydrophilic membranes are the thickness of reaction layers, which is much smaller in the hydrophobic membranes, and the capability of hydrophilic membranes to increase the enzymes activity, hence the immobilized enzymes could retain their full catalytic activities.[121] As described in Table 1, most TSP-EMR use hydrophilic HFM. Sakaki and Itoh[79] found that the biphasic reactor with hydrophobic microfiltration membranes was not well suited to optical resolution by lipase-catalyzed hydrolysis. However, in palm and olive oil hydrolysis, adsorption of lipase was higher in hydrophobic HFM than hydrophilic membranes.[86] Combination of hydrophilic–hydrophobic membranes was also tested.[122] These membranes accommodate high enzyme load. Although hydrophobic membranes are more physically and chemically stable, create high affinity and open-state lipases,[121,123–125] the hydrophilic type provides a thick contact zone, water-wetted interfaces, higher enzyme activity, lower enzyme desorption and lower enzyme concentration.[48,122,126] From the economic

Table 2. The TSP-EMR enzymes immobilization characteristics

Method	Membrane	Solvent	Initial [enzyme]	Time (*h*)	Flow rate	TMP (kPa)	T (°C)	Immobilized enzyme	Ref
Physical adsorption	PA 50	50 mM PO_4 buffer pH 7	3 g L^{-1}	4	ND	40	ND	8.1–13 mg	Giorno *et al.*[21]
Physical adsorption	PAN 50	50 mM PO_4 buffer	2 g L^{-1}	ND	1.5×10^{-2} m/s	35	RT	0.10-0.17 g	Kamaruddin *et al.*[32]
Physical adsorption	PAN 50	50 mM PO_4 buffer pH 8	2 g L^{-1}	ND	300 mL/min	35	RT	0.13-0.17 g	Kamaruddin *et al.*[33]
Physical adsorption	CP 5	Distilled water	7.5 g L^{-1}	0.5	30 mL/min	ND	ND	154.8 ± 5.2 mg/m^2	Knezevic *et al.*[35,38]
Physical adsorption	MPP	50 mM PO_4 buffer pH 7	ND	2	ND	ND	25	68.68–230.32 mg/m^2	Xua *et al.*[48]
Physical adsorption	PA 50	50 mM PO_4 buffer pH 7	0.25, 1 g L^{-1}	~120 mL permeate volume	ND	50	ND	8.1, 35 mg	Giorno *et al.*[52]
Physical adsorption	PA 50	PO_4 buffer	2 g L^{-1}	ND	ND	50-70	RT	3.38 mg	Giorno *et al.*[60]
Physical adsorption	PAN 50	50 mM PO_4 buffer	2 g L^{-1}	ND	300 mL/min	35	ND	0.17 g	Kamaruddin *et al.*[73]
Physical adsorption	PS 10	50 mM PO_4 buffer	0.86-17.12 mL L^{-1}	~80% permeate volume	40	25	RT	0.05 mL/mL	Aboul-Enein *et al.*[75]
Physical adsorption	PAN 30, PS 30, PE -	50 mM PO_4 buffer pH 7	1 g L^{-1}	1 h & 50% permeate	ND	50	ND	ND	Sakaki and Itoh[78]
Covalent Bonding	PA 50	20 mM PO_4 buffer, pH 7.2	0.03 & 0.05 mg cm^{-2}	24	ND	ND	30	ND	Ceynowa and Koter[79]
Physical adsorption	PS 30	100 mM PO_4 buffer pH 7	ND	1	ND	120	RT	1.28 units/cm^2	Sousa *et al.*[85]

ND: No Data.

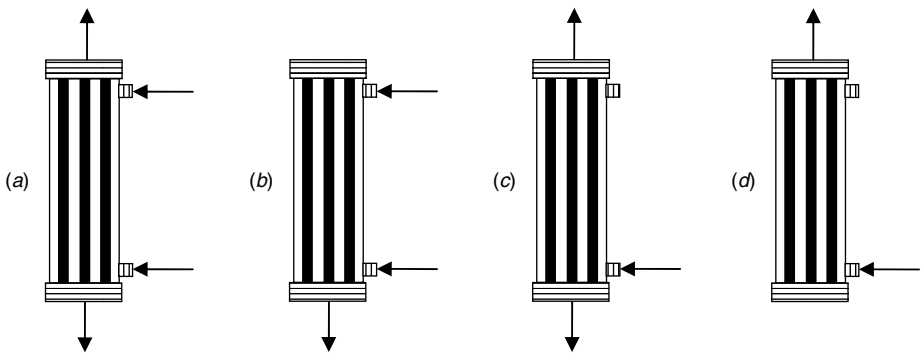

Figure 3. Various procedures for the enzyme loading.

perspective, hydrophilic membranes are preferred since fewer enzymes are required.[48] There may be a significant advantage in employing relatively thin membranes with high loads when the membrane thickness is a critical factor for optimum enzyme loads.[127]

A comparison between polyacrylonitrile (PAN) and polyethersulfone (PES) HFM showed that the PAN gave higher enantioselectivity, but the PES developed higher reaction rates per unit membrane area.[53] Further observation proved that the PES produced higher reaction rates than the PAN, but the enantioselectivities were similar.[79] Higher enantioselectivity was obtained by polyamide than polysulfone HFM.[65,115] The highest process efficiency was found with polypropylene membrane during the adsorption of enzyme, while the cellulose membrane produced higher process efficiency than the polyamide for chemical binding of the same lipase.[66]

Membrane and enzyme size

Membranes are classified into microfiltration membranes (pore sizes 0.1–10 μm), ultrafiltration membranes (10–100 nm), nanofiltration (1–10 nm) and reverse osmosis membranes (<1 nm).[70] Enzymes with molecular sizes 10–500 kDa are retained by ultra filtration membranes, with a typical molecular weight cut-off (MWCO) of 10 kDa. Since the sizes of many enzymes are 10–80 kDa, ultrafiltration membranes with MWCO of 1–100 kDa are the most frequently used.[30] If the enzyme molecules are large compared with the membrane pore sizes, they cannot diffuse through the membrane layers,[86] and will be immobilized easily and no leaks of enzyme will occur from the HFM modules.

Fouling control

Generally a major limitation of membrane operation is loss of performance due to fouling.[128,129] This occurs when membrane flux decreases as a function of time due to increase in hydraulic resistance.[130] The fouling and polarization of concentration during EMR operations could shorten membrane service life and reduce economic benefits, but these limitations can be reduced by improving flows at the membrane surfaces, (which disrupt the boundary layers) by introducing Dean vortices or by surface modifications.[30,128,131] They could also be diminished by a new module design and fluid-dynamic operation.[70]

APPLICATION OF TSP-EMR: IMMOBILIZED ENZYMES

Resolution of racemic compounds

Resolution of racemic drugs, organic acids, amino acids and ester molecules have taken place. Either short-chain racemic esters or their long-chain compounds are resolved by hydrolytic reactions, but esterification of the racemates has also been developed. Performance of the hydrolysis in terms of activity and enantioselectivity is increased with decrease of aliphatic chains of the alcohol group.[72,73] During these processes, the enzyme acts as an enantioselective converting system and the HFM functions as a barrier that separates the enantiomers,[33,43] as illustrated in Fig. 4. Commonly one isomer is transported to the aqueous phase as product and the unreacted enantiomer remains in the organic phase.[78,132,133] Multiphase operation of the TSP-EMR has considerable advantages for enzymatic optical resolution when the substrates are poorly water-soluble and the products dissolve in water.[78]

Drugs and Intermediates

Kinetic resolutions of non-steroidal anti-inflammatory drugs (NSAIDs) give medium to high selectivities at mild temperatures and long reaction times. Although these processes achieved almost maximum resolution values, some conversions are low.

Hydrolysis of racemic *trans*-methylmethoxyphenylglycidate (MMPG), a diltiazem intermediate, is one important application of the TSP-EMR. MMPG has been resolved in an industrial facility. Compound resolution was started at bench-scale where lipase OF 360 was utilized.[43] Using toluene and bisulphite solution at pH 8.5, the process gave 42.6% conversion and product enantiomeric excess of 84.4% under optimized conditions. In a pilot-plant study (100-fold scale-up ratio, 10 pilot-scale modules with 7.5 m^2 membrane effective area), the TSP-EMR produced enantioselectivity of 24.6, product yield of 42.9% and enantiomeric excess of 82% (product) in 22 h at 16–19 °C. A commercial-scale facility was then set-up using twelve 60 m^2 modules, which gave 55 kg $year^{-1}$ m^{-2} of 99% enantiomeric excess of the ester. Similar results were obtained using *Serratia marcescens* lipase.[134]

Several patents on resolution of racemic ibuprofen esters through hydrolysis and esterification reactions were granted to Matson,[40] Matson *et al*.[41] and Lopez and Matson.[74] The racemic ibuprofen trifluoroethyl ester was resolved by *Candida cylindracea* lipase (CCL) under no-solvent operation using potassium phosphate buffer at pH 7.8 and conditions as stated in Table 1. Low yields were obtained after 76 h operating time. Esterification of ibuprofen acid was then developed using the same lipase adsorbed onto polyacrylonitrile multiphase TSP-EMR. Pentanol was used as the organic phase and alcohol as donor. After 68 h, spectrophotometric analysis indicated that the ester was formed. Resolution of sulfomethyl ibuprofen ester using Prozyme 6 protease in an extractive TSP-EMR produced 1.76 g of resolved ibuprofen from 32.3 g of the substrate after 17 h. Later, Seaprose enzyme was cross-linked to an extractive polyacrylonitrile HFM to hydrolyse the racemic ibuprofen sulfomethyl ester.[74] This process was completed after 46 h.

Racemic ibuprofen ester resolution was also studied by Kamaruddin *et al*.[33,73] The ester dissolved in isooctane was circulated continuously in the membrane shell-side, and the products were transported to phosphate buffer at pH 8 flowing in the lumen-side. The polyacrylonitrile reactor was operated for 10 h at 40 °C and 40 K Pa transmembrane pressure to give conversion up to 31% and product enantiomeric excess of 90%. The effect of alkyl length of the ester compounds was also investigated. The short-chain substrate, 2-ethoxy ethyl ibuprofen ester, had higher enantiomeric ratio than 1-heptyl ester. Previously, hydrolysis of the racemic cyanomethyl ibuprofen ester was obtained in

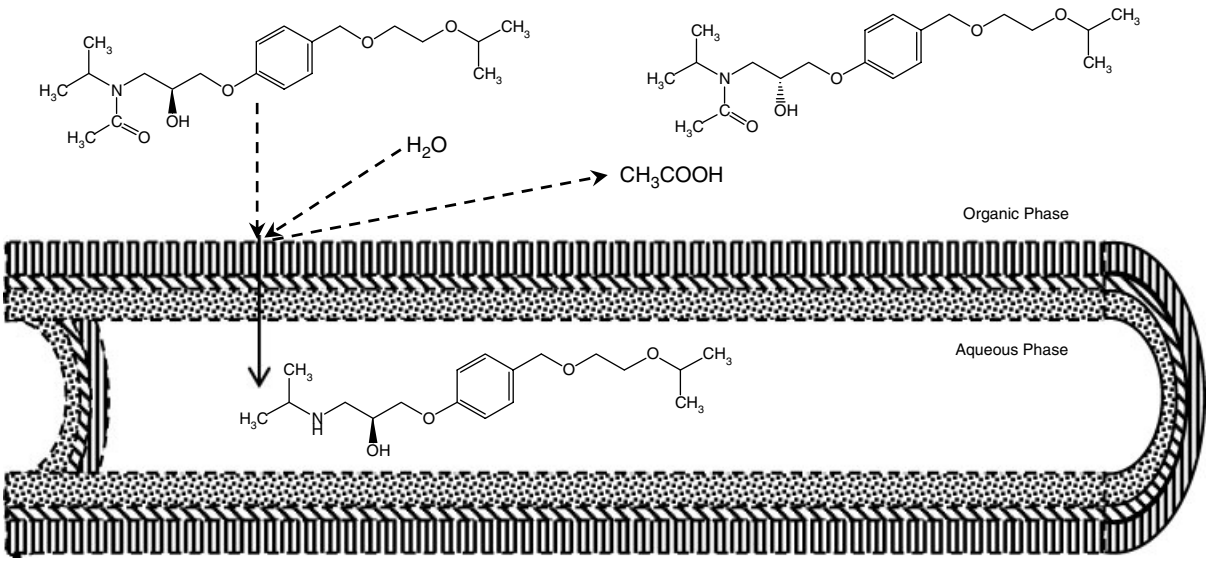

Figure 4. Kinetic resolution of a racemate in the TSP-EMR.

polyamide membranes, which housed CCL.[31] Similar results to those of Kamaruddin were found. The reactor showed no enzyme deactivation up to 16 days. Further, observation of the hydrolysis of racemic cyanomethyl-[2-(4-isobutylphenyl) propionate] indicated that reduction of the immobilized enzyme activity compared with the free-enzyme was mostly due to low mass transport of reagents across the membranes.[77]

Production of (*S*)-naproxen acid obtained excellent product enantiomeric excess (74–100%), but low conversion values (<10%).[21,52,60,65,72] *Candida rugosa* lipase (CRL) immobilized on sponge layers of polyamide membranes was operated to hydrolyse the substrate, (*R*, *S*)-naproxen ester, dissolved in isooctane at 30 °C. Although the conversions were low, the effects of transmembrane pressure and emulsion were revealed. Compared with the batch stirred tank free-enzyme reaction, the enzymatic reactor exhibited higher activity per mass of protein.[65,72] Initially, a high concentration of racemic naproxen methyl ester was resolved by CCL in a polyacrylonitrile multiphase TSP-EMR at high flow rates on both phases.[40] For 36 hs, the hydrolytic rates were 9–14 μmol h^{-1}.

Aboul-Enein *et al*.[75] esterified racemic ketoprofen acid using an alcohol to make (*S*)-ketoprofen acid. (*R*)-ketoprofen acid dissolved in a dichloropropane-hexane mixture (20 : 80 v/v) was converted to an ester by *Candida antarctica* lipase B immobilized on sponge layers of polysulfone HFM. High conversion (73%) and enantiomeric excess of product (87.8%) were obtained after 24 h at 40 °C. The TSP-EMR also gave better performance than a batch stirred tank free-enzyme system.

A cardiovascular drug intermediate, racemic glycidyl butyrate, was treated with porcine pancreatic lipase.[40,56] Subtractive resolution of the racemate recovered 60% of the (*R*)-ester with 96.7% enantiomeric excess after 9.3 h operating at 28 °C using polyacrylonitrile HFM. During the process, both enantiomers were hydrolysed, but the (*S*)-ester reacted faster, and more than 98% of the (*S*)-enantiomer was converted.

Organic acids

The enzymatic resolution of organic acid esters gave high enantiomeric excess, but the technology has rarely been applied. Racemic 2-hydroxy octanoic acid esters were hydrolysed by *Pseudomonas cepacia* lipase immobilized on the spongy layers of three different membranes.[53,78] The process, conducted in isooctane or hexane at 30 °C, gave enantioselectivity of 12–86% and product recovery of 30–67% in which the hydrolysis of the acid butyl ester led to higher enantioselectivity than the acid methyl esters.

Amino acids

Various strategies have been developed to study resolution of tyrosine compounds. These involve type of reaction, multiphase or extractive operation, cross-flowing or cross-linking the enzyme and the solvent type. (*R*, *S*)-*N*-benzoyl-L-tyrosine ethyl ester (BTEE) was hydrolysed in an extractive TSP-EMR by α-Chymotrypsin immobilized on the shell-side of polyacrylonitrile (PAN) HFM by the diffusive method.[40,74] 200 mmol L^{-1} of substrate dissolved in buffer solution at pH 7.8 was pumped through the lumen-side at 1000 mL min^{-1}, and silicone oil was maintained in the shell-side at a constant pressure of 9 psi. The product, *N*-benzoyl-L-tyrosine acid, recovered in the aqueous phase indicated that the module activity was 80 μmole min^{-1}. It was found that lower substrate flow rate produced lower activity. A multiphase TSP-EMR was also used to hydrolyse racemic BTEE using the same enzyme (immobilized by the cross-flow filtration method) and HFM module. In this case, 10 mmol L^{-1} BTEE in amyl acetate was recirculated in the shell-side at 10 mL min^{-1} and a constant pressure of 6.5 psi, and 200 mmol L^{-1} phosphate buffer at pH 7.8 flowed in the lumen side of the TSP-EMR. This process gave a reaction rate of 45 μmole min^{-1}. Later, the BTEE in *n*-octanol (40 mmol L^{-1}) run in the shell-side at 500 mL min^{-1} was hydrolysed by the same enzyme attached to the shell-side of the same TSP-EMR module by cross-linking it with bovine serum albumin and glutaraldehyde. A high reaction rate was obtained (700 μmole min^{-1}). Finally, the same enzyme was cross-linked to surfaces of the PAN HFM with a 2.5% glutaraldehyde solution in 50 mmol L^{-1} phosphate buffer at pH 7.[40] A lower reaction rate than the previous experiments was obtained. Hydrolysis of (*R*, *S*)-*N*-acetyl-L-tyrosine ethyl ester (ATEE) was conducted using the α-Chymotrypsin and polyacrylonitrile module.[40] 2.86 mmol L^{-1} ATEE dissolved in *n*-octanol was flowing at 400 mL min^{-1} on the shell-side of the module, while 100 mmol L^{-1} K_2PO_4 was fed to the lumen-side at the same flow rate. Cross-linking of the enzyme with 2.5% glutaraldehyde in 50 mmol L^{-1} phosphate buffer at pH 7 produced a low reaction rate.

Esterification of racemic *N*-benzoyl tyrosine (BT) was also studied.[40] The α-Chymotrypsin was immobilized by cross-linking it with 2.5% glutaraldehyde in 50 mmol L^{-1} phosphate buffer at pH 7 on the shell-side of the polyacrylonitrile HFM. 200 mL *n*-octanol mixed with 87 mL ethanol was flowed in the shell-side at 400 mL min^{-1}. The reaction was started by introducing a solution of 4.85 g of BT in phosphate buffer at pH 6 in the lumen-side at the same flow rate. The BTEE was produced at 10% conversion after 120 h.

Other racemic esters

Ceynowa and Koter[79,80] applied the TSP-EMR to select particular enantiomers of racemic alcohol. (*R*, *S*)-1-phenylethyl propionate dissolved in *n*-heptane and phosphate solution at pH 8.0 were circulated inside polyamide HFM as shown in Fig. 5. The substrate was hydrolysed to (*R*)-1-phenyl ethanol by *Pseudomonas sp*. with 55% conversion and 99% enantiomeric excess of the substrate.[79] An innovative step was performed in the process: the unreacted compound ((*S*)-enantiomer) was transported across the HFM to the aqueous phase. Later, racemic 1-phenylethyl acetate and (*R*, *S*)-1-phenyl-1-propyl acetate were hydrolysed to their alcohols in polyamide membranes at 30 °C. These multiphase operations produced 40–60% conversion of their (R)-esters.

Hydrolysis of (*R*, *S*)-octyl 2-chloro propionate was done without solvent.[40,41] 210 g of the racemate was recirculated at 400 mLmin^{-1} in the shell-side of the PAN HFM, which housed *Candida* L1754 immobilised by the cross-flow filtration method. 50 mmol L^{-1} K_2PO_4 solution was flowed in the lumen-side at the same flow rate. 41% of the ester was hydrolysed during 6.8 days operation. A higher result was obtained when racemic butyl 2-chloro propionate was hydrolysed using the same module, enzyme and immobilization method.[40]

Reactions of non-chiral substrates

Esterification of decanoic acid

The gas phase was used instead of the aqueous phase during the esterification process. Constant humidity air was flowed in the shell-side of cellulose dialyzer HFM to control water activity (a_W) as illustrated in Fig. 6.[82] After immobilization of the enzyme in the lumen-side, decanoic acid and dodecanol in hexane were

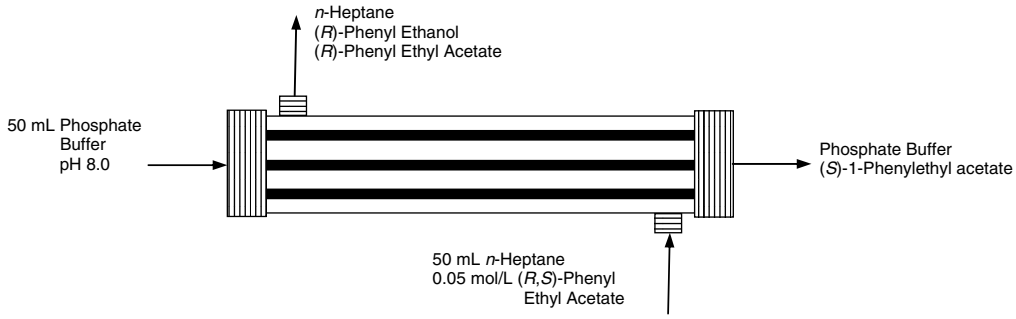

Figure 5. Hydrolysis of (*R*, *S*)-1-phenylethylacetate.

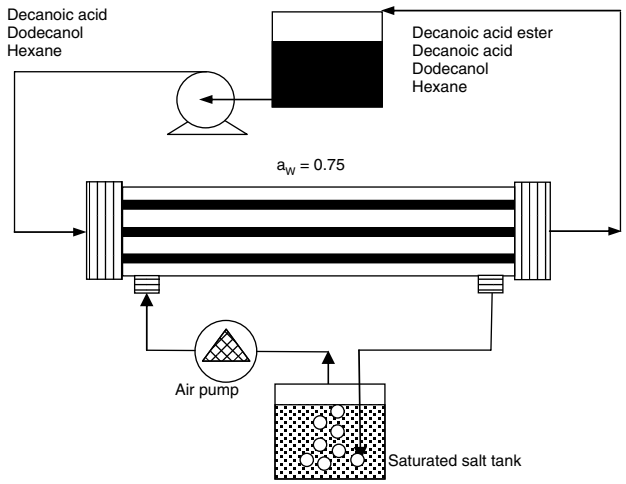

Figure 6. The TSP-EMR for esterification of decanoic acid.

reacted where the formed moisture was delivered to the gas phase and freed into saturated salt solution. By controlling a_W, the ester product dissolved in the organic phase was obtained at 97% yield in 120 h, while the experiments without a_W control produced 84% yield. Under continuous operation, the yield decreased to less than 50% at a constant a_W of 0.75 caused by diffusion limitation at the membranes. Increasing the enzyme load or varying a_W did not affect mass transfer coefficients significantly.[83]

Previously, a unique strategy was developed.[84] The organic phase (decanoic acid in hexadecane, lumen-side) and aqueous phase (water containing D-sorbitol, shell-side) were flowed continuously inside Cuprophane HFM where the enzyme was immobilised in the lumen-side. The reaction occurred when sorbitol contacted the lipase-decanoic acid complex. The sorbitol was transported from the aqueous phase to the membrane surfaces and the ester product was moved to the organic phase, but the moisture dissolved in the aqueous phase. Activity of the immobilized enzyme was stable after 570 h. The esterification took place at higher reaction rates compared with the batch stirred tank free-enzyme system.

Hydrolysis of oils

Hydrolysis of oils in the two-liquid phase membrane reactor was considered a more cost effective tool.[135] Olive oil, palm oil, corn oil, butter oil and babassu oil were successfully converted to fatty acids in the biphasic-EMR.

Recent olive oil hydrolysis produced 225 mol m^{-3} of oleic acid and less than 50 mol m^{-3} of palmitic acid in 15 h using *Mucor miehei* lipase adsorbed on the polysulfone HFM.[76] Song *et al.*[108] obtained 7.1–23.45 mol m^{-3} h^{-1} of oleic acids when the CRL on the polyacrylonitrile was used at 30 °C for 12 h. Previously, 0.074 mol m^{-3} h^{-1} fatty acids were obtained using CRL immobilized onto modified polypropylene surfaces, which was still stable after 10 successive runs.[48] When the oil was hydrolysed by CRL on polyamide membranes,[77,114] lower fatty acids resulted. It was found that, using the same HFM module, the cross-flow CCL produced higher conversion than the cross-link CCL.[40]

Hydrolysis of palm oil gave approximately 6 mol m^{-3} h^{-1} of palmitic acid, but the quantity of oleic acid was almost negligible.[76] Yields of 51.8–99% were obtained with 0.059–0.326 mol L^{-1} oil concentrations by employing CRL attached to the Cuprophane HFM at a reactor residence time of 0.067 h where the immobilised lipase retained 85% of its original activity after 20 operating cycles (137 h).[38] Lower results (40–50% yields) were achieved using the same HFM and lipase after 2 h reaction time, although the lipase was stable for 120 h.[35] Compared with the batch stirred tank free-enzyme system, oil hydrolysis in the TSP-EMR showed higher conversion and reduced back reaction, but faster equilibrium was achieved in the batch system.[38]

The hydrolysis of corn oil generated 50% yield of linoleic acid after 4 h of reactor time, where the presence of the oil in the shell-side of polypropylene membranes increased fatty acids production.[118] Complete conversion of substrate was obtained when babassu oil was hydrolysed by *Mucor miehei* lipase immobilized on the sponge layers of polyetherimide membranes.[136]

Chiral product preparation

Hydrolysis of a stereoisomer molecule, *cis*-cycloxex-4-ene-1,2-dicarboxylate, to an enantiomer, (1*S*, 2*R*)-cyclohex-4-ene-1,2-dicarboxylate, was catalysed by an esterase immobilized on polysulphone membranes.[85,86] This multi-gram scale operation using hexane yielded 100% conversion and more than 97% enantiomeric excess. Activity of the enzyme was retained for 25 days. Higher reaction rates were obtained in the TSP-EMR than the batch stirred tank free-enzyme reactor.

Hydrolysis of ethyl butyrate and amyl acetate

Conversion of ethyl butyrate to butyric acid used a no-added solvent operation. The ethyl butyrate was circulated in the shell-side of the TSP-EMR where porcine liver esterase was immobilized, and 200 mmol L^{-1} PO_4 buffer at pH 8 was pumped in the lumen-side.[40,56,74] The reactor productivity was approximately 450 kg $year^{-1}$ m^{-2} membrane area.[56] During operation, the presence of butyric acid in the aqueous phase decreased pH; hence

6.0 mol L^{-1} sodium hydroxide solution was added continuously. Similar to this process, hydrolysis of amyl acetate was also a no-solvent operation using the polyacrylonitrile membrane to dock *Candida* L1754 enzyme.[40,74]

APPLICATION OF TSP-EMR: SUSPENDED ENZYMES

Kinetic resolution of racemic ibuprofen ester

An extractive type of TSP-EMR was used to resolve the racemic sulfomethyl ibuprofen.[41] The racemate dissolved in sodium phosphate buffer at pH 7 was fed to the shell-side of a cellulose HFM module, while hexane flowed in the lumen-side. The hydrolytic reaction took place at room temperature when 2 g of protease (Prozyme 6) was dissolved in the aqueous phase. After 6.3 h, the quantity of ibuprofen acid recovered in the organic phase related to 50% conversion of the racemic ester. Another attempt used the polyacrylonitrile module where a mixture of cyclohexane–toluene (80 : 20, v/v) was pumped through the membrane shell-side and its lumen-side was filled with sodium phosphate buffer consisting of 48.3 g racemic sulfomethyl ibuprofen. The hydrolysis was run for 6.75 h at 30 °C by adding 3 g Prozyme 6 to the aqueous phase. The reaction gave 48.3% conversion of the racemic ester.

Preparation of aspartame precursor

N-benzyloxycarbonyl-L-aspartyl-L-phenylalanine methyl ester, a precursor of aspartame, was formed from *N*-benzyloxycarbonyl-L-aspartic acid and L-phenylalanine methyl ester in an extractive TSP-EMR using a Sepracor MBR-500 model 10 HFM module operated at 40 °C.[87] The aqueous phase, 50 mmol L^{-1} acetic buffer saturated with butyl acetate pH 5, was fed to the lumen side of the module at 200 mL min^{-1}. The organic phase was recirculated in the shell-side at the same flow rate and a constant pressure of 0.2 kg cm^{-2}. 3 g of thermolysin was added to the aqueous phase to start the reaction. The process yielded more than 70% conversion and the product purity of 91.7% after 24 h.

Dynamic kinetic resolution of racemic mandelic acid

Dynamic kinetic resolution (DKR) of racemic compounds in the TSP-EMR is still a novel technology. However, the idea to use the TSP-EMR to resolve racemic mixtures was proposed by Matson and co-workers who described the DKR of racemic naproxen ethyl ester[40] and (*R*, *S*)-ibuprofen.[41] Choi *et al*.[42] used two enzymes suspended in each phase to carry-out the DKR of racemic mandelic acid as described in Fig. 7. The organic phase, ethylene dichloride, was used to dissolve the CALB and circulated through the shell-side of 1.05 m^2 vertical Hemophan HFM at 60 mL min^{-1}. 100 mmol L^{-1} phosphate buffer at pH 7.2 containing the mandelate racemase and substrate was pumped at 30 mL min^{-1} through the lumen-side. Both phases were flowed counter-currently. Esterification of the (*R*)-mandelic acid with ethanol occurred in the organic phase; therefore the racemate should cross the membranes to the reaction sites. The unreacted enantiomers, (*S*)-mandelic acid, was transported back to the aqueous phase and racemised by the racemase to give the (*R*, *S*)-mandelic acid. The product, the (*R*)-mandelic acid ester, was obtained in 65% isolated yield and 98% enantiomeric excess after 48 h.

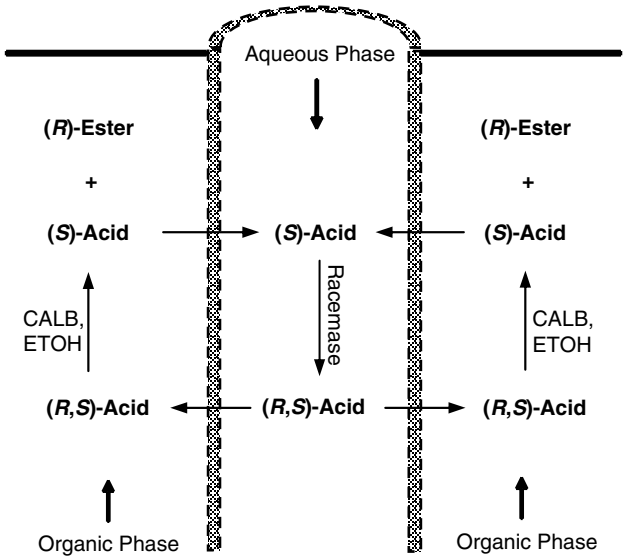

Figure 7. Schematic diagram of mandelic acid DKR.

FURTHER NEEDS

Aspects of the operating conditions, fluid characteristics, substrates and product properties, enzyme and membrane characteristics, type of reaction and type of operations are focused on during the development of the TSP-EMR. Several innovative steps have been introduced such as the use of emulsion and DKR.

Although the DKR has been introduced, the racemic compounds are generally resolved by kinetic resolution (KR). The TSP-EMR still concentrates its KR applications on the hydrolytic reactions. The hydrolytic kinetic resolutions utilize the ester forms of the racemates. Hence, preparation of the raw materials through chemical esterification of the racemates should be conducted before they are fed to the resolution reactions. Although esterification-based TSP-EMR has been introduced, a comparison between the hydrolysis and esterification results has not been stated clearly.

Compared with batch stirred tank processes using free enzymes, the TSP-EMR performs better. It has better operational stability, overall lipase activity and product enantiomeric excess, longer enzyme half-life and lower enzyme load.[33,75] Catalytic activity of the TSP-EMR immobilized enzymes is much more stable than that of the free-enzymes[50,52] and gives higher observed activity per mass of protein.[51] However, the free enzymes generally have higher enantioselectivity than the TSP-EMR immobilized enzymes.[52,115] Higher free enzyme loading was required for the esterification process to achieve performance comparable with the TSP-EMR,[75] but in chiral hydrolysis the free enzyme processes use lower enzyme quantity to achieve 100% product enantiomeric excess.[24,51]

One of the focuses of TSP-EMR operations is distribution of materials. All the existing reactions take place at the organic interfaces, thus the substrates are frequently circulated in the organic phase. After the reaction, the substrates and products are usually distributed in the reaction medium; however, when racemic compounds are used, unreacted enantiomer exists in the medium. Hence, racemic resolution in the TSP-EMR is more difficult than with the non-racemic substrates. The final product(s) should be either the reaction product or the unreacted enantiomer, i.e. the output should be chosen in the beginning.

Many TSP-EMR processes recover the target molecule in the aqueous phase. Since the product transport determines the transmembrane pressure, controlling the materials flow is most important. Some optional strategies can be observed in Table 3.

Table 3. Optional strategies in the TSP-EMR operation

	Description	Solubility: Organic phase	Solubility: Aqueous phase	Transmembrane pressure	Products recovery	Remark(s)
Substrates	Solid: Cannot be used directly. Use dissolved operation	√	√	Organic → Aqueous or Aqueous → Organic	Aqueous phase or organic phase	Preferable: high solubility in organic solvent
		√	X	Organic → Aqueous	Aqueous phase	
		X	√	Aqueous → Organic	Organic phase	Transport substrates to the organic interfaces
	Liquid: Dissolved or no-solvent operation	√	√	Organic → Aqueous or Aqueous → Organic	Aqueous phase or organic phase	With or without organic solvent. High solubility in organic solvent is preferable.
		√	X	Organic → Aqueous	Aqueous phase	
		X	√	Aqueous → Organic	Organic Phase	
	Distribution	Both	X	Organic → Aqueous	Aqueous phase	
		Main	Reagent	Aqueous → Organic	Organic Phase	Reagent is moved to the organic interfaces
		Reagent	Main	Aqueous → Organic	Organic Phase	Main substrate is moved to the organic interfaces
Product(s)		√	√	Organic → Aqueous	Aqueous phase	High solubility in aqueous phase, preferably not dissolved in organic phase
		√	X	Aqueous → Organic	Organic Phase	
		X	√	Organic → Aqueous	Aqueous phase	
Membrane	Hydrophilic	Shell	Lumen	Organic → Aqueous or Aqueous → Organic	Aqueous phase or Organic phase	
	Hydrophobic	Shell	Lumen	Organic → Aqueous	Aqueous phase	
Immobilized enzyme (at the organic interfaces)		Shell	Lumen	Organic → Aqueous or Aqueous → Organic	Aqueous phase or Organic phase	

The substrates, products and membrane characteristics affect development of the flow direction.

It was proved that enzymes immobilized in the TSP-EMR could be used for many cycles of TSP-EMR operation with the enzymes still retaining high catalytic activity. This is, of course, a benefit for the operation, which overcomes the problem of enzymes recycling faced by the batch stirred tank free-lipase system.

It is likely that some factors need further study. Although the TSP-EMR has used emulsion in its operation, which indeed showed better performance than the classic type,[29,113] this innovation still requires more investigation.

Combinations of reactor orientation and fluid direction have been investigated. Vertical orientation requires a high pressure input.[108] As can be observed in Table 1, vertical reactors required a similar TMP to the horizontal type, but no TMP has been operated in the vertical arrangement and further investigation of this should be considered. A comparison of the fluid direction should also be studied further: although counter-current flow gave higher productivity, the co-current TSP-EMR was still favoured for achiral compounds and upflow-type reactors.

Solvent-based operation is preferable as many products are available in crystal and liquid form. However, no-solvent operation could reduce the operational cost, although it can only be applied to liquid non-viscous substrates. Although buffer solutions are frequently used as the aqueous phase, the opportunity exists to develop TSP-EMR based on ionic liquids since they have become alternative media for enzymatic enantioselective reactions.[137,138] These solvents have been associated with resolution in the EMR. A supported liquid membrane containing an ionic liquid was employed to resolve racemic ibuprofen.[139] The ionic liquid allowed enantioselective transport of the ibuprofen enantiomer across the membrane reactor.[132] Ionic liquids increased the selectivity and activity of the enzymatic membrane process.[140]

The cross-flow filtration technique is commonly used to adsorb enzymes onto the HFM surfaces. The technique circulates the enzymes solution only in one direction: from the shell-side to the lumen-side or vice versa as shown in Fig. 4. Efforts have been made to immobilize enzymes on both HFM sides. No explanation is given as to whether both sides immobilization would develop a higher immobilized enzyme quantity.

CONCLUSIONS

Compared with batch stirred tank processes using free-enzymes, the TSP-EMR gave better results. Many factors contributed to a successful TSP-EMR including characteristics of the organic solvents, moisture content and pH, and type of aqueous phase, since they influence the activity of enzymes. The solubility of substrates and product(s) should be taken into account since many enzymatic reactions take place in the organic solvent while the product(s) is generally recovered in the aqueous phase. Use of no-added organic solvents, emulsion environment operation and the gas phase instead of aqueous phase have all been investigated. The preferred method to immobilize the enzyme

is physical adsorption through cross-flow filtration where the enzymes, which can be utilized for many cycles, should be prepared well prior to immobilization because the initial source of the enzymes influences the performance. Most TSP-EMR use hydrophilic hollow fibre membranes, however, the combination of hydrophilic–hydrophobic membranes has beeen developed, to accommodate higher enzyme load. Relatively limited research effort has been applied to date and further study of all relevant factors is necessary.

TSP-EMR have processed racemic and achiral compounds, with applications focused on hydrolytic reactions, but esterification-based TSP-EMR has been introduced. Although a dynamic kinetic resolution process has been used, hydrolytic kinetic resolutions are often applied to racemic esters. Operating is at mild temperatures, relatively long reaction times, low transmembrane pressures and medium flow rates in the shell- and lumen-side to give low–high conversions and medium–high selectivities.

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